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Evaluating Risks of Paramyxovirus and Coronavirus Emergence in China

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DECLARATION

I hereby declare that whilst registered for a research degree at Kingston University London I have not been a registered candidate or an enrolled student at any other academic or professional institution.

I declare that the materials contained in this thesis have not been used in any other submission for an academic award. All the sources of investigation have been duly acknowledged.

I have composed this thesis myself and all results presented in this thesis are from my own investigations.

Aleksei Chmura

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31 March 2017

ABSTRACT

Viruses such HIV, SARS Coronavirus, Ebola, and influenza A virus pose significant burdens globally to human health due to their continued emergence from wildlife reservoirs such as birds, bats, and rodents. In southern China, animal markets, wildlife trade, and human activity create unique opportunities for zoonotic emergence as wild animals frequently come into contact with domestic animals and humans. Influenza A H7N9 virus and SARS emerged from live and wild animal markets in south China where frequent mixing and high volume of species enabled rapid viral evolution and emergence. This research aims to examine zoonotic viral emergence by testing the following hypotheses:

- there are novel Coronaviruses (CoVs) and Paramyxoviruses (PMVs) in wildlife reservoirs in south China
- human behaviour may result in viral spillover from wildlife reservoirs
- market and other wildlife trade activities may be drivers of disease emergence
- CoVs and PMVs circulating in wild animal populations may be closely related with or ancestral to known pathogenic viruses.

Over a five-year period, oral and anal swabs and blood samples were collected from 3,146 wild-caught bats and 559 rodents from more than 30 different species primarily focused on wildlife trade pathways in southern China. RT-PCR assays were performed to screen for CoVs and PMVs. 39 CoVs (1.2%) and 114 PMVs (3.6%) were confirmed from bat samples by sequencing. Of the latter, 80 were novel bat PMVs. No rodent samples were positive for either virus.

An online survey was conducted to assess 2,238 Chinese millennials' attitudes about wildlife consumption and perceived health-risks. The results suggest that although this population is currently the primary driver of demand for wildlife trade in China, it may also be the most effectively targeted with campaigns to educate about zoonotic emergence from wildlife reservoirs.

Diverse mammalian wildlife species including two listed by IUCN as vulnerable and one on China's endangered species list were observed to be maintained in circulation over three years in two of the largest live animal wildlife markets in south China. An overall increase in the volume of wildlife traded in the two markets was also observed. Through 87 ethnographic interviews and 685 structured interviews with rural residents observed to be exposed at some level to wildlife, strong evidence was provided that local consumption of wildlife has reduced, but exposure and awareness

of the commensurate health-risks have not. Additionally, exposure to bats, poultry, and rodents as well as handling, hunting and raising animals were all activities positively correlated with self-reported symptoms of viral infections of unknown aetiology and potentially of zoonotic pathogens.

The information garnered in this study about the current status of wildlife trade, people's attitudes and actions, as well as the ecology of these viruses and their hosts in south China, provides data that may be used towards predicting and preventing emergence of these and other as-yet-unknown viruses. If patterns of human behaviour, wildlife trade, and viral ecology may be quantified, then a relative level of risk may be predicted and evaluated.

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LIST OF ABBREVIATIONS

bp	base pairs
CoV	Coronavirus
EID	Emerging Infectious Disease
EVD	Ebola Virus Disease
HeV	Hendra Virus
HIV	Human Immunodeficiency Virus
IUCN	International Union for Conservation of Nature
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
MojV	Mojiang virus
NCBI	National Center for Biotechnology Information
NiV	Nipah Virus
PCR	Polymerase Chain Reaction
PMV	Paramyxovirus
PPE	Personal Protective Equipment
RMB	Renminbi or the official currency of the People’s Republic of China
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
SL-CoV	SARS-like Coronavirus

1 Introduction

1.1 Emerging Infectious Diseases

The world is increasingly interconnected. A world wherein a pathogen such as a virus, bacterium or other disease causing agent can infect a human, a mosquito, a dog, a snake, or even a plant and then travel by boat, plane, car or some combination thereof from western Africa to London in less than 12-hours. In fact, this has been observed retroactively in early 2003 when Severe Acute Respiratory Syndrome (SARS) coronavirus spread by infected individuals from mainland China to Hong Kong and then as far as Toronto in Canada (WHO 2003b). West Nile virus did this too in 1999 spreading from somewhere in Africa or Europe to the Americas for the first time and likely as a hidden passenger in an infected human host arriving in the New York City area and then being transmitted to mosquito, human, and avian reservoirs (Nash *et al.* 2001; Gubler 2007). Four or more decades earlier the Human Immunodeficiency Virus (HIV) made the same pandemic and initially silent leap out of Africa (Huminer *et al.* 1987; Jones *et al.* 2008; Morens *et al.* 2009; Morse *et al.* 2012).

Diseases such as SARS, West Nile virus, and HIV and others that are changing their patterns of transmission are termed emerging infectious diseases and are defined as having recently increased in incidence, shifted host population, are caused by recently evolved strains, or have changed in their pathogenicity (Henderson 1993; Krause 1994; Daszak *et al.* 2000). Emerging infectious diseases can have significant effects on human mortality and morbidity such as the 1918 influenza pandemic that resulted in over 50 million deaths globally (Johnson & Mueller 2002). Other influenza outbreaks H1N1, H5N1, H2N2, H7N9, and others (all subtypes of the influenza A virus) have occurred since, but none yet with such devastating effects as the 1918 pandemic (Lai *et al.* 2016). Avian influenza A virus subtype H5N1 alone has caused 452 confirmed deaths globally since 2003 and about twice that number in morbidity (WHO 2017).

More recently discovered emerging infectious diseases such as HIV and SARS Coronavirus also threaten human health with high mortality. It is estimated that from its initial identification in the 1980s through 2015 as many as 78 million people have been infected and 40 million people have died from AIDS-related causes (UNAIDS 2016). SARS Coronavirus emerged in late 2002 and 2003 rapidly spreading across 29 countries, infecting almost 7,000 people and resulting in 500 deaths (WHO 2003a). Other emerging infectious disease epidemics such as Nipah virus in Malaysia, Bangladesh, and India (Clayton 2017); Ebola in Western and Central Africa (Peters & LeDuc 1999); and Middle East Respiratory Syndrome (MERS) Coronavirus (Cotten *et al.* 2013), though not pandemic, have also resulted in localised mortality and morbidity.

Emerging infectious diseases such as Chikungunya (Silva & Dermody 2017) and Zika (Bogoch *et al.* 2016) have more recently crossed the Atlantic from the Old World and begun to cause public health issues in the Americas. Still other emerging infectious diseases such as morbilliviruses result in outbreaks and die-offs in marine mammals (Osterhaus *et al.* 1995) and other viruses such as Bluetongue (Sperlova & Zendulkova 2011), Schmallenberg (Beer & Conraths 2013; Wu *et al.* 2014a), and avian Influenzas (Shriner *et al.* 2016) cause significant domestic animal morbidity and mortalities.

In 2008, the first global effort to evaluate regions that had the highest risk of emerging infectious disease was published (Jones *et al.* 2008). This research generated maps of the highest risk locations or 'hotspots' for disease emergence and has since been updated (Figure 1) (Morse *et al.* 2012).By targeting disease surveillance and control efforts in these high-risk regions, public health efforts may pre-empt disease emergence rather than merely react post-emergence (Morse 1995; Daszak *et al.* 2001).

1.2 Economic Impact of Emerging Infectious Diseases

As a group of pathogens, emerging infectious diseases (EIDs) cause a large number of deaths annually and some such as HIV, SARS, influenza A, and Ebola have cost the global economy tens of billions of dollars (Keogh-Brown & Smith 2008) in public health costs, but also in tourism and trade revenues (Figure 2) (Newcomb 2009). Even emerging viral diseases of agricultural crops such as Plum pox virus have caused around USD$10 billion in damage to stone fruits over the past 30 years (Cambra *et al.* 2006). Some studies estimate the total cost of coordinated global efforts to prevent and control emerging infectious diseases as between USD$300 and USD$500 billions (World Bank 2012; Pike *et al.* 2014). Despite the great social, demographic, and economic impact of EIDs and billions of dollars spent on drug and vaccine development to control them (Thanassi & Schoen 2000; Hotez *et al.* 2004), there has been little advance in understanding the underlying process of the emergence of infectious diseases and in developing predictive approaches to their prevention (Daszak *et al.* 2000). In particular, more work is required to detail the ecology of the EIDs and the causes or drivers of their emergence. Plausible, predictive models of current and future disease emergence risk may be built upon knowledge of the ecology and pathogen dynamics of an EID (Galvani 2004). Policy and public health decisions may be informed with the results of this sort of modelling.

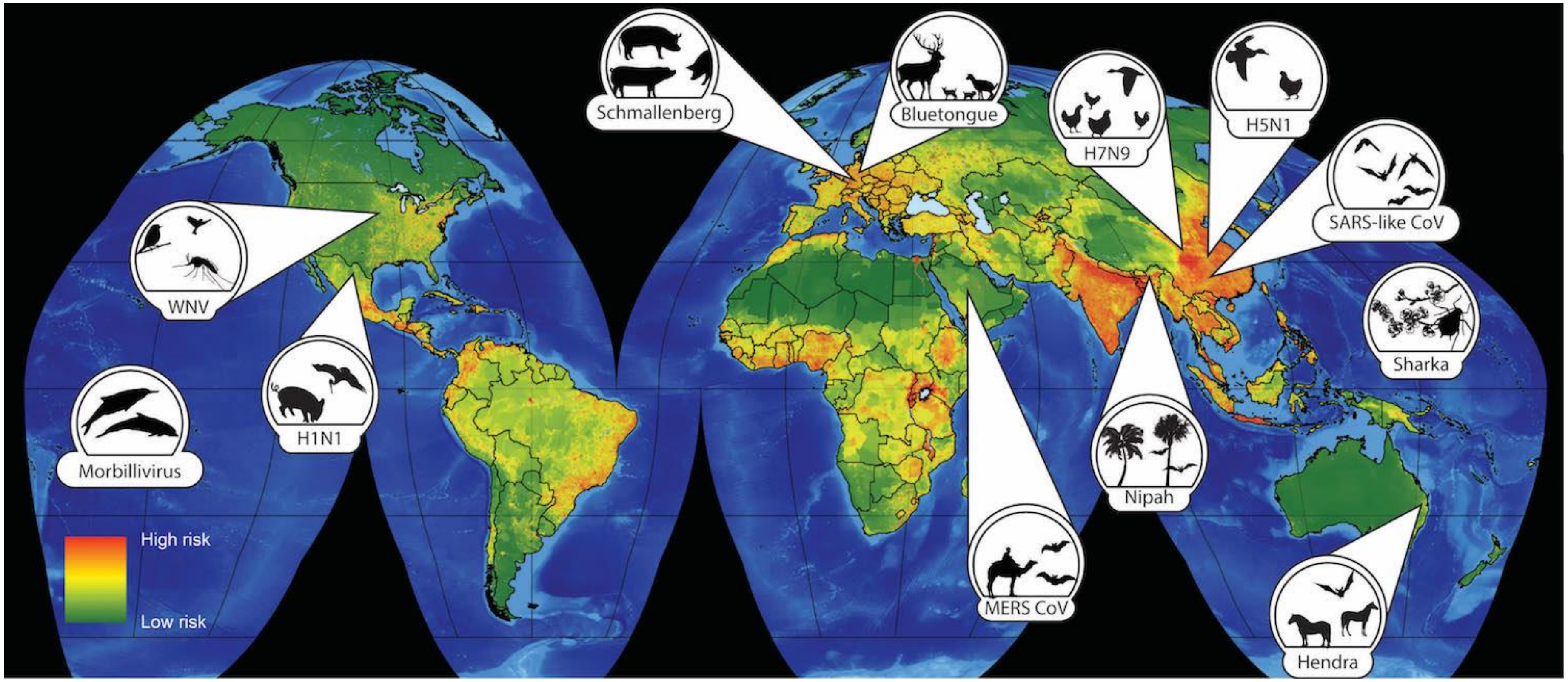


Figure 1. Global Hotspots Map of Emerging Infectious Diseases from Wildlife. Global map with hotspots (red and orange areas) indicative of areas (gridded at 1km^2 resolution) with highest risk of emerging diseases from wildlife modified from Morse *et al*. (2012). The white circles are an overlay of 12 emerging infectious zoonotic diseases of economic, conservation, and public health impact and indicate origins of emergence. From left to right: Morbillivirus, West Nile virus, Influenza A H1N1, Schmallenberg, Bluetongue, MERS coronavirus, Influenza A H7N9, Nipah virus, Influenza A H5N1, Hendra virus, SARS coronavirus and SARS-like coronavirus, and Sharka virus. Details of each are briefly discussed in the text, below.

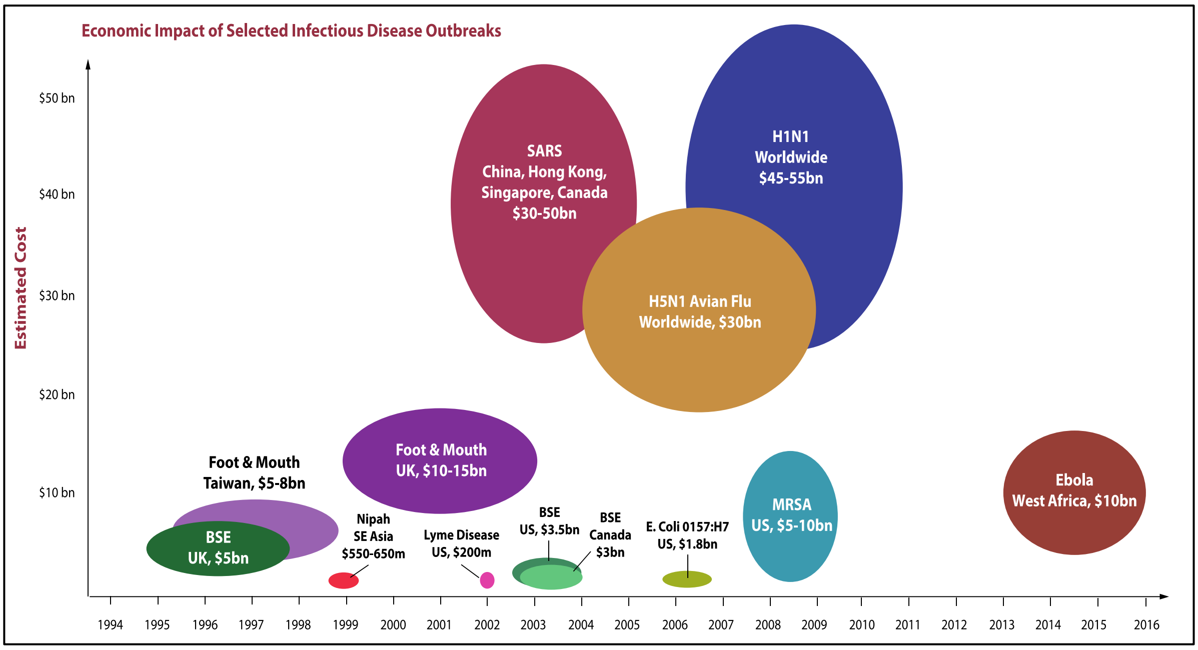


Figure 2. Economic Estimates of Emerging Infectious Diseases. Bubble chart modified from a 2009 bio-era figure (Newcomb 2009) and using World Bank data (World Bank 2012) showing estimated cost in US dollars and duration (years) of Emerging Infectious Disease Outbreaks from Bovine Spongiform Encephalitis (BSE) in the UK in the 1990s to Ebola in West Africa in the 2010s.

1.3 Zoonoses, Public Health, and Security

Compared to endemic diseases such as schistosomiasis, onchocerciasis, and malaria that affect tens of millions of people per year (Hotez 2015; Hotez *et al.* 2015), emerging infectious diseases have relatively low overall morbidity and mortality. Ebola, Nipah virus, Hantavirus, and others, though, can have mortality rates exceeding 50% (Simonsen *et al.* 1995; Epstein *et al.* 2006; Heymann *et al.* 2015). Should these known and unknown emerging infectious diseases with high rates of mortality evolve the ability to spread globally, the results could be potentially disastrous for human health (Morse *et al.* 2012). The majority of emerging infectious diseases are zoonotic (Taylor *et al.* 2001). Of the new emerging and re-emerging infectious zoonotic diseases, 71.8% originated in wildlife (Jones *et al.* 2008). Although emerging zoonoses result from diverse pathogens such as deoxyribonucleic (DNA) viruses (Herpes and Pox viruses), ribonucleic acid (RNA) viruses (HIV, SARS-Coronavirus), bacteria (MRSA), protozoans (Malaria), fungi (cryptococcal meningitis), and helminths (Schistosomiasis), the majority are RNA viruses (Jones *et al.* 2008). RNA viruses' replication strategy is prone to copy-errors or mutations, which results in RNA viruses being rapidly able to adapt to host and environment changes (Elena & Sanjuán 2005).

It has been demonstrated that human-caused or anthropogenic changes are driving the increased emergence and spread of pathogens from wild animals into domestic animals and humans (Daszak *et al.* 2001). Global trade and travel, urbanisation, and agricultural intensification are exponentially increasing interactions among people, wild animals, and domestic animals, which provides not only more, but novel opportunities for pathogens that spill over from animals into humans to then spread globally (Daszak *et al.* 2001). The increased rate of emergence of zoonotic diseases coupled with exponential increases in global travel and trade have resulted in highly pathogenic zoonotic viruses becoming one of the most significant threats to global economy, health, and national security (Cecchine & Moore 2006; Sueker *et al.* 2010).

1.4 Drivers of Zoonotic Emergence

To elucidate the ecology of zoonotic viruses and the likelihood of emergence from their wildlife hosts into domestic animals or humans, it is necessary to develop a framework within which to study the ecology of these viruses in their host species and their interactions with the environment and other potential animal hosts including humans (Wood *et al.* 2012). Understanding the causal factors or the drivers of zoonotic disease emergence (Figure 3) is critical to both predicting and preventing zoonotic emergence or spillover from reservoir hosts to novel host species (Daszak *et al.* 2001; Woolhouse & Gowtage-Sequeria 2005; Morse *et al.* 2012). Repeated opportunities for spillover of zoonotic pathogens are driven by human activities such as agricultural expansion or intensification (Perry *et al.* 2013); deforestation or land conversion; hunting or wildlife trade as part of animal value chains (Smith *et al.* 2017); and urbanisation and global travel (Epstein & Field 2015). Drivers or pathways of zoonotic emergence in human and wildlife populations are “common underlying causal themes” such as climate change, agricultural intensification, deforestation, wildlife trade, and others (Daszak *et al.* 2001). Across a gradient of increasing anthropogenic disturbance from forested areas to intensive livestock production, the risk of zoonotic emergence increases as drivers such as livestock production intensity, land use change, and size of wildlife markets increase (Patz *et al.* 2004; Wolfe *et al.* 2005; Gottdenker *et al.* 2011). Animal value chains have been implicated as the driver of the emergence of SARS coronavirus, HIV, and avian influenza (Webster 2004) with large-scale fowl or pig production playing a key role in the emergence of Avian Influenza (Yassine *et al.* 2013). Animal value chains originate in wild-sourced animals that are brought to consumers and may include animal or wet markets of all sizes; animal warehouses; animal farms; restaurants; and processing sites or abattoirs (FAO 2011). As the animals move through these chains, their value increases from source (wild, forested areas) to sink (end consumer).

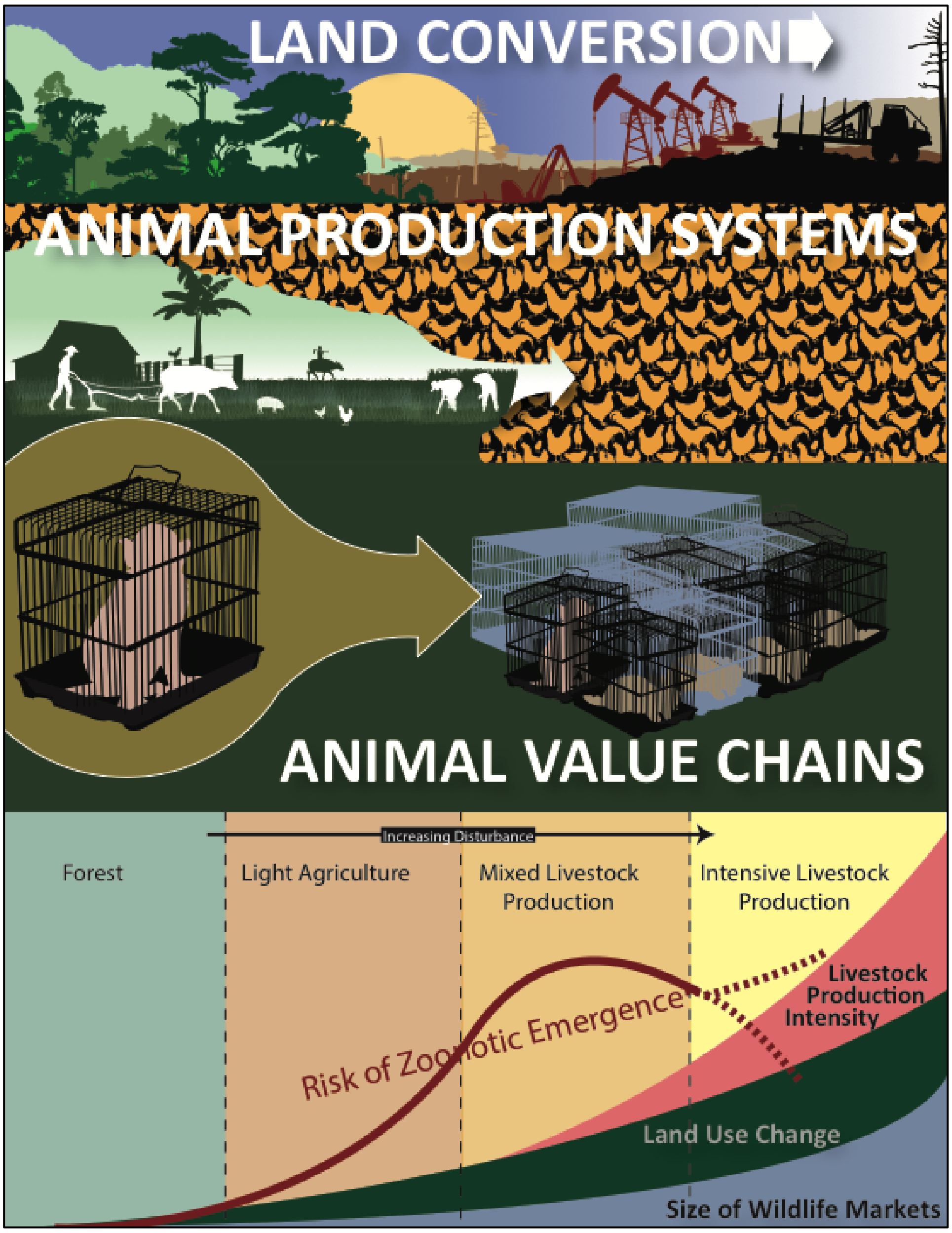


Figure 3. Three pathways of zoonotic emergence. From top to bottom (top three panels) show: land conversion; animal production systems; animal value chains. These pathways drive spillover of zoonoses in different setting and are not mutually exclusive. Bottom panel shows increases in animal production systems (livestock production intensity), land conversion (land use change), and animal value chains (size of wildlife markets) across a gradient of increasing anthropogenic disturbance from forested (left) to highly impacted areas (intensive livestock production). The solid red curved line with two dotted tails indicates the risk of zoonotic emergence. The dotted tails raise the question of what happens to the risk of zoonotic emergence when livestock production, land use conversion, wildlife markets all increase: does zoonotic emergence increase or reduce? Figure created by Chmura and Daszak (unpublished).

The risk of zoonotic emergence from one monkey in one cage in a rural village roadside restaurant is likely different to that from the same monkey species and cage in a closely packed animal market stall along with many other animals including other primates, and in close proximity to humans. Parts of this thesis will examine some of these themes further. Additionally, this thesis will attempt to answer the question of whether the conditions (human behaviour, wild animal mixing, and circulating viruses) that led to the emergence of SARS coronavirus still exist in southern China and whether there may be an ideal setting for the re-emergence of SARS-Coronavirus or some other virus from the same or even another viral family.

1.5 Ecology of Disease Emergence

When viruses emerge or spillover from viral host species these events may result in outbreaks within other species or human populations (Daszak *et al.* 2000). The magnitude and duration of these spillover events or outbreaks depends on the frequency and ease with which the virus is transmitted from host species to novel species (Morse 1995). The escalation from small-scale outbreaks to a broader epidemic is a result of the connectivity between the initially infected individual and other human populations. HIV is a prime example of this sort of emergence. HIV likely emerged in isolated communities in central Africa from chimpanzee reservoirs multiple times throughout human history (Hahn *et al.* 2000) following human contact with chimpanzees as bushmeat (Martin 1983; Kalish *et al.* 2005) or even via chimpanzee predation of humans (Wrangham *et al.* 2000). Despite this low-level, isolated, episodic viral spillover, it was not until early to mid-20th century increases in mining, agriculture, deforestation, international trade, and travel in central and west Africa that HIV began to expand globally and evolve into its present pandemic forms (Wolfe *et al.* 2005; Faria *et al.* 2014).

Understanding the ecology of host species is fundamental to understanding how host and pathogens interact and provides insight to factors that influence infection and transmission dynamics. Figure 4 details the stages of zoonotic emergence. Localised transmission is maintained (bottom panel, left) with endemic viruses transmitted among conspecifics or other species within an ecosystem. Pre-emergence (bottom panel, right) begins when pathogens are transmitted to novel species such as domestic animals or humans following encroachment into wildlife habitat or landuse changes. Minor population level or more major ecosystem level changes can alter the dynamics of microbial transmission and lead to localised pathogen spillover. In the example above, this would be an instance of HIV infecting one hunter after accidental exposure to chimpanzee blood. Highly virulent strains of emerging viruses will burn out once the host population is depleted or immune. Given enough time, emerging zoonotic viruses would be expected to evolve with their novel hosts and natural selection would favour the evolution of low-virulence strains (Murray *et al.* 2015b).

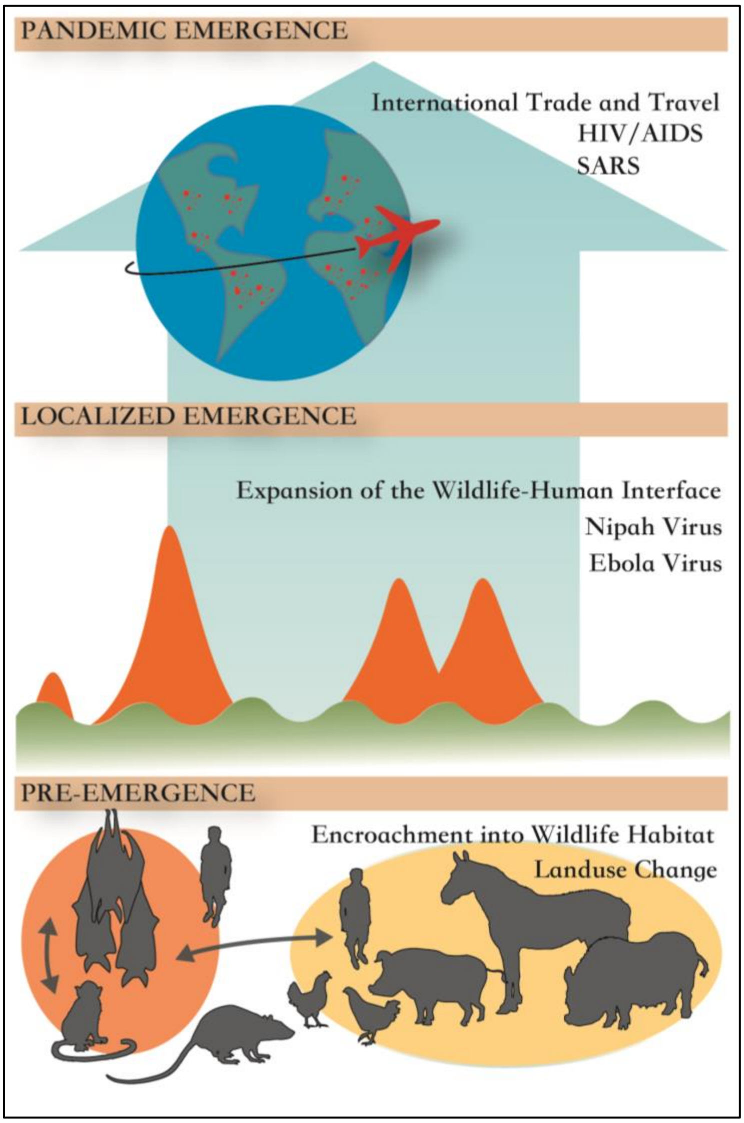


Figure 4. Stages of Zoonotic Emergence. A modified version of this figure was published in Lancet (Morse *et al.* 2012). Original figure credit to Chmura and Daszak (unpublished).

Green peaks and troughs (centre panel) represent the rise and fall in numbers of infected people over time. These localised spillover events or outbreaks are usually self-limiting, but when they are not (orange peaks) then a larger-scale outbreak results in higher numbers of infected people and may lead to some person-to-person transmission of the pathogen. Usually increasing the incidence of contact between humans and wildlife will result in higher incidence of localised emergence resulting in higher numbers of infected individuals and more opportunities for viral evolution in sustained outbreaks. Ebola virus and Nipah virus epidemics are examples of this stage of localised emergence (orange peaks). Some epidemics may spread further resulting in global spread such as with HIV, SARS coronavirus, or influenza A (H5N1); these large-scale epidemics are termed pandemics. Global emergence is usually facilitated by international trade and travel. The arrow indicates the progression from pre-emergence to localised spillover to epidemic to pandemic. The frequency of outbreaks decreases from bottom to top in the figure, so pandemics are infrequent occurrences, but result in high morbidity and mortality. Analysis of the process and drivers of disease emergence may permit a means towards pandemic prediction and aid in implementing preventative measures at the earliest stage where they may be most effective and efficient (Wolfe *et al.* 2007; Morse *et al.* 2012; Pike *et al.* 2014).

1.6 Zoonoses in China

Although there may be more, three broad categories of human activities or drivers of zoonotic disease emergence in China are most notable (Figure 3): land conversion including deforestation to provide timber and agricultural lands (Liu *et al.* 2014; Yang *et al.* 2014a); animal production systems including large-scale poultry farms (Patz *et al.* 2004; Fan *et al.* 2014; Wu *et al.* 2015), and animal value chains with wild animals sourced from forested areas in China or in surrounding countries and transported to different farms, markets, restaurants, and other venues (Daszak *et al.* 2007; Shepherd & Nijman 2007; Zhang *et al.* 2008; Greatorex *et al.* 2016; Lynn 2016). The emergence of pathogens from wildlife and domestic animal hosts is a key concern for public health (Morens *et al.* 2004). The southwest of China is the most rural and biodiverse region of China, yet also has high incidences of neglected tropical diseases (Steinmann *et al.* 2008; Yang *et al.* 2014a). The relatively recent emergence of SARS coronavirus and highly pathogenic avian influenza A subtypes H5N1 and H7N9 has heightened concerns about emerging zoonoses in southeast Asia and especially in China (Subbarao *et al.* 1998; Drosten *et al.* 2003). Other viral zoonotic diseases such as Rabies and Dengue have increased in incidence, perhaps due to increased human population density (for Rabies) and climate change allowing Dengue vectors (mosquitoes) to expand their geographic ranges (Ooi 2015; Zhou *et al.* 2016a). Additionally, emerging diseases including brucellosis, Japanese encephalitis, and other vector (sand fly, tick, or mosquito) borne diseases in China are of increasing concern for global public health (Liu *et al.* 2014).

China is undergoing a rapid economic transformation, with resulting changes to the environment (Wan 1998), demography, sociology, agriculture, and trade – all of which are key drivers of disease emergence (Morse 1995; Morens *et al.* 2004; Weiss & McMichael 2004). China's rapid development has led to increased contact between people and wildlife via expansion of hunting, wildlife trade, forestry, and agriculture (Knight & Ding 2012). From a political and public health perspective, one of the most important emerging zoonotic viruses to have affected China's population has been SARS coronavirus precisely because China and the world were initially so unprepared to respond to the pandemic (Huang 2004).

1.7 Coronaviruses

The *Coronaviridae* is a family of spherical (hence the corona or 'crown' nomenclature) non-segmented positive single strand RNA viruses from the order *Nidovirales* (ICTV 2017b). With genomes of about 30 kilobases in length, these are among the largest and most complex of the RNA viruses yet discovered and are found in a wide range of animal species such as domestic animals, some bird species, rodents, whales, bats, and humans (Siddell *et al.* 1983). In humans, most coronaviruses are respiratory pathogens causing laryngitis (e.g. HCoV-229E, HCoV-OC43, and HCoV-HKU1) and croup (HCoV-NL63) (Fehr & Perlman 2015).

Prior to 2002 and the pandemic emergence of SARS coronavirus and more recently MERS coronavirus, only two human coronaviruses (HCoVs) had been characterised (HCoV- 229E and HCoV-OC43) (Fehr & Perlman 2015). Since then, two more human coronaviruses HCoV-NL63 (van der Hoek *et al.* 2004) and HCoV-HKU1 (Woo *et al.* 2005) were identified in individuals with respiratory infections. These HCoVs may account for up to 30% of respiratory infections in the general population (Fouchier *et al.* 2004; Holmes & Rambaut 2004). Animal and human coronaviruses have been classified into the subfamily *Coronavirinae* (Figure 5) with four genera based on their antigenicity (Rota *et al.* 2003; Mihindukulasuriya *et al.* 2008):

- *Alphacoronavirus*: containing HCoV- 229E, bat, porcine, feline, and canine coronaviruses
- *Betacoronavirus*: with four lineages (A-D) and including SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-HKU1 containing human, bat, murine, bovine, camel, and other mammalian CoVs
- *Deltacoronavirus*: containing avian and porcine CoVs
- *Gammacoronavirus*: containing avian and cetacean CoVs

Coronaviruses have been shown experimentally and in nature to undergo genetic recombination by a genomic template-switching mechanism and to generate genetic point mutations at a rate similar to that of other RNA viruses including influenza A viruses, which suggests a frequency of host switching and zoonotic transmission within the group (Tsunemitsu *et al.* 1995; Saif 2004). A large number of novel CoVs have been discovered in bat species in both the Old and New World (Dominguez *et al.* 2007; Müller *et al.* 2007; Donaldson *et al.* 2010; Quan *et al.* 2010; Rihtaric *et al.* 2010; Wacharapluesadee *et al.* 2015) as well as strains discussed in Chapter 3.

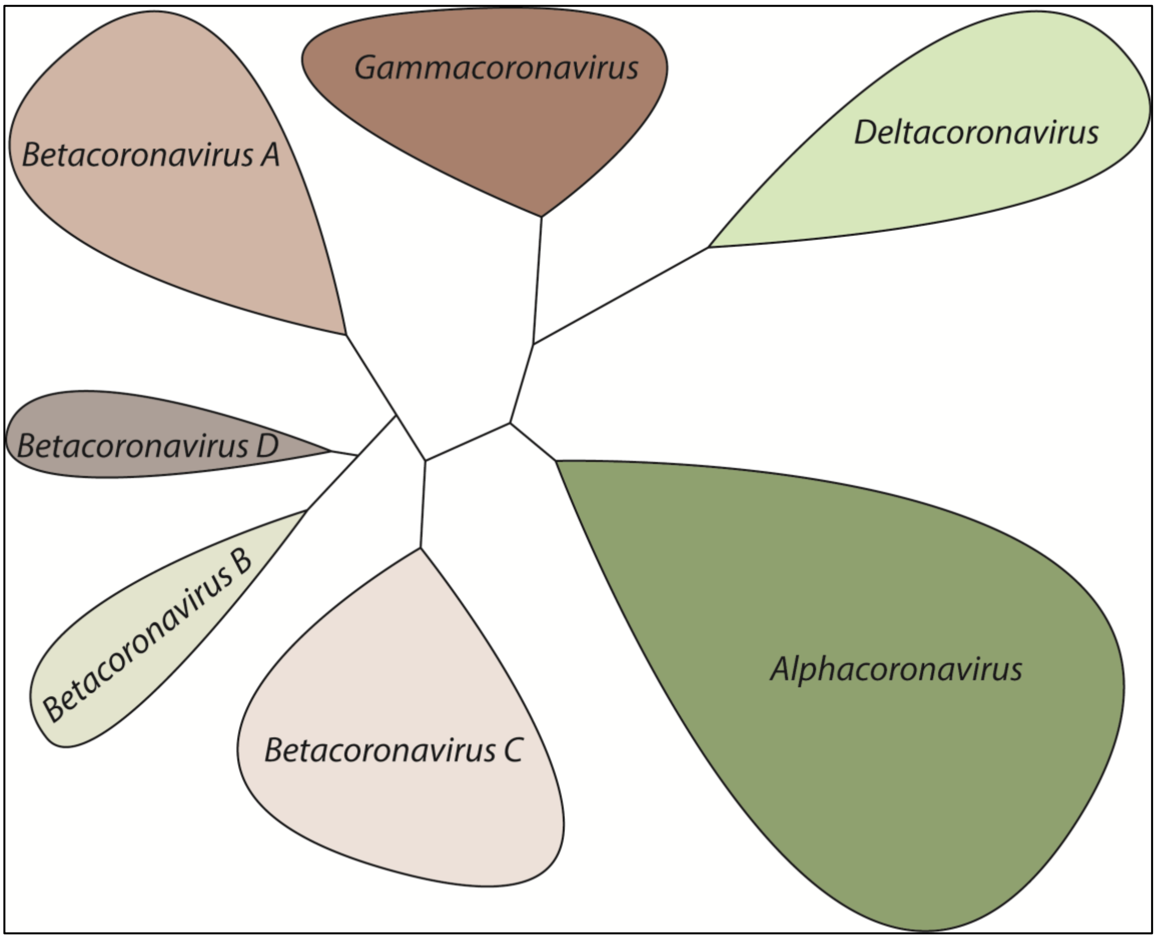


Figure 5. Phylogenetic relationship of the major genera in the *Coronaviridae*family. Modified from Chan *et al*. (2015). Counter clockwise from bottom right: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus.

1.7.1 Severe Acute Respiratory Syndrome Coronavirus

In November 2002, a new respiratory disease, severe acute respiratory syndrome (SARS), was discovered in Guangdong province of south eastern China (Drosten *et al.* 2003). SARS spread rapidly via international travellers, eventually affecting over 8,000 individuals in 32 countries and causing at least 800 deaths in early 2003 (Riley *et al.* 2003; Tsang *et al.* 2003; Anderson *et al.* 2004). In response to this outbreak, Chinese authorities, working with an international collaborative group set up by the World Health Organization (WHO) investigated the outbreak and identified a novel Coronavirus – termed SARS coronavirus (SARS CoV) – as the causative agent (Drosten *et al.* 2003; Guan *et al.* 2003; Ksiazek *et al.* 2003; Kuiken *et al.* 2003; Peiris *et al.* 2003).

During the original SARS-CoV outbreak, the earliest cases reported were from restaurant workers (He *et al.* 2003). By the end of the outbreak in May 2003, assays for IgG antibodies for SARS or SARS-like coronaviruses in humans in the Guangdong region in China yielded highest prevalence among animal market workers, animal traders and particularly in those handling civet cats (*Paguma larvata*) (CDC 2003; Guan *et al.* 2003). These data suggest that those having contact with animals were infected by SARS-CoV first, but that others regularly handling animals had previously been infected with other strains of Coronaviruses, provided strong evidence for animal origins of SARS (Wang *et al.* 2006).

SARS-like coronaviruses (SL-CoVs) were then identified in wild-caught and farm-bred masked palm civets (*Paguma larvata*), one raccoon dog (*Nyctereutes procyonoides*), and one Chinese ferret badger (*Melogale moschata*) in wet markets in Guangdong province, suggesting that these animals (and particularly civets due to their high viral prevalence) were reservoir hosts (Guan *et al.* 2003). However, subsequent surveys did not find virus in wild or farmed civets and experimental infection of civets caused acute clinical signs, which would be unlikely to occur in a wildlife reservoir of a virus (Tu *et al.* 2004; Wang *et al.* 2006).

Since the outbreak, substantial evidence has been published demonstrating that bats are the natural reservoirs of CoVs. This includes (a) high seroprevalence of a wide diversity of SL-CoVs in wild-caught bats; (b) these viruses being phylogenetically close to SARS-CoV; and (c) a phylogeny suggesting that SARS-CoV originates from within this diverse group of SL-CoVs (Li *et al.* 2005; Ge *et al.* 2013; Ge *et al.* 2016a). However, the precise mechanisms, in other words the mix of viral and host ecology as well as human behaviour, by which SARS emerged are still unknown (Graham *et al.* 2013). It is possible that other animals mixing in wet markets played a role either as reservoir or amplifying hosts for SL-CoVs (Tu *et al.* 2004; Shi & Hu 2008). The aim of this thesis is to uncover some of these mechanisms.

The emergence of SARS-CoV is clearly linked to live animal trade in mixed species in wet markets in Guangdong province of south China where there was a rapid expansion of the economy and the recent growth of a large population of relatively wealthy people (Woo *et al.* 2006a). It is unclear whether the trade regulations and changes in human activities have sufficiently altered so that the re-emergence of SARS-CoV or emergence of a SL-CoV is still likely. The dynamics of wildlife trade in southern China need to be evaluated to determine the potential risk of coronavirus re-emergence. Chapter 3 will delve further into the emergence of SARS-CoV, the recent discoveries of novel coronaviruses from work conducted under this thesis, and the ecology of both the virus and its chiropteran host species.

1.8 Paramyxoviruses

Paramyxoviridae are a family of negative single strand RNA viruses of the order *Mononegavirales* with genomes between 15-19 kilobases in length (ICTV 2017a). *Paramyxoviridae* or paramyxoviruses are subdivided into 7 genera: *Aquaparamyxovirus*; *Avulavirus*; *Ferlavirus*; *Henipavirus*; *Morbillivirus*; *Respirovirus*; and *Rubulavirus*. Paramyxoviruses infect a broad range of species and exhibit high viral diversity, which increases the possibility of spillover (Anderson & Wang 2011; Johnson *et al.* 2015). Newcastle disease virus (genus *Avulavirus*) and Rinderpest virus (genus *Morbillivirus*) are pathogenic with high rates of mortality in domestic and wild animals (Alexander 2001; Morens *et al.* 2011). Some paramyxoviruses have high rates of human morbidity such as measles (genus *Morbillivirus*), mumps (genus *Rubulavirus*), and human parainfluenza viruses (genus *Respirovirus*) (Drexler *et al.* 2012).

Other lesser known and recently discovered zoonotic paramyxoviruses such as Nipah virus and Hendra virus in the *Henipavirus* genus pose significant burdens to livestock and human health, with mortality rates of 40% and higher in humans during outbreaks or spillover events (Marsh & Wang 2012). These viruses have been shown to originate in bats, as their reservoir hosts (Halpin *et al.* 2000; Chua *et al.* 2002b).

1.8.1 Hendra Virus and Nipah Virus

In 1994, a novel Paramyxovirus called Hendra virus (HeV) first emerged from fruit bats into infected horses in Queensland, Australia, causing severe respiratory and neurological disease, and occasionally infecting people who work with or were exposed to these horses (Selvey *et al.* 1995). There have been multiple outbreaks of HeV in Australia since, but no evidence of human-to-human transmission and the number of human cases has been low (Field *et al.* 2011). The mechanism of transmission is likely via bat excreta or sputum dropped from roosting or feeding trees into areas where horses are present (Field *et al.* 2016). The reservoir hosts of HeV were shown to be flying foxes of the *Pteropus* genus (Halpin *et al.* 2000). The urbanisation of the *Pteropus* species has been hypothesised to be the driver for the emergence of HeV (Plowright *et al.* 2011). Prevalence of HeV has been shown to be generally low in bats with a winter-time peak in some regions corresponding to equine cases (Field 2016). In 2012 an equine vaccine was developed and has proven highly effective in preventing further spillover of HeV (Field *et al.* 2016), although the challenge now is for veterinary and public health authorities to enforce vaccination of all horses (Peel *et al.* 2016).

Nipah virus (NiV) is closely related to HeV and was first discovered with an outbreak in pigs in Malaysia in 1998 (Chua *et al.* 2000) which caused encephalitis and respiratory disease, as well as abortion, with a mortality rate of approximately 2.5% (Nor *et al.* 2000). During this outbreak, 283 people, mostly (70%) those directly involved with pig farming, were reported infected with a 38.5%

mortality rate (Chua 2003). *Pteropus vampyrus* and *Pteropus hypomelanus* bats were identified as the reservoir for NiV in Malaysia (Rahman *et al.* 2013).

In the outbreak of Nipah virus in Malaysia, pigs were an amplifying host shedding virus in droplets of saliva expelled when coughing (Chua *et al.* 2001). There were no documented cases of human-to human transmission and only humans exposed to pigs were infected, nor was there any evidence of direct bat-to-human transmission (Chong *et al.* 2003; Tan & Wong 2003). Agricultural intensification (pig farming and fruit cultivation) has been implicated in the emergence of NiV in Malaysia (Pulliam *et al.* 2012). Chua et al. (2002a) hypothesise that fruit trees were planted hanging over pig enclosures and their fruits attracted fruit bats. In the process of feeding, the bats would let fall pieces of fruit contaminated with their saliva and potentially with NiV. Pigs then would consume the fallen fruits leading to exposure to NiV. Pulliam *et al*. (2012) showed that repeated spillover from bats and structured intensive production of pigs resulted in a prolonged outbreak and high number of human cases. Since the 1998 outbreak there has been no observed reoccurrence in Malaysia and this is likely due to government enforced surveillance, prohibition of fruit tree planting near livestock, and reduced sizes of pig farms (Lam & Chua 2002).

Viruses similar to NiV were identified in other *Pteropus* species in Australia (Halpin *et al.* 2011), in Asia (Olson *et al.* 2002; Sendow *et al.* 2006; Hasebe *et al.* 2012) as well as in African bats in the same family (Drexler *et al.* 2012; Pernet *et al.* 2014). Despite these viruses and their hosts being endemic across a broad geographic range, Bangladesh is, to date, the only other location where NiV transmission to humans has been recorded, with the first report of spillover near Dhaka in 2001 (Hsu *et al.* 2004; Luby 2013). Outbreaks of NiV encephalitis have since been recorded annually in Bangladesh with mortality between 75% and 100% (Luby *et al.* 2009b; Homaira *et al.* 2010; Pulliam *et al.* 2012). More recently, two confirmed NiV outbreaks were been reported in West Bengal India (Chadha *et al.* 2006).

Unlike in Malaysia, the route of transmission from bats to humans in Bangladesh and India is direct: without an intermediary, amplifying host. In the region, date palm sap is a traditional drink tapped by affixing collecting pots to the trunks of date palm trees (*Phoenix sylvestris*). Using thermal imaging or infrared cameras, Khan *et al.* (2010) recorded *Pteropus* bats feeding from collecting pots at night. The contaminated sap is harvested and consumed by unsuspecting locals (Luby *et al.* 2006). Recent evidence has shown that there are multiple HiVs present both in host species and in humans in the region (Anthony *et al.* 2013; Chowdhury *et al.* 2014).

Paramyxoviruses like Hendra and Nipah virus are of concern to human and domestic animal health because they have repeatedly emerged from wildlife reservoirs, bats, into humans, and other animals. NiV is associated with high mortality and has the ability to be transmitted person-to-person (Luby *et al.* 2009a; Luby *et al.* 2009b). Elucidating the ecology of these zoonoses is crucial in order to develop targeted prevention and predict emergence. Given the right conditions, these viruses or as yet unknown Paramyxoviruses may emerge and result in a larger epidemic or like SARS quickly spread globally and potentially become pandemic.

1.8.2 Paramyxoviruses in China

This research aims to identify novel paramyxoviruses in Chinese bats and test the hypotheses that some of these diverse viruses are not only present in their chiropteran hosts, but may be closely related or even ancestral to known paramyxoviruses of concern to human and animal health such as NiV or HiV. A diversity of paramyxoviruses has already been identified in China (Figure 6) predominantly in bats, but also in rodents, birds, and cats (Li *et al.* 2006; Lau *et al.* 2010b; Woo *et al.* 2012b; Wu *et al.* 2014b; Yuan *et al.* 2014). Chapter 4 will delve further into paramyxoviruses in China including recent discoveries of novel paramyxoviruses from work conducted within this thesis; the ecology of both the paramyxoviruses and the chiropteran host species; and the potential risks of paramyxovirus emergence in human populations.

1.9 Host species

Some wild animals have become well adapted to anthropogenically challenged environments such as urban or agricultural landscapes and these species can be reservoirs for known zoonoses as well as undiscovered or emerging zoonoses. Foxes and raccoons are reservoir species for rabies in North America (Blanton *et al.* 2010). Deer, rats, and monkeys are hosts for various vectors (ticks and fleas) of diseases ranging from Lyme disease, plague, and Kyasanur Forect disease respectively (Kruse *et al.* 2004; Gage & Kosoy 2005; Holbrook 2012). Other animals including bats (NiV) and civets (SARS-CoV) are implicated in viral spillover resulting in epidemic or pandemic emergence.

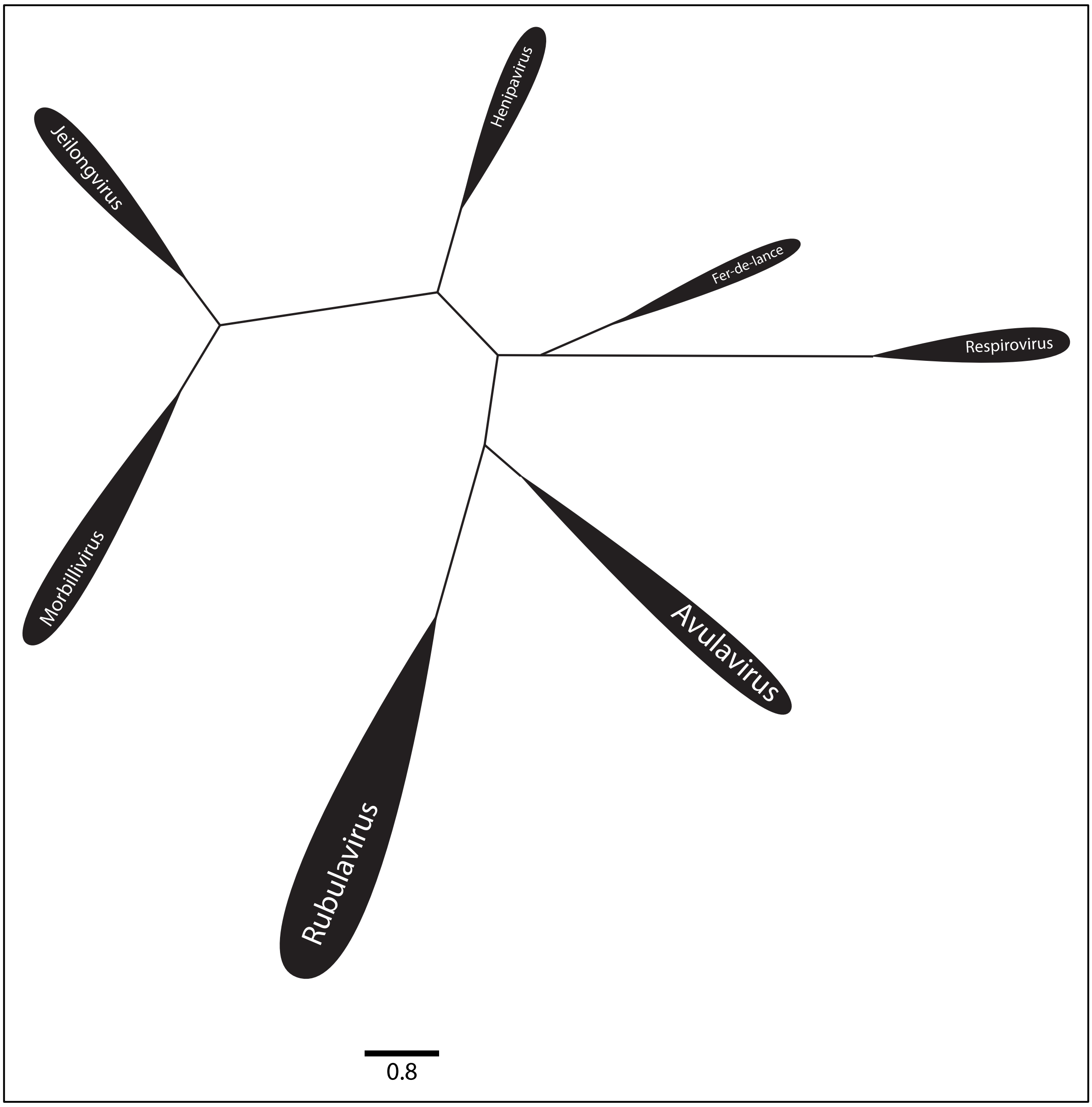


Figure 6. Unrooted, radial phylogenetic tree showing *Paramyxoviridae* genera. Branch lengths represent relative evolutionary distances. *Jeilongvirus* is not a currently recognised genus, but has been recently proposed by Woo *et al*. (2016) and therefore included for illustrative purposes only.

Recent research has demonstrated that viruses with the ability to infect a diverse range of host species are more likely to spread geographically and should be considered at a higher risk of pandemic emergence (Johnson *et al.* 2015). Research suggests that due to anthropogenic drivers an ‘ecological threshold’ has been crossed and this accounts for the global emergence of zoonoses particularly such as coronaviruses and henipaviruses from bats (Field 2009). In Chapter 5, these risks and the wildlife trade as a driver of viral emergence will be examined further.

1.10 Bats, Rats, Primates, and Other Animal Viral Reservoirs

Since most emerging infectious diseases are zoonotic in origin (Taylor *et al.* 2001; Jones *et al.* 2008), and most of these emerge from mammals (Woolhouse & Gowtage-Sequeria 2005), discovery and surveillance efforts to predict and prevent disease emergence should focus on mammalian species. The orders *Rodentia* (rodents), *Chiroptera* (bats), and *Soricomorpha* (shrews and moles) comprise 70% of mammalian species. With the addition of *Primata* (primates, of which humans number), these four orders comprise over 75% of mammalian species and diversity (Wilson & Reeder 2005).

Bats and rodents are the most speciose of mammals and are present on every continent excluding Antarctica and are found in or proximate to every environment in which humans may occur (Wilson & Reeder 2005). Some bat and rodent species are commensal, exploiting human dwellings, constructs, and food resources, leading to direct and indirect contact amongst humans, domestic animals and rodents and bats (Reperant & Osterhaus 2014). Rodent, bat, shrew and primate species are hosts to most of the known zoonoses (Han *et al.* 2016a). Rodents harbour diverse known zoonotic pathogens, are a potential health risk for humans (Mills 2006), and are estimated to host 85 or more viral species, almost three times as many as bats (Han *et al.* 2016a). Bats, though, have been shown to host more zoonotic viruses per species than rodents and due to their volant nature have overlapping and larger home ranges (Luis *et al.* 2013). One example of this is recent evidence showing that bat species with overlapping ranges from Africa to Asia are reservoirs of filoviruses including Ebola and Marburg viruses as well as uncharacterised filoviruses (Han *et al.* 2016b). Other studies suggest that bats are unique among mammalian orders, since they are reservoir hosts to many recently emerged and significant zoonoses such as Ebola virus, SARS-CoV, MERS-CoV, and NiV (Brierley *et al.* 2016; Zhou *et al.* 2016b). This may be a result of the interplay of both intrinsic (bat and viral ecologies) and extrinsic (anthropogenic) factors leading to zoonotic emergence (Han *et al.* 2016a).

Bats inhabit a wide range of ecosystems, and while many roost in large colonies in caves or trees, some are more tolerant of human activities and roost in buildings and other human structures (Nowak 1994; Hutson *et al.* 2001). Ecologically, bats fall into two distinct groups – those that are insectivorous and those that are frugivorous. The latter include large species commonly called fruit bats or flying foxes and are often colonial and roost in trees across pristine and anthropogenically dominated environments (Nowak 1994; Epstein *et al.* 2009; Hahn *et al.* 2014a; Hahn *et al.* 2014b). Some bat species are solitary. Frugivorous bats forage for fruit or flowers from a variety of sources including commercially cultivated fruit trees, which may heighten the likelihood of viral spillover to people (Field *et al.* 2001; Plowright *et al.* 2015).

Direct human-bat contact may be incidental, for example bats that roost in buildings sometimes enter living areas and attempts to catch and remove them may result in accidental scratches or bites, which is a common mechanism for rabies and other bat lyssaviruses to directly infect humans (McCall *et al.* 2000; De Serres *et al.* 2008). Direct human-bat contact may also be due to human hunting and consumption of bats, which is a common practice in some countries, particularly in southeast Asia and Africa (Mickleburgh *et al.* 2009; Harrison *et al.* 2011; Kamins *et al.* 2011). Indirect contact with bats may be through exposure to bat excreta due to close proximity of roosting or feeding sites, or through behavioural practices that bring people into contact with feeding or roosting sites, e.g. date palm sap collection in Bangladesh (Khan *et al.* 2010) or guano collection from bat caves (Suwannarong & Schuler 2016). Exposure to bat excreta – primarily urine and saliva – is thought to be the mechanism of viral spillover of Nipah virus and Hendra virus from bats to humans or other animals (Luby *et al.* 2006; Field *et al.* 2007; Halpin *et al.* 2011).

The proximity of a diversity of mammal species including bats in live animal markets in southern China, the general lack of biosecurity practices in handling and butchering animals, and sheer volume of animals being traded, shipped, and stored is thought to have been responsible for the emergence of SARS-CoV in 2003 (Woo *et al.* 2006a). Non-bat species including civets (*Paguma larvata*), raccoon dogs (*Nyctereutes. procyonoides*), and ferret badgers (*Melogale moschata*) showed evidence of SARS-CoV infection and all were initially suspected as being the reservoir species (Guan *et al.* 2003). The subsequent discovery of SL-CoVs in bats and more recently of a strain capable of directly infecting people (Li *et al.* 2005; Ge *et al.* 2013) highlight the importance of surveillance to identify wildlife reservoirs of zoonotic viruses.

1.11 Human and Non-Human Animal Interactions

The emergence of other zoonotic diseases has been associated with contact and the consumption of infected animals as part of traditional hunting practices (Leroy *et al.* 2004). In 2013, an outbreak of Ebola Zaire virus in West Africa resulted from a single introduction from an animal reservoir into the human population (Gire *et al.* 2014). Human behaviour, rather than repeated spillover from an animal reservoir, was responsible for the uncontrolled, rapid spread of Ebola virus disease through the neighbouring countries of Guinea, Sierra Leone, and Liberia. Without information about a reservoir species and the associated human behaviours resulting in spillover, public health efforts towards preventing re-emergence will be ineffectual.

1.12 Wildlife Trade in China

With both a diversity of habitats and an equally diverse number of species within its borders, China is considered “megadiverse” (Mittermeier & Mittermeier 1997). About 10% (556/5,416) of the world’s mammals may be found in this region (Smith *et al.* 2010a). This natural resource, China’s biodiversity, has become a component of both domestic (Zhang *et al.* 2008) and international wildlife trade (Rosen & Smith 2010; Smith *et al.* 2017), although much of it is illegal and is difficult to quantify (Karesh *et al.* 2005). Recent research has suggested that because of the volume of trade that passes through China it also plays a key role in illegal wildlife trade (Patel *et al.* 2015).

Wildlife trade routes in southern China have been documented (Yiming & Dianmo 1996; Zhang *et al.* 2008), however, the practices and behaviours of those involved in the wildlife trade at the local level have not been explored in detail. Among the diverse species traded are bats, rodents, and nonhuman primates and these are reservoirs for the many of the zoonotic diseases infecting humans (McFarlane *et al.* 2012). In southern China large animal markets, wildlife trade, and human migrations create opportunities for zoonotic emergence as wild animals such as bats, rodents, civets and others come into frequent contact with each other, domestic animals, and humans (Morse 1995; Karesh & Cook 2005; Lau *et al.* 2005).

In southern China wildlife has long been utilised for food, medicine, pets, and as raw material such as clothing and ornaments (Zhang *et al.* 2008; Chow *et al.* 2014). Recent globalisation of trade and increasing wealth have resulted in higher demand for wildlife foods, both for general consumption and for the health benefits attributed by traditional medicine to their consumption (Yiming & Wilcove 2005). The animal markets such as those in Guangdong province grew to accommodate this demand (Li *et al.* 1996; Yiming & Dianmo 1998). Trade and farming in masked palm civets (*Paguma larvata*) is a good example of this. Before 2003, it has been estimated that over 660 farm were raising at least 40,000 civets to supply demand in southern China (Shi & Hu 2008). After SARS-CoV emerged and it seemed as if civets were the source of the virus, the Chinese government prohibited the sale of civets ensuring their disappearance from the markets (Zhao 2007).

SARS has not to date re-emerged since the last and minor outbreak in 2004, but the wildlife trade in China continues and civets and other farmed and wild animals still may be seen in farms and in the wet markets (personal observations). There has been little available data concerning wild animal trade and markets in China since. If these wet markets are not closed, the conditions for SARS re-emergence may persist. The practice of consuming wildlife represents a substantial risk for zoonotic disease spillover, given the diversity of wildlife traded as well as the density of the human population in southern China. Surveillance efforts and changes in practice may be able to reduce the risk of spillover or to contain the next outbreak. This is discussed further in Chapter 5 with data presented on humans and non-human animals surveyed along south China's wildlife trade networks.

1.13 Surveillance

Within the last decade, there has been a growing awareness of the interdependence between humans and animals as it relates to global health (Daszak *et al.* 2007; Alexander *et al.* 2010). The human-animal interface has been singled out as one of the most important risk factors to human health, well-being, and the emergence of infectious disease (King 2008). With approximately 75% of emerging infectious diseases of zoonotic origin (Taylor *et al.* 2001), a truly multidisciplinary approach is key to appropriately address health concerns on a global scale (Daszak *et al.* 2000). Research has been limited in its ability to describe this interface. One of the greatest strengths of an anthropological perspective is its interdisciplinary nature in attempting "to understand what it means to be human" (Peters-Golden 2008). A number of anthropological theorists have sought to move beyond the dichotomies of human-nature and biological-cultural (Kirksey & Helmreich 2010; Fuentes 2015). Recent multispecies ethnographies engage with ecology to explore how the relations among different actors in an environment provide additional context and meaning (Kirksey & Helmreich 2010). Anthropological and ecological surveillance methods can quantify the ways in which the human-animal interface is constructed and experienced. This interdisciplinary approach can improve health outcomes especially around areas of disease emergence and anthropogenic environmental change (Abel 1998). Further, this type of in-depth research is critical as many of the identified hotspots of emerging zoonotic diseases are in regions of the world with poorly documented indigenous knowledge (Hurn 2012; Han *et al.* 2016a).

In Guangdong province, restaurant and food service workers and food animal handlers comprised the majority of early cases of SARS infection (Zhong *et al.* 2003; Xu *et al.* 2004). Following these index cases, SARS-CoV spread rapidly via international travellers, eventually affecting over 8,000 individuals in 32 countries, and caused at least 800 deaths in early 2003 (Riley *et al.* 2003; Tsang *et al.* 2003). The highest incidence occurred in Guangzhou city, with 12.5 cases per 100,000 people (Xu *et al.* 2004). During the outbreak "superspreaders", patients with unusually high numbers of contact infections, were the primary mechanism for expansion of case numbers (Tu *et al.* 2004). During the

SARS-CoV outbreak, healthcare workers were infected at unusually high rates, despite following standard precautions (Gopalakrishna *et al.* 2004; Svoboda *et al.* 2004). Research also supports the roles of unrecognised cases of SARS, long incubation periods (~14 days), and high aerosolisation in the extensive spread of the disease, especially in hospitals (Ofner *et al.* 2003; Varia *et al.* 2003; Chow *et al.* 2004; Shen *et al.* 2004). Chapter 6 will investigate current human behaviour as surveyed throughout south China in regions where humans and wild animals frequently are in contact. Human attitudes, perceptions of risk, and current activities such as travel and exposure to wild animals all may be predictive of future zoonotic emergence.

In the wet markets of south China, the high degree of contact among people, bats, and other mammals due to handling and butchering activities may still result in conditions that allow viruses to emerge and spread. To test these assumptions, this thesis reports on results of assays for paramyxovirus and coronavirus from samples collected from both wild and captive animal species in the wildlife trade pathways in southern China. Behaviour and potential for spillover to humans with high occupational exposure to bats and other wildlife are also evaluated.

Qualitative research (ethnographic interviews and observational surveys) conducted with individuals living in Guangxi and Yunnan provinces in rural southern China is analysed. These provinces bordering Vietnam are known hotspots of faunal diversity (Myers *et al.* 2000) and have long been integral to trading routes from Vietnam to Guangdong (Yiming & Dianmo 1996) as well as home to protected forests and other areas where wildlife has been traditionally hunted and captured (Yiming & Wilcove 2005). This study was conducted to better understand the local wildlife value chain and explore the following four areas:

- the types of wildlife exposures experienced by people living and working in an environment known for its wildlife trade
- the socioeconomic drivers of the local wildlife trade, as well as the classification and value placed upon different wild animals
- the potential risk factors for zoonotic disease transmission associated with exposure to wild animals particularly bats, rodents, and nonhuman primates
- reported incidences of unusual illnesses.

1.14 Aims and Objectives

In the immediate aftermath of SARS, Li *et al* (2005) and others (Tang *et al.* 2006; Wang *et al.* 2006) called for urgent research in three areas: (1) the ecology and genetic diversity of reservoir species such as bats and civets that have an important role in the ecology and evolution of CoV, (2) the mechanisms of disease emergence in the wet-market system, and (3) a predictive model of disease emergence integrating both (1) and (2). Zoonotic viruses such as coronaviruses and paramyxoviruses are a threat to health globally. Wildlife reservoirs of both viruses have been identified as various bat species found globally and since then hundreds of novel CoVs have been discovered. These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating significant opportunity for bats and people to come into contact, thereby increasing the risk of coronavirus transmission.

The goal of this thesis is to evaluate the risk of emergence of two families of viruses, coronaviruses and paramyxoviruses, found in bats (Chapters 3) along the animal value chain in China and how that links to wildlife trade (Chapter 4) and other human behaviours (Chapter 5).

Detailing the current status of trade and the ecology of these viruses and their hosts provides invaluable data that may be used to predict and prevent the risk of emergence of these and other as-yet-unknown viruses. This research will evaluate the risk of infectious viral diseases of high interest to human and non-human animal health, in the coronavirus and paramyxovirus families, emerging in south China. The studies detailed in the following chapters have looked at whether current anthropogenic practices, viral and host ecology, human and non-human animal behaviour in markets and the wild through the trade networks of south China, as well as the diversity and detection rates of viruses of these two families, may result in future disease emergence. One aim is to assess the nature and frequency of contact among animals and people in two critical environments in southeast Asia: 1) live animal markets and trade routes and 2) rural areas where people hunt bats. This information will be used to determine whether viruses discovered in this research have the potential to cause large-scale epidemics. If patterns of human behaviour, wildlife trade, and viral ecology may be quantified, then a relative level of risk may be predicted and evaluated.

2 Materials and Methods

2.1 Overview of Methods

The central aim of this research was to evaluate the diversity of coronaviruses and paramyxoviruses that may be circulating in human and non-human mammals within the live animal market and trade pathways in China. The work will examine the risks of these viruses infecting humans and the potential of pandemic threats. In order to achieve these aim, it was necessary to collect biological samples from live non-human animals and humans and to assay these samples for coronavirus and paramyxovirus ribonucleic acids. The majority of data in this study were derived from surveillance efforts designed to identify and characterise these viruses circulating in animals present in the wild, wildlife markets, farms, and trading routes in southern China. In addition to assessing the risk of the emergence of these viruses, ethnographic data were collected from people working in the wildlife trade and communities from which the animals were sampled.

2.2 Field Sampling Locations

Bats, rodents, other mammals, and humans were sampled in 20 provinces and the capitol district (Beijing) of China in this study (Figure 7). Initially samples were collected opportunistically and then as behavioural, ecological, observational, and laboratory results became available, sampling efforts were prioritised in southern China (a) along wildlife trade pathways and (b) where humans, wildlife, and domestic animals were in regular contact. These priority sites were in three southern China provinces, from east to west, of Guangdong, Guangxi, and Yunnan. Population-level variables were recorded for humans in the farms and in markets and for non-human animals in farms, markets, and in the wild. These included data on production of domesticated and farmed wild animals, population sizes (of non-human animals) in the wet markets, as well as the temporal and spatial dynamics of these.

The project sites are those where both large bat populations were found to exist and the bats screened positive for viruses in this study. For the human behavioural surveys, community sites were selected based on previous research conducted throughout southern China wherein bat populations were identified and tested for existing and novel viruses such as paramyxoviruses and coronaviruses (Li *et al.* 2005; Li *et al.* 2010; Ge *et al.* 2012; Yuan *et al.* 2014).

Figure 7. Map of China. The 20 Provinces and Beijing shaded in darker grey indicate where human and non-human animal biological specimen sampling were conducted for this study. Observational, ethnographic, and qualitative behavioural surveys were conducted in southern China in Guangdong, Guangxi, and Yunnan Provinces.

Table 1. Human behavioural field sampling and surveillance sites. Sampling and surveillance conducted at farms and markets in Guangdong, Guangxi, and Yunnan Provinces in China including Latitude and Longitude in decimal degrees.

Province	Type of Site	Lat	Long
Guangdong	Conghua Market	23.548852	113.586605
	Foshan Market	22.640484	112.258051
Guangxi	Wei Farm	24.487824	110.395287
	Shi Farm	24.488242	110.373373
	Jiang Market	24.478321	110.405044
	Lipu Market	24.486367	110.392084
Yunnan	Zhou Farm	25.112958	102.076721
	Dali Old Town Market	25.705578	100.153770
	Dai Agricultural Village	24.943728	101.527404

The community sites (Table 1) were selected in Guangdong, Guangxi, and Yunnan provinces based on the following eligibility criteria where:

- large bat populations exist in caves and other natural or manmade roosting sites
- bats and other animals including humans were found to be positive for paramyxoviruses or coronaviruses
- regular contact had been observed among wild animals, domestic animals, and humans
- wild animal farming, consumption, and trade was known or found to occur

Sites in each province included wet markets where wildlife was sold, farms that breed wildlife species for consumption and trade, hunting areas, wildlife restaurants, wildlife holding areas where animals were kept on the way to larger markets, caves where people dwell or collect guano, and residential areas with known bat roosts. In addition, data and samples were collected from traditional healers who use animals and animal by-products as part of Traditional Chinese Medicine (TCM).

2.3 Field Sampling Methodology

Three types of field sampling methodology were conducted: observational, biological specimens, and ethnographic interviews.

2.3.1 Biosafety, Security, and Approvals

All field team members were trained in correct use of PPE, hygiene, safety, and veterinary animal handling techniques to minimise potential exposure or injury. Additionally, all team members were required to pass the Collaborative Institutional Training Initiative (CITI) Research Team Member training modules in Animal Care and Use, Human Subjects Research, Healthcare Ethics Committee, and Biosafety and Security (about.citiprogram.org/en/series/animal-care-and-use-acu/). All field team members who worked with animals were required to have current rabies and tetanus vaccinations.

For all bat, rodent, and other non-human animal capture and sampling, personal protective equipment (PPE) was used due to the potential for exposure to both known and unknown zoonotic agents. For this research, minimum PPE requirements for field team members were:

- N95 Respirator – fit tested (sized) per each field team member

- Goggles, face shield, or protective glasses
- Nitrile gloves – when directly handling animals (extracting from cages, or restraining so other team members may collect samples), leather gloves were worn on top of Nitrile gloves to protect from risk of bites or scratches
- PPE overalls or other field-sampling, dedicated clothing and washable shoes

2.3.2 Non-Human Animal Sampling Approval

To ensure the safety and protection of both non-human animals and sampling teams, full non-human animal sampling field techniques were written up and submitted to the Tufts University & Tufts Medical Center and the Human Nutrition Research Center on Aging Institutional Animal Care and Use Committee (http://viceprovost.tufts.edu/iacuc/) for approval. The Tufts Institutional Animal Care and Use Committee (IACUC) approved non-human animal sampling protocols as per protocol numbers G968-08 (2008 – 2014) and G2017-32 (2014 – present). Annual updates and reporting were supplied to the IACUC committee as per the Institution's annual renewal requirements. Some animal sampling for this thesis was also conducted under the approval of both the Animal Ethics Committee of the Wuhan Institute of Virology (WIVH05210201) in Hubei China and by the Institutional Animal Care and Use Committee at the University of California at Davis (UCDAVIS16048). The latter institution is the prime contractor on one of the sources of funding for this work. Sampling of non-human animal species was conducted under agreements between EcoHealth Alliance, Inc. and the following Chinese Institutions: East China Normal University Joint Institute for Zoonoses and Wildlife in Shanghai; the Yunnan Center for Disease Control and Prevention in Dali; and the Guangdong Entomological Institute based in Guangzhou. All of these Chinese Institutions regularly conduct bat and other wild animal sampling projects in China.

2.3.3 Human Sampling Approval

To ensure the safety and protection of both human subjects interviewed and field teams, the full protocol for quantitative and qualitative human sampling field techniques was written up, submitted to, and approved by the USA-based Hummingbird Institutional Review Board (2014-23 and 2016-55) (http://hummingbirdirb.com/index.asp), the Wuhan University School of Public Health Institutional Review Board (http://en.sph.whu.edu.cn), and the Yunnan Institute for Endemic Disease Control and Prevention Ethics Review Board (http://www.yiedc.com). Annual updates and reporting were supplied to the IRB committees as per each Institution's annual renewal requirements (See Sections 7.7 and 7.8). Human sampling was conducted under agreements between EcoHealth Alliance, Inc., the Wuhan School of Public Health, and the Yunnan Center for Disease Control and Prevention in Dali.

2.3.4 Biological Specimen Sampling

In this study, a conservative estimate of a 10% detection rate was used to calculate sampling target sizes. Published detection rates of coronaviruses identified in bats and rodents are between 10% and 38% (Tang *et al.* 2006; Osborne *et al.* 2011) and similar ranges have been identified for paramyxoviruses (Wacharapluesadee *et al.* 2010; Drexler *et al.* 2012; Young & Olival 2016). A 10% detection rate in wild populations of bats or rodents requires a sampling of a minimum of 30 individuals per species (Wobeser 2013).

Sampling locations in Guangdong, Guangxi, and Yunnan China were selected based upon site assessments of proximity to human activities and domestic animals as well as upon prior results from species sampled in these regions (Zhang *et al.* 2009b; Zhu *et al.* 2012; Ge *et al.* 2013). Sampling of bats was conducted annually between April and October from 2009 to 2014.

Prior to all field sampling, cold chains were set up to maintain sample preservation, prevent deterioration, and ensure rapid transport from the field to the laboratory.

2.3.5 Non-Human Sampling Procedures

Bats were trapped in natural habitat in China (See Chapter 3 and Chapter 4). Free-ranging bats were captured using a single mist net, series of mist nets, or a telescoping hand or fish net. The mist net system was monitored by two or more people during the entire capture period. Bats were removed from the nets as soon as they became entangled to minimise stress and prevent injury. A maximum of 30 bats were set per trapping period. Duration of trapping depended upon rate and method of capture. Following capture, bats were placed in clean, porous, unbleached and undyed cotton bags, sealed with a drawstring, and hung from a branch or post until all samples were collected. Bats and bags were monitored constantly to ensure ventilation and prevent bats from escaping. Bats were held for a maximum of three hours and immediately released on site following sample collection (See Sections 7.2 and 7.3).

Bats were manually restrained during sampling. Sterile paediatric swabs with polyester tips and aluminium shafts were used to collect samples from the oropharynx, urogenital tract, and rectum. A rectal swab was not collected if fresh faeces were excreted. Blood was collected from bats either from the brachial or femoral artery or vein using a 25-gauge needle and 1cc syringe. Up to 6µL of blood to 1g of bat body weight (Table 2) was collected. Collection did not exceed 10% of circulating blood volume calculated at 6.25% total body weight (Morton *et al.* 1993; Smith *et al.* 2010b). Serum was separated on-site by centrifugation at 3000 x g for 15 min within 24 h and preserved at 4.0˚C. Urine was collected opportunistically using sterile swabs to soak urine from the exterior urogenital opening during urination. Most bats upon handling freely excreted urine and faeces.

Table 2. Circulating Blood Volume (CBV) of mammals sampled. CBV was calculated at 6.25% of body weight in grams. A 10% of CBV maximum blood draw (in millilitres) from mammals was permitted. This table was used by field teams to rapidly calculate 10% ranges for small mammals such as bats and rodents. Modified from published methods (Morton *et al.* 1993; Smith *et al.* 2010b).

Body weight (g)	Circulating Blood Volume (ml)	10% CBV (ml)
20	1.10 – 1.40	0.11 – 0.14
25	1.37 – 1.75	0.14 – 0.18
30	1.65 – 2.10	0.17 – 0.21
35	1.93 – 2.45	0.19 – 0.25
40	2.20 – 2.80	0.22 – 0.28
125	6.88 – 8.75	0.69 – 0.88
150	8.25 – 10.50	0.82 – 1.0
200	11.00 – 14.00	1.1 – 1.4
250	13.75 – 17.50	1.4 – 1.8
300	16.50 – 21.00	1.7 – 2.1
350	19.25 – 24.50	1.9 – 2.5

Throat and faecal swab samples were collected in viral transport medium (VTM) composed of Hank's balanced salt solution, pH 7.4, containing bovine serum albumin (1%), amphotericin (15 mg ml/l), penicillin G (100 U ml/l) and streptomycin (50 mg ml/l) (Li *et al.* 2010).

Free-ranging rodents were captured with standard procedures as described by Mills et al. (1995) using small (7.62 x 8.89 x 22.86 cm), perforated, folding, aluminium Sherman traps (H. B. Sherman Traps, Inc., Tallahassee, Florida; https://www.shermantraps.com). As permitted by terrain, traps for free-ranging rodents were placed along transects and checked a minimum of every 6 hours. Traps were not set, if weather conditions were adverse, such as abrupt changes in precipitation intensity resulting in flash flooding or extreme heat spells. Traps were always placed beneath vegetation or in areas protected from direct sunshine to prevent heat stress. In areas without any vegetative or other cover, traps were closed between sunrise and sunset.

Wild and farmed rodents were docile enough to permit direct, manual restraint for sampling. Captive bred and wild rodent sampling procedures involved the same manual restraint, venepuncture, mucosal swabs, faecal, and urine sample collection as those described above for bats.

Blood samples were collected by making a small puncture to the submandibular or facial vein. Blood was collected in a small heparinised tube or capillary tube and the bleeding was stopped by applying pressure to the puncture. Saphenous or femoral venepuncture was used for larger rodents such as bamboo rats (*Rhizomys* spp.). In all rodents and as with bats, blood of no more than 10% of total circulating blood volume (6.25% of body weight) was withdrawn (Table 2). For example, 125µL blood was collected from a 20-gram rodent (Lee & Blaufox 1985; Morton *et al.* 1993).

Since sampled animals on farms were ultimately intended for human consumption anaesthesia was not permitted. Animals in farms were manually restrained. Blood as drawn from the femoral artery or saphenous vein using the same method of calculating maximum volume (Table 2) as for bats and rodents (Lennox & Bauck 2012).

All animals survived the sampling process and were immediately released back into their habitat or, for farmed animals, into their cages or confinement areas. All sampling was performed by veterinarian-trained team members (See Section 2.3.1).

2.4 Observational Research

Observational research was conducted in wild animal farms and wild animal markets in Guangdong, Guangxi, and Yunnan provinces in southern China. Sites were identified based upon previous, repeated observations of contact among humans, wild animals (both farmed and wild-caught), and domestic animals. Sites included both wild animal farms and markets (Table 1).

The following checklist was developed for observational research:

- Assess facilities and condition, e.g. toilet, hand washing, waste disposal, and drainage
- Assess animal and livestock conditions
- Estimate the number of cages; the number of animals in each cage; how many animals there are in the market; and how cages and animals are stacked.
- Map: draw the site including all buildings, roads, and other features
- Look for signs of health department inspections, sanitation conditions, and hand washing
- Look for butchering activities, de-feathering, etc.

- Assess customer and vendor population: age, sex, ethnicity, and relationship
- Observe ventilation conditions and infrastructure including number of floors
- Observe if people use any personal protective equipment (PPE)

Field observations including drawings (Figure 8). Notes were taken electronically or manually on site when possible or otherwise recorded immediately following exit from the site. Drawings, notes, photographs, and audio recordings were used to add context to quantitative data collection and analyses. Drawings of markets were invaluable tools for recollection and providing supplemental details.

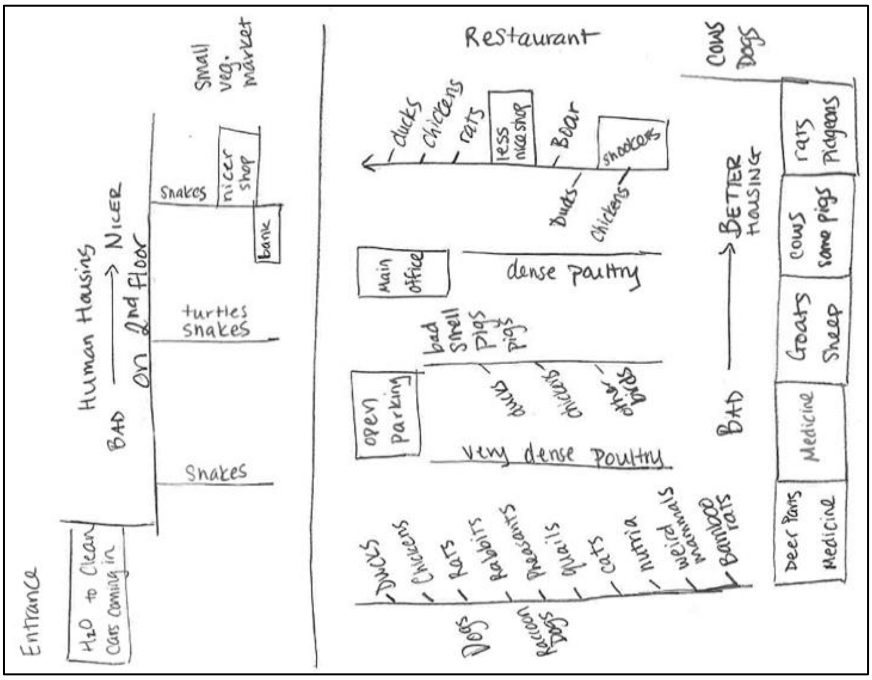


Figure 8. Post-site-visit hand-drawn diagram of the Conghua Shichang wild and domestic animal marketwetmarket is located in Conghua City, Guangdong Province, China. The drawing from a visit on 05 April 2015, shows the layout of stalls; groupings of species and domestic animals; as well as administrative, housing, and other organisational structures of the market. (Cf. Figure 32).

From 2014 to 2016, six direct observational market surveys each were conducted in two large animal markets in Guangdong Province: Foshan and Taiping Markets (Table 1). In order to be as unobtrusive and inconspicuous as possible, animal count estimates were made by the same two members of the field team and recorded immediately upon leaving the market using methodology modified from Gray (2009). Counts were compared and averaged when dissimilar.

2.5 Human Sampling

The number of humans exposed to wildlife at any site is difficult to enumerate, so an adaptive cluster sampling method was utilised (Thompson 1990). Adaptive cluster sampling is a standardised sampling methodology used when it is not possible to compile an exhaustive list of the elements (e.g., individuals) that make up the target population. The site specific/cluster approach, in which well-defined sites are identified for sampling is widely used in infectious disease research (Seber & Salehi 2014). In this study, site specific clusters consisted of well-defined groups of highly exposed individuals (e.g., wildlife market vendors, hunters, people who live near known bat roosts). If the population size of the cluster was equal to or less than 50 individuals, then a one-stage cluster sampling method was employed wherein the entire cluster of potential respondents was approached to be included in the sample survey. A two-stage cluster sampling method was employed, if the population size of the cluster were over 50 individuals wherein only a subset of respondents from the site-specific cluster were selected to be surveyed (Thompson 1990; Seber & Salehi 2014).

2.6 Human Behavioural Surveillance

Both qualitative and quantitative surveys were conducted. Qualitative research is used to understand the social and environmental context in which infectious disease spillover occurs between animal reservoirs and human populations. The two distinct qualitative methodologies utilised in this study to better understand this context include observational research and ethnographic interviews. Important behavioural information from different sociocultural levels was collected using each methodology. Following observations and qualitative or ethnographic interviews, standardised quantitative questionnaires were administered to collect data on living circumstances; income or resource generating activities; experience with unusual illnesses; and a range of human-animal contacts.

The provinces bordering Vietnam are known hotspots of faunal diversity (Myers *et al.* 2000) and have long been integral to trading routes from Vietnam to southern China and especially to Guangdong province (Yiming & Dianmo 1996). They are also home to protected forests and other areas where wildlife has been traditionally hunted and captured (Yiming & Wilcove 2005). Qualitative or ethnographic interviews were conducted with individuals living in the border provinces of Guangdong, Guangxi, and Yunnan in rural southern and southwestern China. The ethnographic survey methods consisted of (i) targeted interviews (see section 2.6.1), (ii) observational records, and (iii) field notes (Brewer 2000).

All surveys, consent forms, and protocols are included in Sections 7.11-7.14 and 7.16-7.23 in both English and Chinese.

2.6.1 Ethnographic Interviews

Targeted, in-depth ethnographic interviews were conducted with participants in rural Southern China along wildlife trade routes that have been documented {Zhang, 2008 #128}. Individuals were required to be able to provide informed consent in order to participate. As part of the consent process, participants were advised of the voluntary and anonymous nature of the study, the kinds of questions that would be asked, the fact that the interview would be audiotaped, and that precautions would be taken to protect their data.

In an effort to collect consistent qualitative data concerning factors relevant to zoonotic disease spillover, standardised qualitative and quantitative methods were first developed and pilot tested in live animal markets and abattoirs in New York USA. The methods were designed to provide a framework to gain a rapid understanding of the human-animal interactions that may lead to zoonotic disease spillover. Additionally, protocols and training materials were developed for interviewers with a range of skills and familiarity with social science methodology.

Five core themes were developed to guide the ethnographic interviews: 1) human movement, 2) socioeconomics, 3) biosecurity in human environments, 4) illness, medical care/treatment and death, and 5) human animal contact (See Section 7.15). An ethnographic interview guide was developed with examples of questions that could be asked around each theme. A 2.5-day qualitative research training workshop for interviewers was developed that covered observational research methods, ethnographic interviews, and preliminary analysis. All interviewers attended the training workshop. Workshop sessions included abstracting or creating appropriate thematic questions as well as conducting, recording, reviewing, and discussing practice interviews. In addition, field based participant-observation was ongoing throughout the study and involved site observations and informal conversations with people in a natural setting (Agar 1996; Spradley 2016a, b) Detailed field notes were maintained of all observations and discussions.

Recruitment sites in each province included forested areas or preserves, wildlife farms, hunting areas, wildlife restaurants, live animal markets, and residential areas or farms near known bat caves or roosts. Participants were recruited primarily through local contacts developed as part of wildlife conservation and health research conducted by team members over the past decade. Contacts facilitated introductions and provided referrals. Time available, resources, and the aims of a study best determine the optimal qualitative sample size (Patton 1990; Morse 1994; Creswell 2013). To achieve a sample with sufficient representation of categories of interest, participants were recruited using purposive sampling, which provided minimum quotas in terms of sex, age and wildlife exposure setting e.g., live animal market, forest preserve.

Surveys were conducted throughout 2015 and early 2016 by the same 3 trained interviewers from regional Centres for Disease Control and Prevention in Yunnan and Guangdong Provinces as well as Wuhan School of Public Health personnel. Interviews were audio-recorded, transcribed verbatim, and then translated into English. All participants received a token gift of cooking oil valued at US$10 in appreciation of their time.

Ethnographic and qualitative surveys may have been subject to intentional or unintentional variations in responses. Interview target numbers were chosen so as to achieve a level of responses sufficient to yield either a consensus or lack of consensus for specific responses. Other types of questions in surveys were too subjective for this approach.

2.6.2 Quantitative Research

Risks for disease transmission and spread likely differ according to behavioural factors. In addition to understanding the contributions of ethnicity/cultural group, occupation, age, gender, the aim of this research is to understand contact with wild and domesticated animals and the factors motivating those behaviours, such as occupational exposure in markets or extractive industry zones, or hunting, preparation, consumption and other exposures to wild and domestic animal meat, in order to develop an evidence base for identifying appropriate interventions and messages to decrease exposure to emerging infections. This research aims to improve understanding of the drivers and host-pathogen dynamics, including which human behaviours and practices increase risk, and under what circumstances these behaviours facilitate spillover of zoonotic viruses.

As with the qualitative survey above (Section 2.6.1), research subjects enrolled were those living, working, or visiting the locations where previous studies have isolated several SARS-like coronaviruses with potential pathogenicity to humans (Zhang *et al.* 2006; Ge *et al.* 2012; Yang *et al.* 2015) and where observational studies (Section 2.4) indicate high levels of human-wildlife contact and expected high risk for zoonotic disease emergence. Recruitment began with visits to existing contacts at local CDCs. During site visits, informal discussions were held to educate, sensitise, and inform people about zoonotic pathogens and potential pathways for disease spread/emergence. Cluster sampling (See Section 2.3.4) was performed where concurrent or past sampling of animals in this study was conducted.

A full questionnaire (See Sections 7.20 and 7.21) was administered and the duration of this was between 20-30 minutes. Questions included addressed contact with animals, travel, health, disease, and hygiene. Interview locations were identified before the interviews and conducted in a quiet, private locations without other individuals present.

2.6.3 Attitudes to Wildlife Trade Survey

An online survey was developed and conducted to assess Chinese people's attitudes towards wildlife trade in China and their awareness of whether or not any direct or indirect risk to themselves may be involved (See Section 7.10). The survey included questions about involvement in wildlife trade and awareness of health risks. Additionally, there was an informative or educative component to the survey. Since this was an online survey, sample size was estimated using the current, official number of online users of approximately 100,000,000 as provided by the China Internet Network Information Center (2016). Minimum sample size was calculated (Charan & Biswas 2013) at 1,500 respondents (95%±2.5). The survey was translated into Chinese and conducted online from August to September 2016.

2.7 Laboratory Methodology

All laboratory work was conducted at the Wuhan Institute of Virology, Chinese Academy of Sciences (http://english.whiov.cas.cn) based in Wu Chang, Wuhan, Hubei Province, China under the supervision and collaboration with the laboratory director Dr. Zhengli Shi. Laboratory protocols were tested and developed at Wuhan Institute of Virology and the Centre for Infection and Immunity at Columbia University in New York City USA (https://www.mailman.columbia.edu/research/center-infection-and-immunity).

2.7.1 RNA Extraction

All RNA extraction was conducted under dedicated, sterile RNase free hoods using all efforts to avoid RNase contamination of samples including suitable lab coat, disposable gloves, and protective goggles. Field samples were removed from -80˚C freezers, sorted by sample number, and placed in ice baths. Using Qiagen RNeasy kit and as per the manufacturer's instructions (https://www.qiagen.com/gb/resources/download.aspx?id=14e7cf6e-521a-4cf7-8cbc-bf9f6fa33e24&lang=en) RNA was extracted from each sample type (whole blood, oral swab, and anal swab) and pooled by individual sampled. Briefly, 170µl was pipetted from sample cryovials and into 1.5mL sterile RNase free centrifuge tubes, centrifuged at 4˚C and 5,000rpm for 5 minutes, and stored on ice. RNase free tubes were prepared with 5.6µl carrier RNA (Qiagen). To these 554.4µl of a viral lysis buffer was added along with 140µl of supernatant from samples. Samples were then buffered in two rounds in order to ensure complete binding of viral RNA to the QIAamp membrane (Qiagen) and then loaded into spin columns (Qiagen). Once RNA was bound to the QIAamp membrane, contaminants were washed away through the spin column in successive centrifuging. The final, extracted RNA was eluted with the RNase-free buffer (Qiagen) and stored in RNase-free 1.5ml vials, labeled, and kept at -80˚C.

2.7.2 RT-PCR

Extracted and pooled RNA was reverse transcribed (RT) to cDNA using Invitrogen Superscript III One-Step RT-PCR System with Platinum *Taq* DNA Polymerase (https://tools.thermofisher.com/content/sfs/manuals/superscriptIII_onestepRTPCR_man.pdf) according to manufacturer's instructions and then sensitive and broadly reactive Polymerase Chain Reaction (PCR) assays were performed to screen for novel coronaviruses and paramyxoviruses using respectively and specially designed primer sequences for the highly conserved RNA dependent RNA polymerase (RdRp) gene motifs in Coronaviruses (Xu *et al.* 2003; de Souza Luna *et al.* 2007) and in Paramyxoviruses (Tong *et al.* 2008).

2.7.2.1 Coronavirus cDNA Extraction and RT-PCR

RT-PCR protocols for Coronavirus assays were developed at the Wuhan Institute of Virology Laboratory in China and the Center for Infection and Immunity at Columbia University in the USA and based upon published protocols (Guan *et al.* 2003; Xu *et al.* 2003; de Souza Luna *et al.* 2007) targeting the highly conserved RNA-dependent RNA Polymerase (RdRP) gene, which contains short amino acid motifs (A and C) that are 100% identical to all known coronaviruses(de Souza Luna *et al.* 2007).

RNA was extracted from 140µl of swab samples with QIAamp Viral RNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. RNA was eluted in 60µl Qiagen buffer AVE (RNase free water containing 0.04% NaN_3) and stored at -80˚C until RT-PCR assays were conducted.

Amplification was conducted on an automated Applied Biosystems Veriti^{TM} 96-Well Fast Thermal Cycler.

One-step RT–PCR (Invitrogen) was used with a round 1 mixture consisting of 12.5µl reaction mixture (dNTPs, $MgSO_4$, and buffer solution); 1.0µl BSA (bovine serum albumin); 1.0µl of each of the two forward primers TTATGGGTTGGGATTATC and TGATGGGATGGGACTATC; 1.0µl of each of the two reverse primers TCATCACTCAGAATCATCA, TCATCAGAAAGAATCATCA; 0.5µl $MgSO_4$; 1.0µl RNA polymerase inhibitor; 1µl Platinum *Taq* enzyme; and 5.0µl extracted RNA template.

The round 1 amplification procedure comprised cycling of 30 minutes at 50°C; 2 minutes at 94°C; 10 cycles of 15 seconds at 94˚C; 15 seconds at 62˚C (decreasing by 1˚C each cycle); 40 seconds at 68˚C; 35 cycles of 15 seconds at 94°C, 15 seconds at 52˚C; and 40 seconds at 68˚C; with a final extension at 68°C for 5 minutes.

Round 2 mixture consisted of 34.3µl of ddH_2O; 5.0µl PCR reaction buffer ((Invitrogen, Karlsruhe, Germany); 1µl deoxynucleoside triphosphate (dNTP); 2.5µl $MgCl_2$; 1µl of each of the three forward primers CTTATGGGTTGGGATTATCCTAAGTGTGA, CTTATGGGTTGGGATTATCCCAAATGTGA, and TKATGGGWTGGGAYTATCCYAARTGTGA; 1µl of the reverse primer CACACAACACCTTCATCAGATAGAATCATCA; 0.2µl Platinum *Taq* DNA polymerase, and 2.0µl of DNA product from round 1 PCR.

The round 2 amplification procedure comprised cycling of 3 minutes at 94°C; 40 cycles of 15 seconds at 94°C; 15 seconds at 52°C; 40 seconds at 72°C; with a final extension of 72°C for 10 minutes.

SARS Coronavirus cDNA was used as a positive control and a blank (double distilled water – ddH_2O) as the negative control throughout. No false-positive signal was observed in the negative controls. Laboratory procedures were designed to avoid contamination. Designated UV cabinets were used for each step: mix, RT-PCR step one, RT-PCR step-two.

2.7.2.2 Paramyxovirus cDNA Extraction and RT-PCR

RT-PCR protocols for broadly reactive *Paramyxovirus* assays were developed at the Wuhan Institute of Virology Laboratory in China and the Center for Infection and Immunity at Columbia University in the USA and based upon published protocols (Tong *et al.* 2008) targeting the highly conserved RNA-dependent RNA Polymerase (RdRP) gene, which contains short amino acid motifs that are 100% identical in all known Paramyxoviruses (Tong *et al.* 2008).

RNA was extracted from 140µl of swab samples with QIA amp Viral RNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. RNA was eluted in 60µl Qiagen buffer AVE (RNase free water containing 0.04% NaN_3) and stored at -80˚C until RT-PCR assays were conducted.

Amplification was conducted on an automated Applied Biosystems Veriti™ 96-Well Fast Thermal Cycler.

One-step RT–PCR (Invitrogen) was used with a round 1 mixture consisting of 12.5µl reaction mixture (dNTPs, $MgSO_4$, and buffer solution); 1µl Bovine Serum Albumin (BSA); 2.3µl of forward primer GAAGGITATTGTCAIAARNTNTGGAC; 2.3µl of reverse primer GCTGAAGTTACIGGITCICCDATRTTNC; 0.4µl $MgSO_4$; 0.5µl RNA polymerase inhibitor; 1.0µl Invitrogen Superscript IV/Platinum *Taq* Enzyme; and 5.0µl extracted RNA template.

The round 1 amplification procedure comprised cycling at 1 minutes at 60˚C; 30 minutes at 50°C; 2 minutes at 94°C; 40 cycles of 15 seconds at 94°C, 30 seconds at 50°C, and 50 seconds at 68°C; with a final extension of 72˚C for 10 minutes.

Round 2 mixture consisted of 29.8µl of ddH_2O; 5.0µl PCR reaction buffer (Invitrogen, Karlsruhe, Germany); 1.0µl deoxynucleoside triphosphate (dNTP); 2.0µl $MgCl_2$; 5.0µl of forward primer GTTGCTTCAATGGTTCARGGNGAYAA; 5.0µl reverse primer GCTGAAGTTACIGGITCICCDATRTTNC, 0.2µl Platinum *Taq* DNA polymerase; and 2.0µl DNA product from Round 1 PCR.

The round 2 amplification procedure comprised cycling at 3 minutes at 94°C; 40 cycles of 15 seconds at 94°C, 30 seconds starting at 50°C, 40 seconds at 72°C; with a final extension of 72°C for 7 minutes.

Paramyxovirus cDNA was used as a positive control and a blank as the negative control (double distilled water – ddH_2O) throughout. No false-positive signal was observed in the negative controls. Laboratory procedures were designed to avoid contamination. Designated UV cabinets were used for each step: mix, RT-PCR step one, RT-PCR step-two.

2.7.3 Gel Electrophoresis, DNA Extraction, and Sequencing

PCR products were size fractionated by electrophoresis on gel moulds comprised of 1% agarose gel, ethidium bromide, and TAE buffer. Bands of gene fragments of 450 base pairs for Coronaviruses and 561 base pairs for Paramyxoviruses were removed from the gel and DNA was extracted using Omega

Bio-Tek E.Z.N.A. Gel Extraction Kit. Purity of the samples was confirmed by running another gel with 6µl of extracted DNA and if confirmed, the DNA was sequenced commercially using ABI 3730 PRISM Big Dye Terminator 1.1 Cycle Sequencing kits (Life Technologies, Carlsbad, CA, USA) at the Genomic Sequencing Commercial Facility of the Biological Engineering Co., Ltd., Wuhan, China.

DNA barcoding was used to confirm field species identification for positive samples. DNeasy Blood and Tissue Kit (Qiagen) was used to extract DNA from field samples. Extracted DNA was amplified using QIAGEN Fast Cycling PCR Kit according to manufacturer's instructions and then Polymerase Chain Reaction (PCR) assays were performed to amplify bat cytochrome b gene sequences. Forward primer GTHACHGCYCAYGCHTTYGTAATAAT and reverse primer CTCCWGCRTGDGCWAGRTTTCC were used. Amplification was conducted on an automated Applied Biosystems Veriti^{TM} 96-Well Fast Thermal Cycler. PCR cycling was as follows: 10 minutes at 95°C followed by 38 cycles of 60s at 95°C, 30s at 60°C, and 30s at 72°C with a final extension step of 10 minutes at 72°C. DNA products were visualised on 2% agarose gels and extracted and sequenced using the same methods as described for RT-PCR above.

2.8 Wildlife Trade Analysis Methodology

Data were acquired from multiple sources including (a) personal observations in wet markets and farms in southern China; (b) the China TRAFFIC data base (http://www.trafficchina.org) of reports of wildlife trade confiscations, (c) peer-reviewed published reports on wildlife trade in the region (See Chapter 5), (d) direct market observations by trained field team members, and (e) a viral-mammalian cross-referenced database (Olival *et al.* 2017). The collected data were statistically analysed by Wizard 1.9.2 and Microsoft Office Excel 15.29.1. Standard statistical analyses were used to summarise the respondents' data.

2.9 Viral Sequences

To assess the phylogenetic relationship of coronaviruses and paramyxoviruses identified in this study, full and representative nucleotide reference sequences of known viral species were downloaded from National Center for Biotechnology Information (NCBI) GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Nucleotide sequences were then aligned and translated by using Geneious 10 (Kearse *et al.* 2012). The best-fit model of the phylogenetic relationship was determined using CIPRES (https://www.phylo.org) {Miller, 2010 #1087} and Maximum Likelihood phylogenetic trees were constructed with both reference and outgroup sequences using 1,000 bootstrap replicates using PhyML with Smart Model Selection (Guindon & Gascuel 2003).

Correlations between both geographic locations and variation in genetic polymorphisms within and between populations (Fst) were calculated using Microsoft Office Excel Version 15.29.1.

2.10 Statistical and Risk Analyses

Quantitative data from human behavioural surveys were analysed for statistical significance using Chi-Squared test and Fisher's exact test to show association between categorical variables based upon gender, age, or education and behaviours listed in the questionnaires; i.e. eating or hunting wild animals. Using R software package (R Core Team 2016) and within the context of disease spill-over or emergence, data were classified and analysed to generate heat maps of highest risk animals, viruses, activities, and geographic locations (See Chapter 5).

2.11 Coding of Ethnographic Surveys

A thematic analysis framework was used to code and analyse the data from the human ethnographic interviews (Braun and Clarke 2006). Individual interviews and field notes were analysed to allow complete familiarity with the data set in its entirety and to confirm narrative consistency within individual interviews. The process of coding relied on the specific themes such as Human Movement, Socioeconomics, Human-animal Contact (See Section 7.15). Themes were further subdivided into topics (Human Movement -> Home, Work, Travel, Observed Environment) for coding. These subdivisions or codes allowed for a directed and consistent coverage of the domains that were the focus of the ethnographic interviews. Qualitative data were used to develop additional theoretical categories or typologies and develop the quantitative questionnaire. Central to the qualitative analysis was an assessment of the individual perceptions, knowledge, and participation in the wildlife trade as well as observed changes over time (such as agricultural expansion, urbanisation, etc.). The data were coded for factors associated with wildlife consumption, the socioeconomic drivers of the local wildlife trade, conservation and legal efforts, the prevalence and types of wildlife observed, and wildlife exposures that could transmit disease to humans.

All coding and qualitative data analyses and abstraction of quotes were performed using Quirkos (2017), Microsoft Word, and Excel (2016).

3 Coronaviruses and Paramyxoviruses in Chinese Wildlife

3.1 Introduction

Several viruses have recently spilled over from wildlife reservoir hosts to become pandemic with significant global health and economic impacts, including Human immunodeficiency viruses (HIV-1 and HIV-2), Zaire ebolavirus in West Africa, and Severe acute respiratory syndrome coronavirus (SARS-CoV) (Keogh-Brown & Smith 2008; Gire *et al.* 2014; Katz *et al.* 2014). Outbreaks of novel zoonoses are usually infrequent events that have been, so far, difficult to predict (Murphy 1998). A better understanding of how zoonotic viruses and their hosts interact may aid in predicting and preventing emergence or future pandemics. However, the complexity of their ecosystems and the rapidity with which they are changing presents a challenge, because these changes have been causally linked to disease emergence (Morse *et al.* 2012). Understanding of the viral-host system dynamics can take years, decades, or even longer, because of challenges in collecting field data to estimate host abundance, distribution, and infection status, and because our understanding of the mechanisms of zoonotic spillover is rudimentary (Morse *et al.* 2012; Daszak *et al.* 2013; Pike *et al.* 2014). Additionally, the requisite laboratory analyses are expensive, and for viruses that have low prevalence, they yield few results per assays or effort expended (Anthony *et al.* 2013). Consequently, the ecology of many emerging zoonoses still is poorly understood and this hinders the prediction and prevention efforts to mitigate outbreaks (Karesh *et al.* 2012; Morse *et al.* 2012; Daszak *et al.* 2013; Murray *et al.* 2015a).

Recent studies suggest that the risk of zoonotic emergence increases with factors such as livestock production intensity, land use change, and size of wildlife markets (See Figure 3), and that these are related to landscapes such as when forested areas are urbanised or changed to intensive livestock production (Patz *et al.* 2004; Wolfe *et al.* 2005; Gottdenker *et al.* 2011). In 2003, SARS-CoV spilled over from bat reservoirs in live animal markets of southern China and rapidly spread globally becoming pandemic (Li *et al.* 2005). This was likely driven by the volume and proximity of diverse mammal species as well as poor sanitary and biosecurity practices particularly in handling and butchering animals (Ka-Wai Hui 2006). In these markets, SARS-CoV and spread to humans and several other vertebrate species including Raccoon dogs (*Nyctereutes procyonoides*), Civets (*Paguma larvata*), and Ferret badgers (*Melogale moschata*) (Guan *et al.* 2003). Since the SARS-CoV outbreak, many SARS-like Coronaviruses (SL-CoVs) have been discovered in bats in southeast Asia including a SL-CoV capable of binding to human cells (Ge *et al.* 2013).

The emergence of many zoonotic diseases has been associated with contact and the consumption of infected animals as part of traditional hunting practices (Leroy *et al.* 2004). Human contact with animals is thought to be responsible for the spread of Ebola virus disease in west Africa (Gire *et al.* 2014). Without information about a reservoir species and associated viruses, efforts towards preventing emergence cannot be effectively targeted (Morse *et al.* 2012).

Around 60% of emerging infectious diseases are zoonotic in origin (Taylor *et al.* 2001; Jones *et al.* 2008) and most of these emerge from mammals (Woolhouse & Gowtage-Sequeria 2005). Bats and rodents are the most speciose of mammals and present on every continent excluding Antarctica (Wilson & Reeder 2005). Some bat and rodent species are commensal in the sense that they exploit human dwellings, constructs, and food resources, leading to direct and indirect contact amongst humans, domestic animals and rodents and bats (Reperant & Osterhaus 2014). Rodents harbour diverse zoonotic pathogens, and their close contact with people and livestock make them a significant health risk for humans (Mills 2006). Viral surveys to date have identified around 85 viral species from rodents, almost three times as many as have been identified in bats (Luis *et al.* 2013; Han *et al.* 2016a).

3.2 Bat and Rodent Reservoirs of Zoonotic Viruses

Recent evidence shows that bat species from Africa to Asia are reservoirs of filoviruses including Ebola viruses and Marburg virus, as well as other uncharacterised filoviruses (Han *et al.* 2016b). Filoviruses have been discovered in bats in southwestern China (Yuan *et al.* 2012; He *et al.* 2015). Other viruses with pathogenic potential for humans such as Adenoviruses (Li *et al.* 2010) and Henipaviruses (Li *et al.* 2008) have been discovered in Chinese bats. Other studies have shown that diverse Paramyxoviruses are found in Chinese bats and in rodents (Magoffin *et al.* 2007; Li *et al.* 2008; Lau *et al.* 2010b; Wu *et al.* 2014b; Yuan *et al.* 2014).

Bats are reservoirs of many recently emerged and significant zoonotic viral pathogens such as Ebola and Marburg viruses, SARS-CoV, Middle East respiratory syndrome Coronavirus (MERS-CoV), Nipah and Hendra viruses (Brierley *et al.* 2016; Zhou *et al.* 2016b). Recent evidence suggests bats are reservoir hosts to a disproportionately high number of zoonotic viruses (Olival *et al.* 2017). This may be a result of the interplay of both intrinsic (bat and viral ecologies) and extrinsic (anthropogenic) factors leading to zoonotic emergence (Han *et al.* 2016a; Olival *et al.* 2017). This chapter will examine whether Coronaviruses and Paramyxoviruses circulating in bat and rodent wildlife reservoirs are potential sources for zoonotic emergence in South China.

Bats in China inhabit a wide range of ecosystems, and while many roost in large colonies in caves and trees, some tolerate human activities and roost in buildings and other human structures (Nowak 1994; Hutson *et al.* 2001; Smith *et al.* 2010a). Other species are distributed across natural and anthropogenically dominated environments (Nowak 1994; Epstein *et al.* 2009; Hahn *et al.* 2014a; Hahn *et al.* 2014b). Frugivorous bats forage for fruit or flowers from a variety of sources including commercially cultivated fruit trees, which may heighten the likelihood of viral spillover to people. In Bangladesh bat reservoirs of Nipah virus regularly feed at palm sap collection sites and contaminate collection vessels leading to Nipah virus spillover (Khan *et al.* 2010). Direct human-bat contact may be incidental and indirect. For example, bats that roost in buildings sometimes enter living areas and attempts to catch and remove them may result in accidental scratches or bites, which is a common mechanism for Rabies and other bat Lyssaviruses to directly infect humans (McCall *et al.* 2000; De Serres *et al.* 2008). Exposure to bat urine, faeces, and saliva is thought to be the mechanism of viral spillover from bats to humans or other animals (Leroy *et al.* 2005; Luby *et al.* 2006; Field *et al.* 2007; Towner *et al.* 2009; Halpin *et al.* 2011; Openshaw *et al.* 2015).

Like bats, rodents in China also are found across varied ecosystems (Wilson & Reeder 2005; Smith *et al.* 2010a). Some rodent species (*Rattus rattus* and *Rattus norvegicus*) are commensal and have been the cause of much human morbidity and mortality including Plague, Hantaviruses, and other pathogens (Meerburg *et al.* 2009; Blasdell *et al.* 2011; Goeijenbier *et al.* 2013; Morand *et al.* 2015; Van Cuong *et al.* 2015).

In Asia, direct human contact with wild animals occurs due to hunting and consumption of rodents and bats, which has long been, and still is, a common practice in countries in these regions (Mickleburgh *et al.* 2009; Ripple *et al.* 2016). In southern China large animal markets, the wildlife trade, and largescale consumption of wildlife as food create unique opportunities for zoonotic emergence as diverse wild animals such as bats, rodents, and civets come into novel or increasing contact with each other, domestic animals, and humans (Morse 1995; Karesh *et al.* 2005; Lau *et al.* 2005). Both volume and frequency of the transportation of wildlife from their respective sources in the wild to markets, abattoirs, and restaurants, along animal value chains, are potential and likely drivers for viral spillover events (Johnson *et al.* 2015).

3.3 Coronaviruses

The *Coronaviridae* is a family of spherical non-segmented positive single strand RNA viruses from the order *Nidovirales* (ICTV 2017b). With genomes of about 30 kilobases in length, these are among the largest and most complex of the RNA viruses yet discovered and are found in a wide range of animal species such as domestic animals, some bird species, rodents, whales, bats, and humans (Siddell *et al.* 1983). In humans, most coronaviruses are respiratory pathogens causing laryngitis (e.g. HCoV-229E, HCoV-OC43, and HCoV-HKU1) and croup (HCoV-NL63) (Fehr & Perlman 2015). Prior to 2002 and the emergence of SARS-CoV and MERS-CoV, only two human coronaviruses (HCoVs) had been characterised (HCoV- 229E and HCoV-OC43) (Fehr & Perlman 2015). Since then, two more human coronaviruses HCoV-NL63 (van der Hoek *et al.* 2004) and HCoV-HKU1 (Woo *et al.* 2005) were identified in individuals with respiratory infections. These human coronaviruses may account for up to 30% of respiratory infections in the general population (Fouchier *et al.* 2004; Holmes & Rambaut 2004). Animal and human coronaviruses have been classified into the subfamily *Coronavirinae* (Figure 9) with four genera based on their antigenicity (Rota *et al.* 2003; Mihindukulasuriya *et al.* 2008):

- *Alphacoronavirus*: containing HCoV- 229E, bat, porcine, feline, and canine coronaviruses
- *Betacoronavirus*: with four lineages (A-D) and including SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-HKU1 containing human, bat, murine, bovine, and camel CoVs
- *Deltacoronavirus*: containing avian and porcine CoVs
- *Gammacoronavirus*: containing avian and cetacean CoVs

Coronaviruses have been shown experimentally and in nature to undergo genetic recombination by a genomic template-switching mechanism and to generate genetic point mutations at a rate similar to that of other RNA viruses including influenza A viruses, which suggests that there may be frequent host switching and zoonotic transmission (Tsunemitsu *et al.* 1995; Saif 2004). A large number of novel CoVs have been discovered in bat species in both the Old and New World (Dominguez *et al.* 2007; Müller *et al.* 2007; Donaldson *et al.* 2010; Quan *et al.* 2010; Rihtaric *et al.* 2010; Wacharapluesadee *et al.* 2015).

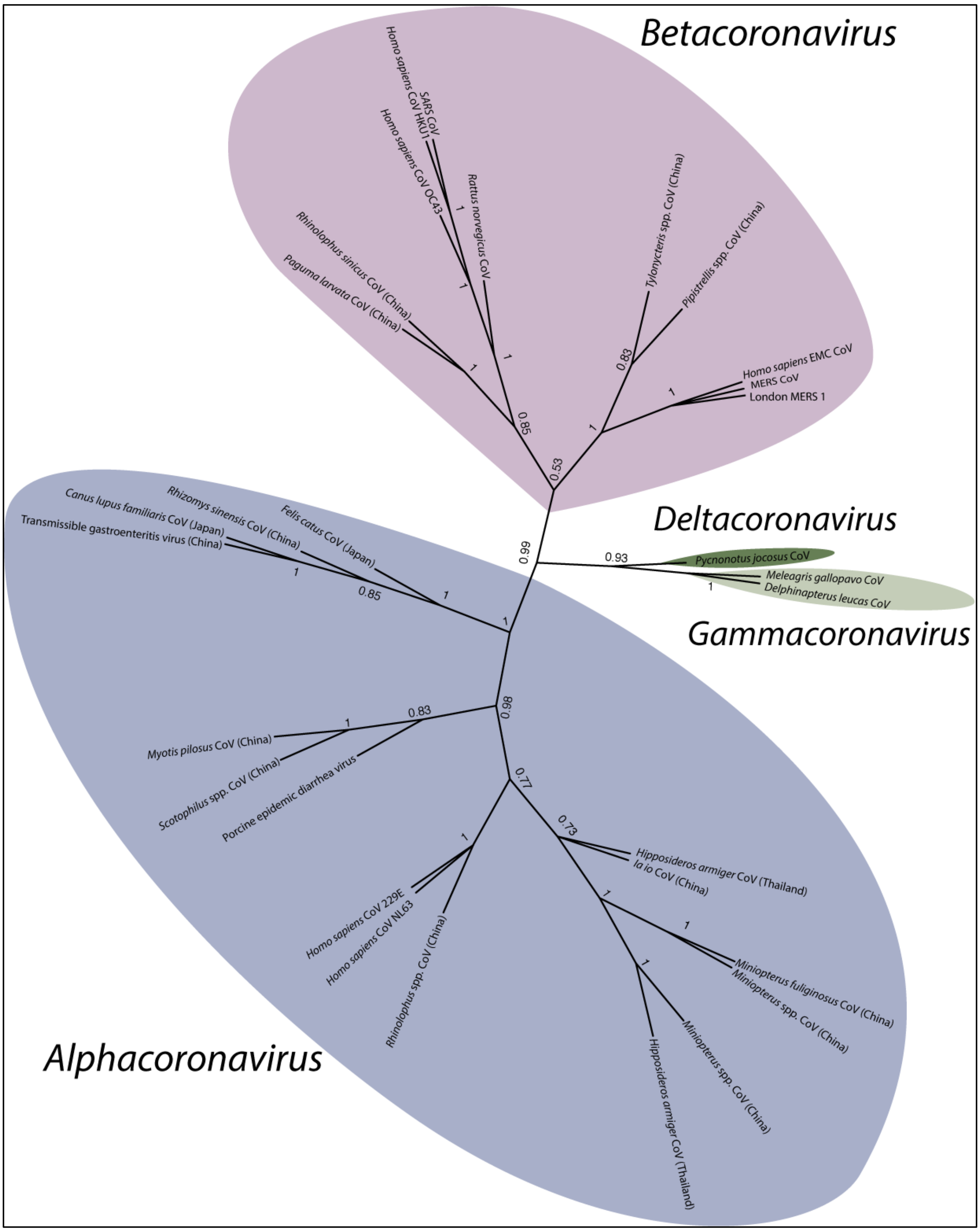


Figure 9**.** *Coronaviridae* family. Phylogenetic analysis of the four recognised (ICTV 2017), major genera in the *Coronaviridae* family clockwise from lower left: *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus, Gammacoronavirus*. Figure above was created using Geneious 10.1.3 and a Jukes-Cantor Neighbour-joining un-rooted consensus tree. Bootstrap values from 1,000 resampled data sets are indicated. Distance is not shown, since all branch lengths are equal. See Table 27 and Table 28 for additional sequence data.

3.3.1 Alphacoronaviruses

Alphacoronaviruses infect various mammal species including humans, pigs, cats, and bats (Pedersen *et al.* 1984; Kusanagi *et al.* 1992; van der Hoek *et al.* 2004; Chu *et al.* 2008). Among the 11 currently established species within the genus *Alphacoronavirus,* four have been identified in Chinese insectivorous bats: *Miniopterus bat coronavirus 1*, *Miniopterus bat coronavirus HKU8*, *Scotophilus bat coronavirus 512,* and *Rhinolophus bat coronavirus HKU2* (Tang *et al.* 2006; Lau *et al.* 2007; Chu *et al.* 2008). The first bat Alphacoronavirus designated bat CoV 1 was reported from three different *Miniopterus* bat species found in Hong Kong (Poon *et al.* 2005). The high prevalence rate (63%) of the virus in *Miniopterus pusillus* suggests that it might be a commonly circulating CoV in this species (Poon *et al.* 2005). In two subsequent studies of Hong Kong bats, four distinct Alphacoronaviruses (CoV 1A, 1B, HKU7, and HKU8) were found in *Miniopterus* sp. These viruses were demonstrated to be closely related genetically and derived from a common ancestor (Chu 2006; Woo *et al.* 2006b). Co-infections of bat CoV 1B and HKU8 were observed in *Miniopterus pusillus* (Chu *et al.* 2008). The presence of genetically diverse but related Alphacoronaviruses in *Miniopterus* sp. bats within a relatively small geographical region suggested that Alphacoronaviruses had adapted to this bat genus for a long time (Chu 2006). Bats of *Myotis*, *Scotophilus*, and *Rhinolophus* genera in China have been shown to harbour Alphacoronaviruses (Tang *et al.* 2006; Woo *et al.* 2006b; He *et al.* 2014).

Alphacoronaviruses discovered in *Miniopterus fuliginosus* in Japan show a close relationship to those from *Miniopterus magnater* in Hong Kong (Shirato *et al.* 2012). In the Philippines, two Alphacoronaviruses were found in *Scotophilus khulii* and *Hipposideros diadema* with high nucleotide sequence identities (95% and 80% respectively) to the strains previously described in China (Watanabe 2010; Tsuda *et al.* 2012). In Spain, France, and Germany, a number of Alphacoronaviruses with a wide diversity and distribution were reported from multiple bat species including *Myotis* sp., *Pipistrellus* sp. and *Nyctalus lasiopterus* (Gloza-Rausch *et al.* 2008; Falcon *et al.* 2011; Goffard *et al.* 2015). Some European bat Alphacoronaviruses are related to those found in Asia while others are distinct and clustered in new monophyletic clades (Gloza-Rausch *et al.* 2008; Drexler 2011; Falcon *et al.* 2011). A great diversity of bat Alphacoronavirus is also present in Africa. Alphacoronaviruses (*Miniopterus bat coronavirus 1*, BtKY22, BtKY41, and BtKY43) were identified from *Chaerephon* sp. and *Cardioderma* sp. bats in Kenya (Tao *et al.* 2012). In North America, Alphacoronaviruses were found in *Eptesicus fuscus* and *Myotis occultus* bats with significant dissimilarity from Alphacoronaviruses of Asian bats (Dominguez *et al.* 2007). More recently, a novel Alphacoronavirus was discovered in guano of New Zealand *Mystacina tuberculata* bats with 80%

nucleotide identity to bat CoV HKU8 and despite the geographic and evolutionary isolation of the host species, the virus had not diverged significantly from Chinese bat Alphacoronaviruses (Hall *et al.* 2014). Although some studies suggest host species restriction of bat Alphacoronavirus, different bat species from the same colony have been shown to harbor Alphacoronaviruses of the same genetic lineage, which indicates some complexity of the ecology of this viral genus in bats (Tang *et al.* 2006; Falcon *et al.* 2011). The first evidence for interspecies transmission of Alphacoronavirus (CoV HKU10) between different suborders of bats (*Rhinolophus leschenaulti* to *Hipposideros pomona*) has recently been described (Lau *et al.* 2012).

There have been reports of bat Alphacoronaviruses closely related to human pathogenic coronaviruses. An Alphacoronavirus was detected in *Hipposideros caffer ruber* bats in Ghana with 92% nucleotide identity to HCoV 229E and predicted to share a common ancestor only 200 years ago (Pfefferle *et al.* 2009). Another bat Alphacoronavirus discovered in North American tricolored bats (*Perimyotis subflavus*) was also predicted to share common ancestry with HCoV NL63 (Huynh *et al.* 2012).

3.3.2 Betacoronaviruses and other Coronaviruses

Compared with bat Alphacoronaviruses, bat Betacoronaviruses have been identified from fewer host species and show lower genetic diversity (He *et al.* 2014). Bat Betacoronaviruses are distributed among three of the four betacoronavirus lineages. *Betacoronavirus* lineage B contains diverse bat SARS-like coronaviruses while lineage C Betacoronaviruses include diverse MERS-related coronaviruses that have been identified in bats. The other bat-associated Betacoronavirus species, *Rousettus bat coronavirus HKU9*, was first discovered in bats in China in *Rousettus leschenaultii* in Guangdong Province (Woo *et al.* 2012a) as well as in *Hipposideros* sp. from Yunnan Province (Ge *et al.* 2012). Although not as abundant or diverse as bat Alphacoronaviruses, bat Betacoronaviruses are of special importance, since they include SARS-CoV, MERS-CoV, and other as yet unknown zoonotic viruses that may threaten human health (Hu *et al.* 2015). The huge diversity of Alphacoronaviruses and Betacoronaviruses in bats supports the hypothesis that bats as ideal hosts fuel the evolution and dissemination of these two genera (Woo *et al.* 2012a). Currently, the sole recoded bat Gammavirus (PgCoV-4) was discovered in a bat (*Pteropus giganteus*) in Bangladesh (Anthony *et al.* 2013).

3.4 Paramyxoviruses

The *Paramyxoviridae* is a family of negative single strand RNA viruses of the order *Mononegavirales* with genomes between 15-19 kilobases in length (ICTV 2017a). *Paramyxoviridae* or Paramyxoviruses comprise 7 genera: *Aquaparamyxovirus*; *Avulavirus*; *Ferlavirus*; *Henipavirus*; *Morbillivirus*; *Respirovirus*; and *Rubulavirus* (Figure 10). Less than 20 years ago only three genera had been described in this family (Enders 1996). Paramyxoviruses that have recently been discovered such as Beilong virus (Li *et al.* 2006), J virus (Jun *et al.* 1977), Mossman virus (Miller *et al.* 2003), Tailam virus (Woo *et al.* 2011), and Tupaia virus (Tidona *et al.* 1999) are not yet classified within any of these seven genera. All have been isolated from wild animals. Novel Paramyxoviruses are still being discovered and an additional genus (*Jeilongvirus*) proposed to the International Committee on Taxonomy of Viruses (ICTV) may incorporate some of these as yet unclassified Paramyxoviruses (Kurth *et al.* 2012; Woo *et al.* 2016).

Horizontal transmission from one conspecific to another via viral particles in faeces, urine, or saliva seems to be the main mechanism of Paramyxovirus infection and the likely means of zoonotic spillover (Plowright *et al.* 2008).

Paramyxoviruses have been discovered in a broad range of species and they exhibit high viral diversity, which may increase the likelihood of their spillover to people because there is a greater chance of some of these diverse lineages having receptor binding domains able to bind to human cells (Anderson & Wang 2011; Johnson *et al.* 2015). Newcastle disease virus (genus *Avulavirus*) primarily affecting poultry and other birds and Rinderpest virus (genus *Morbillivirus*) affecting cattle and other grazing animals (and now eradicated in the wild) are both pathogenic with high rates of mortality in domestic and wild animals (Alexander 2001; Morens *et al.* 2011; Roeder *et al.* 2013). Some Paramyxoviruses such as measles (genus *Morbillivirus*), mumps (genus *Rubulavirus*), and human parainfluenza viruses (genus *Respirovirus*) have high rates of human morbidity (Drexler et al. 2012). *Aquaparamyxovirus* and *Ferlavirus* (Woo *et al.* 2014) genera contain viruses affecting salmon and reptiles respectively.

Emerging zoonotic Paramyxoviruses such as Nipah virus and Hendra virus in the *Henipavirus* genus pose significant burdens to livestock and human health, with mortality rates of 40% and higher in humans during outbreaks or spillover events in Australia and Asia (Marsh & Wang 2012). These two viruses have been shown to originate in bats (Halpin *et al.* 2000; Chua *et al.* 2002b). Viruses similar to Nipah virus have been identified in other bat species in Australia, Asia, and African (Olson *et al.* 2002; Sendow *et al.* 2006; Halpin *et al.* 2011; Drexler *et al.* 2012; Hasebe *et al.* 2012; Pernet *et al.* 2014). Paramyxoviruses such as Hendra virus and Nipah virus are of concern to human and domestic animal health because they have repeatedly emerged from wildlife reservoirs (Han *et al.* 2015). In

Bangladesh, there is growing evidence that Nipah virus can be transmitted person-to-person (Luby *et al.* 2009a; Luby *et al.* 2009b). Elucidating the ecology of these zoonoses is crucial in order to develop targeted prevention programs. Given the right conditions, these viruses or as yet unknown Paramyxoviruses may emerge and result in a large epidemic or like SARS-CoV quickly spread globally and become pandemic. A diversity of Paramyxoviruses has already been identified in China, predominantly in bats, but also in rodents, birds, and even cats (Li *et al.* 2006; Li *et al.* 2008; Shi *et al.* 2008; Lau *et al.* 2010b; Wu *et al.* 2014b; Yuan *et al.* 2014; Awu *et al.* 2015). The research reported in this chapter aims to identify novel Coronaviruses and Paramyxoviruses in Chinese bats and rodents. Using sequence data from these and other described species of these viruses, the hypothesis that some of these diverse viruses are closely related or even ancestral to known Coronaviruses and Paramyxoviruses of concern to human and animal health may be tested.

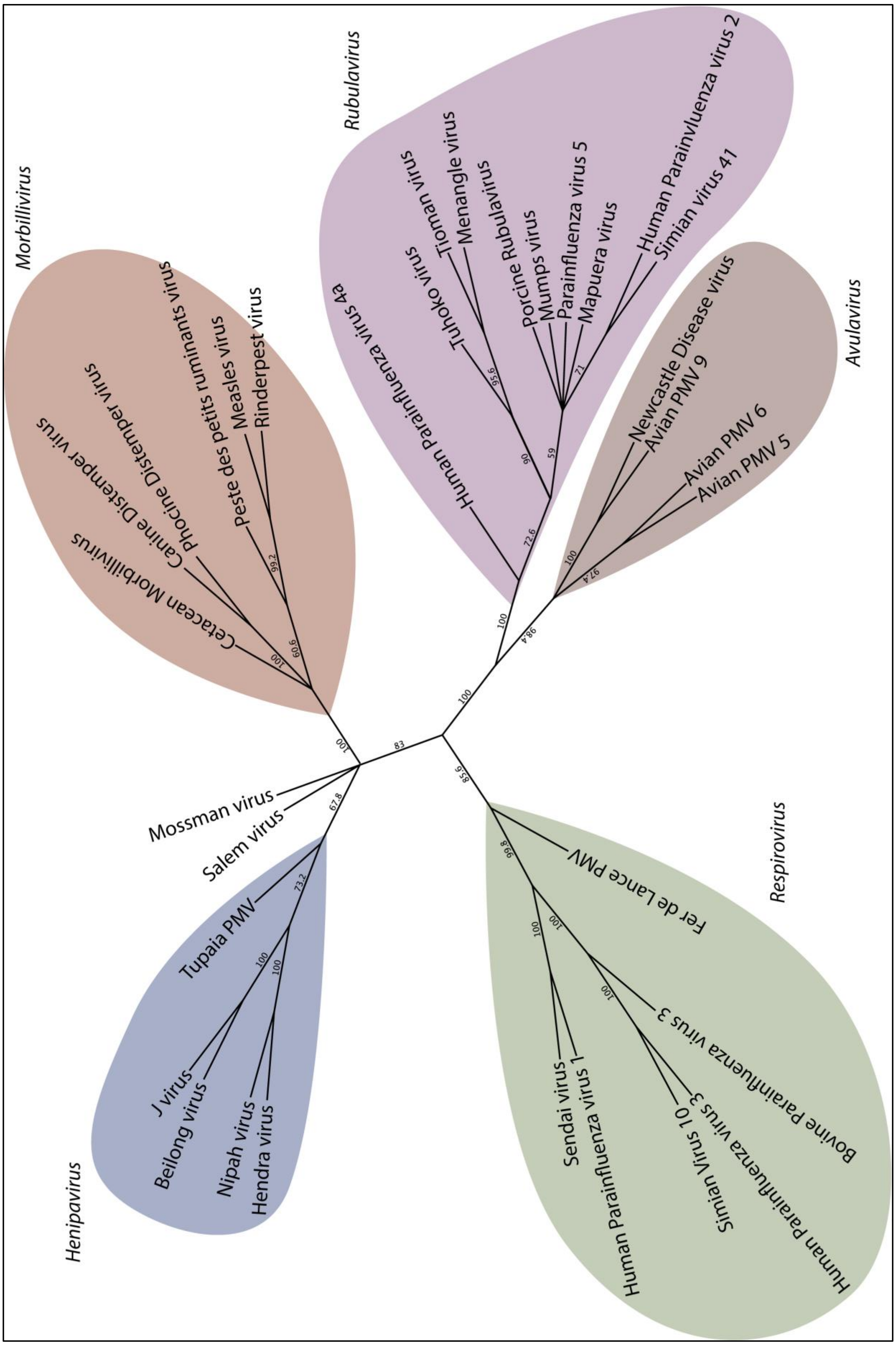


Figure 10. Paramyxoviridae family. Phylogenetic analysis of the five recognised (ICTV 2017), major genera in the Paramyxoviridae family clockwise from the lower left: Respirovirus, Henipavirus, Morbillivirus, Rubulavirus, and Avulavirus. Figure above was created using Genious 10.1.3 and a Jukes-Cantor Neighbor-joining un-rooted consensus tree. Bootstrap values from 1,000 resampled data sets are indicated. Distance is not shown, since all branch lengths are equal. See Table 29 and Table 30 for additional sequence data.

3.5 Methods

The field collection period for this study was between April 2009 and September 2015. Pilot surveys were conducted from 2008 to 2010 across China. Based upon initial surveys and results from other assays for Coronaviruses and Paramyxoviruses in bats and rodents in China (Li *et al.* 2008; Shi *et al.* 2008; Lau *et al.* 2010b; Ge *et al.* 2013; Ge *et al.* 2016a), field survey efforts were focused along wildlife trade pathways (Figure 3) particularly in Yunnan, Guangxi, Guangdong, and Hainan provinces (Figure 11).

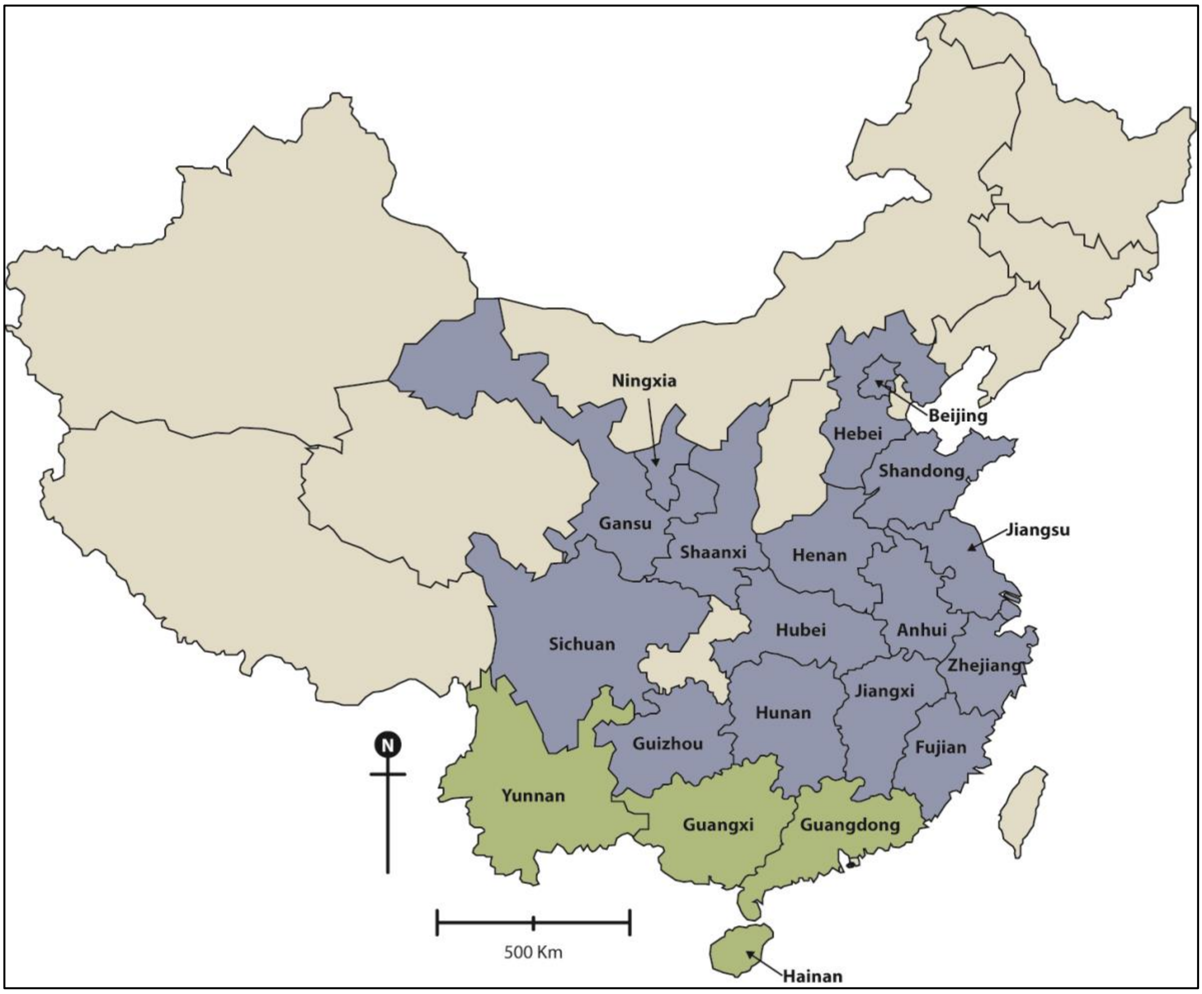


Figure 11. Map of China with sampling locations. The 20 provinces, autonomous regions, or national central cities are indicated where bats and rodents were sampled from 2009 to 2015. The four green shaded provinces (Yunnan, Guangxi, Guangdong, and Hainan) were where 70.2% of samples were collected along the wildlife trade pathway in southern China. The fewest samples were collected in Ningxia (17) and Gansu (12) in the north and Hunan (4) in the south. Bats sampled in 13 regions (65%) yielded samples positive for Paramyxoviruses.

Sampling sites were then identified based on the available information about bat roosts; bat and rodent proximity to human activities; human interactions with wildlife; and field observations of flying and foraging bats. The project sites were selected where both bat populations were found to exist and previously screened positive for viruses in this study (Table 3). Sites for rodents (Table 4) were selected based upon previous studies demonstrating Paramyxoviruses (Li *et al.* 2006) and other viruses circulating in these animal populations in southern China (Lei *et al.* 2007; Blasdell *et al.* 2011; Zhang *et al.* 2011). Sites for bats were selected with the same methodology (Table 3) (Li *et al.* 2008; Lau *et al.* 2010b). Field sites for rodents and bats included a total of 26 villages. 27 caves, 8 public parks or university campuses, 1 market, 4 bridges (bats roost beneath), and 5 wild-animal farms (rodents). Twelve locations (all caves) were relatively inaccessible to people and domestic animals. All other (non-cave) locations (59) had varying levels of humans and domestic animals present. Repeated sampling efforts were conducted between April and October each year. A total of 3,705 individual animals (3,146 bats) and (559 rodents) were sampled. All bats were sampled in locations ranging from undisturbed, forested habitat (caves) to urban environments (parks and university campuses). Sampling trips lasted a maximum of four weeks. No more than 30 individuals were sampled per each 24-hour sampling period. The sampling protocols of animals in this study were approved by Tufts University Institutional Animal Care and Use Committee (USA) and the Animal Ethics Committee of the Wuhan Institute of Virology (China) (See Section 7.1).

Field team support was provided through agreements between EcoHealth Alliance, Inc. and the following Chinese Institutions: East China Normal University Joint Institute for Zoonoses and Wildlife in Shanghai; the Yunnan Center for Disease Control and Prevention in Dali; and the Guangdong Entomological Institute based in Guangzhou. To capture bats, field teams used a single mist net, series of mist nets, or a telescoping hand or fish net depending upon location and accessibility of roosting site. Hand nets were used in the caves and human dwellings. Mist nets were used in roosts or locations where bats were foraging or otherwise difficult to catch by hand. Free-ranging bats were captured either while roosting or at crepuscular times when returning or exiting roosts. Following capture, bats were placed in clean, porous, unbleached, and undyed cotton bags, sealed with a drawstring, and hung from a branch or post until all samples were collected. Bats were held for a maximum of four hours and immediately released on site following sample collection.

Free-ranging rodents were captured with standard procedures as described by Mills *et al.* (1995) using small (7.62 x 8.89 x 22.86 cm), perforated, folding, aluminium Sherman traps (H. B. Sherman

Table 3. Bat sampling locations. Bats sampled in 20 provinces, autonomous regions, or national central cities in China from 2009 to 2015.

Province and Sampling Location	No. Sampled	Latitude	Longitude
Anhui	*45*		
Jade Dragon Cave, Qingyang County	42	30.3419	117.836
Zuyun Village, Sanxi Town, Jing County	3	30.4464	118.413
Beijing	*66*		
Bat Cave, Xiayunling Town, Fangshan District	36	39.7380	115.724
Sihe Village, Fangshan	30	39.7380	115.724
Fujian	*98*		
Qixian Cave, Fukou town, Sha County, Sanming City	14	26.4197	117.655
Rong Cave, Laiyuan town, Longyan City	43	26.4196	117.655
Yinghua Cave, Jiangle County, Sanming City	41	26.6553	117.576
Gansu	*12*		
Pingliang County	12	35.5356	106.698
Guangdong	*616*		
Shuili and Wendao Caves, Nanling Natural Reserve, Shaoguan City	162	24.9287	113.016
Banshan Cave, Dongkeng Village, Chengjia Town, Yangshan County	121	24.6500	112.750
Biluo Cave, Yingde County	45	24.1158	113.353
Kuang and Lutian Caves, Chenjia, Yangshan County	77	24.7749	112.831
Paishui Yanaian Qiao Caves, Ruyuan County	50	24.9308	113.084
Shanxin and Shitazi Caves, Tianjingshan, Nanling Natural Reserve	59	24.7302	112.999
Huidong and Zhuao Bridge, Qiao Island, Zhuhai	102	22.434	113.649
Guangxi	*184*		
Bawang, Gaoxiang, Liming Viliege, Longzhou County, Chongzuo City	70	22.3847	106.762
Bidi Cave, Guilin City	9	25.2901	110.388
Fenkeng Cave, Guilin City	10	25.2855	110.386
Jishui Cave, yaoshan Town, Guilin City	50	25.2817	110.388
Kuang Cave, Hezhou City	37	24.4991	113.552
Lipu Village Near Farm A	8	24.4646	110.405
Guizhou	*58*		
Feilongdong, Xingyi Town	58	27.7166	109.183
Hainan	*871*		
Helai and Jiacha Villages, Qiongzhong County	68	18.9974	109.677
Longhe Town, Dingan County	8	19.3829	110.219
Luqiao Cave, Chenpo, Qiongzhong County	44	18.8976	110.015
Nanxi Cave and Power Station Diaoluo Shan, Lingshui	210	18.6700	109.929
Shibian Village, Fengguoshan, and Maogan Baoting County	215	18.6352	109.705
Shikuang, Changzheng, and Mengqianling, Qiongzhong County	58	19.0830	109.552
Xianren Cave, Wenling, Dafeng, and Lingshan, Haikou	155	19.9480	110.259
Yinggeng Town and Park Qiongzhong	113	19.0500	109.830
Hebei	*48*		
Heilong Cave, Suobao Village, Suobao Town, She County	35	36.6613	113.602
Lianhua Cave, Jingyu Village, Huoshui Town,Wuan City	13	36.8370	113.914

Table 3. Bat Sampling Locations (continued).

Province and Sampling Location	No. Sampled	Latitude	Longitude
Henan	*41*		
Shengxian Cave, Donggou Village, Yingyang City	20	34.6301	113.263
Yunhua Cave, Xixia County	21	33.3133	111.427
Hubei	*72*		
Meikuang Cave, Tiangou Village, Zhushan County, Shiyan City	33	32.4258	110.150
Yeren Cave, Fang County, Shiyan Ctiy	39	31.9190	110.732
Hunan	*4*		
Jiangjundong, Xinqiao Town, Zhangjia Jie	4	29.1705	111.487
Jiangsu	*44*		
Linggu Cave, Yixing City	44	31.2225	119.740
Jiangxi	*89*		
Longhu Shan, Yingtan City	66	28.0964	116.970
Yingtan City, Jiefangdong	23	26.1121	116.988
Ningxia	*17*		
Haikou Village, Gucheng Town, Pengyang County	2	35.8413	106.354
Plaza of Wang Luobin, Liupan Town, Longde County	15	35.6904	106.233
Shaanxi	*133*		
Daluping Village, Wuming Town, Anzhong County	6	33.0320	107.313
Shanhuai Village, Yangdi Town, Shanyang County	11	33.3312	109.758
Xiaozhang Village, Bin County	26	35.0580	108.096
Yulongdong, Huaping Village, Zhenoing Town, Ankang County	44	31.7504	109.384
Zhashui Rongdong Fengdong, Zhashui Village, Shangluo County	46	33.6174	109.159
Shandong	*49*		
Chaoyang Cave, Laiwu City, Shandong Province	23	36.1217	117.666
Kuzi Cave, Xiaoshankou Village, Liancheng Town, Menglian County	26	35.7281	117.839
Sichuan	*119*		
Bridge in Leshan County	48	29.4335	103.861
Longdong Bridge, Emeishan County,	15	29.5876	103.861
Machongkou, Zigong County, Sichuan	39	29.3773	104.771
Wutong Qiao, Leshan, Zigong	17	29.4337	103.862
Yunnan	*539*		
Huize Town, Qujing	5	25.4997	103.694
Jinning Cave, Kunming	75	24.7025	102.575
Natural Bridge of Xianggelila	59	27.7998	99.8455
Tropical Botanic Garden at Menglun of Xishuangbanna	84	21.6830	101.417
Xianrendong, Longlin Town	24	24.3571	99.036
Yuanjiang, Xishuangbanna	247	23.3500	101.570
Xishuangbana, Menglun Town, Pingfeng Cave and Tiansheng Qiao	45	21.9367	101.258
Zhejiang	*41*		
Shihua Cave, Shiruiheqiao Town, Linan County	14	30.0948	119.097
Tianzidilao Cave, Luojiang Village, Tonglu County	27	29.7798	119.361

Table 4. Rodent sampling locationssampled in four provinces or autonomous regions in China from 2010 to 2015.

Province and Sampling Location	No. Sampled	Latitude	Longitude
Guangdong			
Wendao Cave, Gaojia Village, Ruyuan County, Shaoguan City	3	24.9421	113.105
Guangxi			
Wei Shangzheng Farm, LiPu Village, Guilin County	161	24.6160	110.465
Xiao Wei Farm, Lipu Village, Guilin County	66	24.5497	110.404
Dongwu Shichang Market, Nanning City	20	22.9177	108.355
Hainan			
Jiege cave, Lingshui County	4	18.6434	109.961
Yunnan			
Luzi Village, Huangshan Town, Yulong County, Lijiang City	144	26.7384	100.149
Yaping Village, Fugong County, Nujiang Prefecture	161	27.2115	98.706

Traps, Inc., Tallahassee, Florida; https://www.shermantraps.com). As permitted by terrain, traps for free-ranging rodents were placed along transects and checked a minimum of every 6 hours. Traps were not set, if weather conditions were adverse, such as abrupt changes in precipitation intensity resulting in flash flooding or extreme heat spells. Traps were always placed beneath vegetation or in areas protected from direct sunshine to prevent heat stress. In areas without any vegetative or other cover, traps were closed between sunrise and sunset.

Wild bats, rats, and farmed rodents were docile enough to permit direct, manual restraint during sampling. Swabs were taken from the oropharynx, urogenital tract, and rectum. Urine was collected opportunistically using sterile swabs to soak urine from the exterior urogenital opening during urination. No more than 6µL of blood to 1g of bat body weight was collected from bats and rodents either from the brachial or femoral artery or vein. Blood samples were centrifuged on site.

Adult and subadult animals (estimated from body size) were opportunistically collected. All animals were identified to species visually and sexed (M/F) upon sampling.

All biological samples were stored in 1.2ml internally threaded silicon O ring sealed Cryovial® storage cryovials. Serum was stored in cryovials without viral transport medium (VTM). All other samples were stored, separately in cryovials pre-loaded with VTM. All cryovials with samples were put immediately into dewars or dry shippers containing liquid nitrogen. Within 7-days of collection, all samples were transported directly to the Wuhan Institute of Virology Laboratory where they were rapidly catalogued and transferred to ultralow (-80°C) freezers for long-term storage and analysis.

All laboratory work for this research was conducted at the Wuhan Institute of Virology, Chinese Academy of Sciences (english.whiov.cas.cn). RNA was extracted using Qiagen RNeasy kit and as per the manufacturer's instructions at dedicated vertical fume cupboards with protection panels, airflow monitors, and UV light. RNA was extracted from each sample type (whole blood, oral swab, and anal swab) and stored in RNase-free 1.5ml vials and kept at -80˚C. Extracted RNA was pooled by animal and reverse transcribed (RT) to cDNA as detailed in Section 2.7.2.

Coronavirus and Paramyxovirus amplification was conducted as detailed in Sections 2.7.2.1 and 2.7.2.2. Bat RNA-dependent RNA polymerase (RdRp) nucleotide sequences were compared to RdRp gene sequences (Table 30 and Table 28) available in the National Center for Biotechnology Information database (NCBI 2016) using the algorithm blastn from the standard nucleotide BLAST program (Altschul *et al.* 1990). Sequences identified in this study were then aligned with 33 and 31 homologous reference sequences from other Paramyxoviruses and Coronaviruses respectively using Geneious 10 (Kearse *et al.* 2012). The best-fit model of the phylogenetic relationships was determined by constructing both Bayesian and Maximum Likelihood trees. Newcastle virus (*Avulavirus*) was used as the outgroup for Paramyxoviruses and Turkey Coronavirus for Coronaviruses. To generate Bayesian trees sequences were analysed by MrBayes 3.2.6 (Miller *et al.* 2010) run on the Cyberinfrastructure for Phylogenetic Research server (CIPRES: https://www.phylo.org). Maximum Likelihood trees were constructed with 1,000 bootstrap replicates using PhyML 3.0 with Smart Model Selection (Guindon & Gascuel 2003) on the ATGC South of France Bioinformatics Platform (http://www.atgc-montpellier.fr). FigTree 1.4.3 (tree.bio.ed.ac.uk) was used to produce the phylogenetic tree figures.

Correlations between both geographic locations and variation in genetic polymorphisms of the RdRp gene within and between populations (the fixation index: Fst) were calculated using Microsoft Office Excel Version 15.29.1 and DnaSP 5.10.1 (Librado & Rozas 2009).

Field species identification of positive samples was confirmed by DNA barcode and detailed in Section 2.7.3. Detection rate data along a latitudinal gradient and by gender were analysed with a chi-square analysis calculated using Microsoft Office Excel Version 15.29.1.

3.6 Results

Animals were sampled from seven bat families and four rodent families (Table 5).

Table 5. Rodents and Bats sampled by family.

Rodents	559
Cricetidae	118
Hystricidae	39
Muridae	194
Spalacidae	208
Bats	3,146
Emballonuridae	89
Hipposideridae	662
Miniopteridae	323
Molossidae	25
Pteropodidae	147
Rhinolophidae	807
Vespertilionidae	1,093

Individual rodents from 15 species were sampled (Table 6) including Malayan porcupines (*Hystrix brachyura*), voles (*Eothenomys* sp.), bamboo rats (*Rhizomys* sp.), rats (*Rattus tanezumi* and *Niviventer* sp.), field mice (*Apodemus* sp.), and one climbing mouse (*Vernaya fulva*). Of the rodents sampled, 250 (45%) were sourced from wild-animal farms where they (only *Rhizomys* sp.) were being raised for sale. Rodents were trapped and sampled in the wild totalled 309 (55%). No *Rhizomys* sp. were trapped in the wild. Of the rodents sampled, 543 (97.1%) were adults. Juvenile rodents sampled were all *Rhizomys sinensis*.

Individual bats from all 7 known Chiroptera families in China (Table 5) were sampled (Smith *et al.* 2010a). Most (92%) were from four families: Vespertilionidae, Rhinolophidae, Hipposideridae, and Miniopteridae. Samples were collected from 58 (48.3%) (Table 7) of the 120 known bat species in China (Smith *et al.* 2010a).

Table 6. Family, genus, and species for 559 rodents sampled.

Cricetidae	
Eothenomys cachinus	92
Eothenomys proditor	26
Hystricidae	
Hystrix brachyura	39
Muridae	
Apodemus chevrieri	123
Apodemus draco	1
Apodemus latronum	7
Apodemus peninsulae	28
Niviventer confucianus	1
Niviventer coxingi	2
Niviventer eha	27
Niviventer fulvescens	2
Rattus tanezumi	2
Vernaya fulva	1
Spalacidae	
Rhizomys pruinosus	20
Rhizomys sinensis	188

No rodent samples collected in this study were positive for Coronaviruses or Paramyxoviruses. Field identification of 89% (136/153) of host species (bats) with samples that tested positive for Coronaviruses or Paramyxoviruses was independently confirmed in the laboratory by commercial barcoding using the cytochrome b gene of bat mitochondrial DNA as a marker. Barcoding to identify species of 17 (11%) specimens was not possible due to insufficient sample quantity remaining after RT-PCR assays. Of the 89% tested and confirmed, the field identification was 100% accurate providing assurance that the unconfirmed species (by barcoding) had also have been accurately identified.

All Coronavirus positive samples were collected in four south China provinces (Hainan, Guangdong, Guangxi, and Yunnan), therefore no north-south gradient was discernible. Paramyxovirus positive samples were collected across a greater geographic range in China. Along a north-south gradient, the detection rate was significantly highest ($X^2 = 7.8372$, $p = 0.005118$) in the southern provinces of Fujian, Guangdong, Guangxi, Guizhou, and Yunnan. No correlation was evident between bat gender and detection of either virus ($X^2 = 0.2638$, $p = 0.607534$).

Table 7. Family, genus, and species for 3,146 bats sampled.

Emballonuridae	
Taphozous melanopogon	*89*
Hipposideridae	
Aselliscus stoliczkanus	*23*
Coelops frithi	*4*
Hipposideros armiger	*175*
Hipposideros cineraceus	*31*
Hipposideros larvatus	*158*
Hipposideros pomona	*134*
Hipposideros pratti	*137*
Miniopteridae	
Miniopterus fuliginosus	*160*
Miniopterus pusillus	*72*
Miniopterus schreibersii	*91*
Molossidae	25
Chaerephon plicata	*14*
Tadarida plicata	*6*
Tadarida teniotis	*5*
Pteropodidae	147
Cynopterus sphinx	*48*
Eonycteris spelaea	*28*
Rousettus leschenaultii	*71*
Rhinolophidae	807
Rhinolophus affinis	*204*
Rhinolophus ferrumequinum	*109*
Rhinolophus huananus	*6*
Rhinolophus lepidus	*21*
Rhinolophus luctus	*13*
Rhinolophus macrotis	*12*
Rhinolophus pearsonii	*37*
Rhinolophus pusillus	*133*
Rhinolophus rex	*11*
Rhinolophus sinicus	*261*

Vespertilionidae	1093
Barbastella beijingensis	*25*
Hypsugo alaschanicus	*9*
Ia io	*57*
Myotis altarium	*18*
Myotis annectans	*1*
Myotis blythii	*15*
Myotis bombinus	*1*
Myotis brandtii	*10*
Myotis chinensis	*28*
Myotis daubentonii	*97*
Myotis davidii	*24*
Myotis emarginatus	*1*
Myotis fimbriatus	*1*
Myotis longipes	*18*
Myotis myotis	*10*
Myotis pilosus	*223*
Myotis siligorensis	*14*
Nyctalus plancyi	*30*
Pipistrellus abramus	*10*
Pipistrellus mimus	*4*
Pipistrellus pipistrellus	*151*
Plecotus ognevi	*13*
Scotomanes ornatus	*2*
Scotophilus heathii	*34*
Scotophilus kuhlii	*145*
Scotozous dormeri	*1*
Tylonycteris pachypus	*80*
Tylonycteris robustula	*21*
Vespertilio murinus	*3*
Vespertilio sinensis	*8*
Vespertilio superans	*39*

3.7 Coronaviruses

In this study, 39 bat Coronaviruses (CoVs) were identified (Table 27). The percentage of identical bases in the sample sequences (identities) ranged from 100% to 57%. BLAST results for all 39 sequences had identities of 93.3% or higher to known bat CoV sequences in GenBank (Table 28). Coverage was 100% for 35 sequences (90%) and 93.54% or higher for the other 4 sequences (10%). The total detection of CoV positive samples from bats in this study was 1.2% (39/3,146) (Table 7). Coronaviruses were most often confirmed in samples from Hipposideridae (0.3%), Miniopteridae (3.1%), Rhinolophidae (1.4%), and Vespertilionidae (1.5%) (Table 8). Detection of CoV was highest in the following species *Ia io* (3.5%), *Rhinolophus rex* (63.6%), *Tylonycteris pachypus* (7.5%), and *Miniopterus fuliginosus* (6.3%) (Table 9).

Table 8. Coronavirus detection rate by family for bats sampled.

Family	Positive	Sampled	Percent
Hipposideridae	2	662	0.3%
Miniopteridae	10	323	3.1%
Rhinolophidae	11	807	1.4%
Vespertilionidae	16	1039	1.5%
Other Families	0	261	0.0%
Total	39	3146	1.2%

Table 9. Coronavirus detection rate by bat family, genus, and species sampled.

Family and Species	Positive	Sampled	Percent
Hipposideridae			
Hipposdieros armiger	1	175	0.6%
Hipposideros pratti	1	137	0.7%
Other Hipposideridae	0	350	0.0%
Miniopteridae			
Minopterus fuliginosus	10	160	6.3%
Other Miniopteridae	0	163	0.0%
Rhinolophidae			
Rhinolophus rex	7	11	63.6%
Rhinolophus sinicus	4	261	1.5%
Other Rhinolophidae	0	535	0.0%
Vespertilionidae			
Ia io	2	57	3.5%
Myotis pilosus	5	223	2.2%
Pipistrellus pipistrellus	3	151	2.0%
Tylonycteris pachypus	6	80	7.5%
Other Vespertilionidae	0	582	0.0%
Total	39	3146	1.2%

Table 9 total (3,146) in the final row includes an additional 261 bats sampled from other families for which there were no positive samples.

Bayesian (Figure 12) and Maximum Likelihood (Figure 13) trees corresponded generally in topography with the majority of sequences (59%, 23/39) aligning within the Alphacoronavirus genus and the rest (41%, 16/39) within the Betacoronavirus genus. Sequences identified in this study were distributed only in the Alphacoronavirus and Betacoronavirus two clades and these corresponded between both Bayesian and Maximum Likelihood trees as indicated in Figures 12 and 13. The relationships between the 39 sequences from this study and proximate Coronavirus genera and all Coronavirus genera are also detailed these two figures. Diversity tests run in DNAsp showed a high level of genetic variation in nucleotide diversity for Coronavirus sequences.

3.8 Paramyxoviruses

In this study, 114 bat Paramyxoviruses were identified (Table 29). The number of identical bases in the sample sequences (identities) ranged from 99% (PAR-4287) to 68% (PAR-4274) both isolated from samples from *Rhinolophus affinis*. Of the 82 sequences (72%) had 80% identity or less. Only five sequences (4%) had identities higher than 90%. Half (50%) of query reference sequences had a coverage of 100% and 99% of query reference sequences had a coverage of over 92%. Total detection of Paramyxovirus positive samples from bats sampled in this study was 3.6% (114 out of 3,146) (Table 10). Hubei (40%), Ningxia (12%), and Guizhou (41%) provinces had the highest rate of detection. Paramyxoviruses were found mostly in Emballonuridae (2.2%), Hipposideridae (10.6%), Rhinolophidae (2.5%), and Vespertilionidae (1.5%) (Table 10). Detection of Paramyxovirus was highest in the following species *Aselliscus stoliczkanus* (26.1%), *Hipposideros pratti* (27.7%), *Hypsugo alaschanicus* (11.1%), *Plecotus ognevi* (15.4%), and *Vespertilio sinensis* (37.5%) (Table 11).

Table 10. Paramyxovirus detection by family for bats species sampled in China.

Family	Positive	Sampled	Percent
Emballonuridae	2	89	2.2%
Hipposideridae	70	662	10.6%
Miniopteridae	6	323	1.9%
Rhinolophidae	20	807	2.5%
Vespertilionidae	16	1093	1.5%
Other Families	0	172	0.0%
Total	114	3146	3.6%

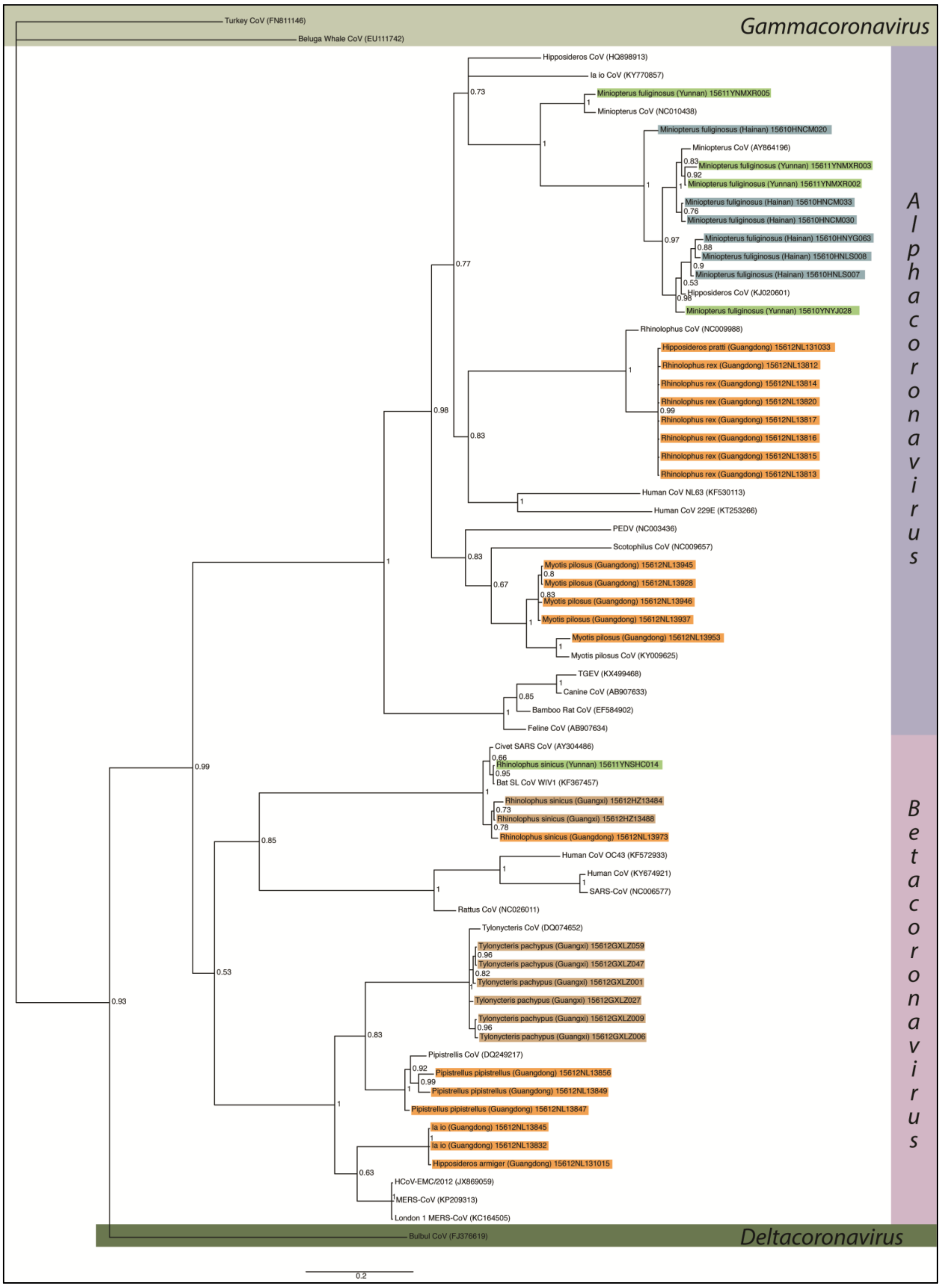


Figure 12. Coronavirus Bayesian inference phylogenetic tree. All Coronavirus sequences from this study are highlighted corresponding to geographic location or province: Guangdong Province (orange), Guangxi (brown), Yunnan (green), and Hainan (blue). All other (un-highlighted) sequences are reference sequences from GenBank (Table 28). Coronavirus sequences group into two major clades (shaded in light and dark purple) corresponding to the Betacoronavirus and Alphacoronavirus genera, respectively. Gammacoronavirus and Deltacoronavirus are also indicated. Scale bar indicates 0.2 nucleotide substitutions per site. Tree was constructed using MrBayes 3.2.6 under assumption of GTR model, using 3,000,000 trees sampled every 100 steps. Node support values are indicated. Tree is rooted on Turkey Coronavirus.

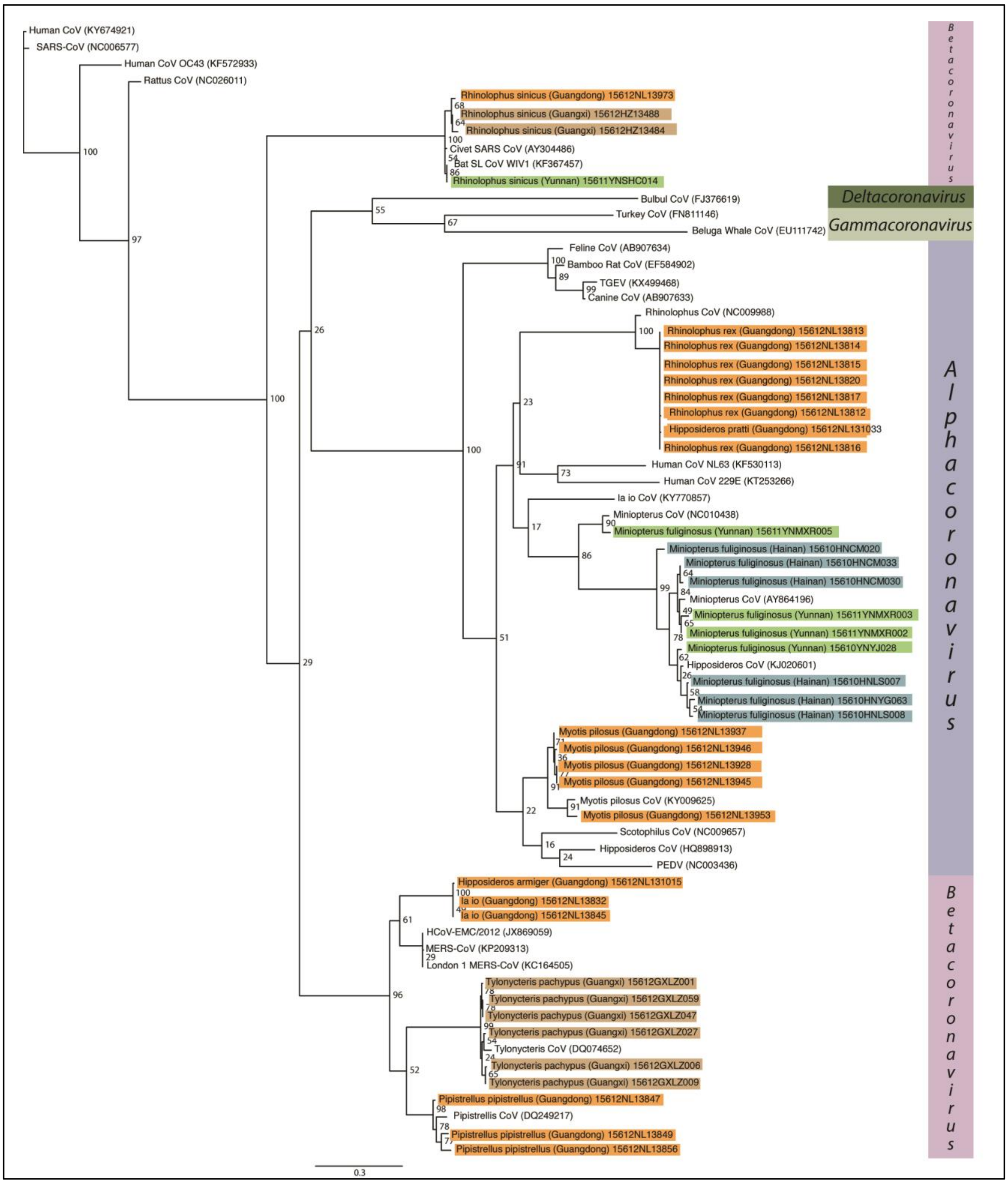


Figure 13. Coronavirus Maximum Likelihood phylogenetic tree. Sequences identified in this study are highlighted corresponding to geographic location or province: Guangdong Province (orange), Guangxi (brown), Yunnan (green), and Hainan (blue). All other (un-highlighted) sequences are reference sequences from GenBank (Table 28). Coronavirus sequences group into two major clades (shaded in light and dark purple) corresponding to the Betacoronavirus and Alphacoronavirus genera, respectively. Gammacoronavirus and Deltacoronavirus are also indicated. Scale bar indicates Scale bar indicates 0.3 nucleotide substitutions per site. The tree was constructed by Maximum Likelihood method using PhyML with Smart Model Selection with bootstrap values calculated from 1,000 trees and unrooted to emphasise the diversity of sequences identified

Table 11. Paramyxovirus positive samples by bat family and species.

Family and Species	Positive	Total	Percent
Emballonuridae			
Taphozous melanopogon	2	89	2.2%
Hipposideridae			
Aselliscus stoliczkanus	6	23	26.1%
Hipposideros armiger	16	175	9.1%
Hipposideros larvatus	1	158	0.6%
Hipposideros pomona	9	134	6.7%
Hipposideros pratti	38	137	27.7%
Other Hipposideridae	0	35	0.0%
Miniopteridae			
Miniopterus fuliginosus	4	160	2.5%
Miniopterus schreibersii	2	91	2.2%
Other Miniopteridae	0	160	0.0%
Rhinolophidae			
Rhinolophus affinis	9	204	4.4%
Rhinolophus pearsonii	2	37	5.4%
Rhinolophus pusillus	1	133	0.8%
Rhinolophus sinicus	8	261	3.1%
Other Rhinolophidae	0	172	0.0%
Vespertilionidae			
Hypsugo alaschanicus	1	9	11.1%
Ia io	2	57	3.5%
Myotis altarium	1	18	5.6%
Myotis davidii	2	24	8.3%
Myotis pilosus	5	223	2.2%
Plecotus ognevi	2	13	15.4%
Vespertilio sinensis	3	8	37.5%
Other Vespertilionidae	0	741	0.0%
Total	114	3146	3.6%

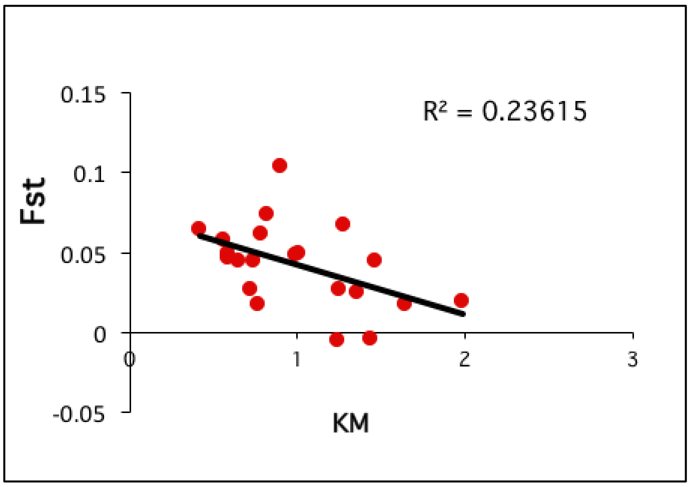


Figure 14. Correlation of Fst and distance. Graph of the fixation index or a measure of genetic polymorphism within populations vs between populations and distance between populations (sampling locations) in 1,000 kilometres. This figure suggests a negative association between genetic diversity and distance between populations.

Diversity tests run in DNAsp showed a high level of genetic variation in nucleotide diversity for Paramyxovirus sequences. Of the 114 positive samples, a total of 88 Paramyxovirus haplotypes were identified. Southern provinces had higher genetic diversity than northern provinces. An analysis of variation in sequence polymorphism or genetic variance within bat populations versus between bat populations (Fst) showed a negative correlation with genetic variance increasing as distance decreased (Figure 14).

Bayesian (Figure 15) and Maximum Likelihood (Figure 16) trees corresponded generally in topography (Figure 17) showing a 'superclade' including Feline Paramyxoviruses from Japan and Hong Kong, rodent and shrew Paramyxoviruses from Africa and Asia, Beilong virus, and J virus. *Henipavirus* is monophyletic to this 'superclade'. Sequences identified in this study are distributed in two clades; these corresponded between Bayesian and Maximum Likelihood trees and are indicated in the green and yellow shaded regions in Figure 15 and Figure 16. The relationships between the 114 sequences from this study and proximate Paramyxovirus genera and all Paramyxovirus genera are shown in Figure 17 and Figure 18 respectively.

Based upon the cytochrome B gene, a phylogeny was constructed of all species tested positive from this study along with rodent genera sampled, as well as domestic cat (*Felis catus*), raccoon dog (*Nyctereutes procyonoides*), and other species found to either harbour or have been infected with SL-CoV or SARS-CoV (Figure 19).

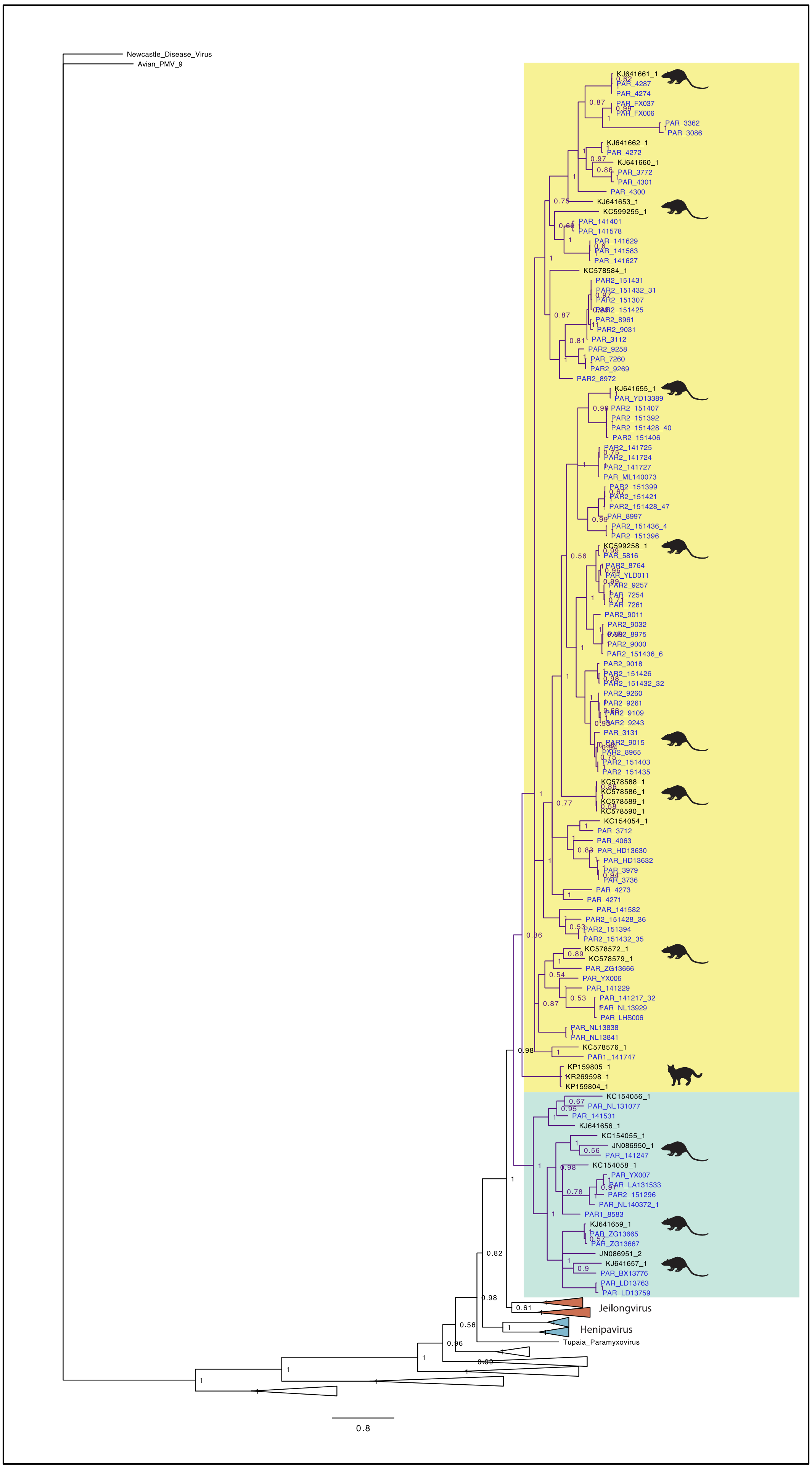


Figure 15. Bayesian inference phylogenetic tree with all Paramyxovirus. Sequences from this study are indicated in purple and all reference sequences from GenBank in black. Sequences group into two major clades (shaded in yellow and aqua) that are paraphyletic with the *Henipavirus* genus. Scale bar indicates 0.8 nucleotide substitutions per site. Tree was constructed using MrBayes 3.2.6 under assumption of GTR model, using 3,000,000 trees sampled every 100 steps. Node support values are indicated. Tree is rooted on Newcastle disease virus. Clades in red include J virus and Beilong virus; clades in blue include Nipah virus and Henipa virus. Rodents and cat indicate respective reference sequences within the clades. Unmarked clades in white at bottom of the tree represent the following genera from top to bottom: Morbillivirus (3 clades), Rubulavirus (1 clade), and Avulavirus (1 clade).See Figure 17 for more discussion.

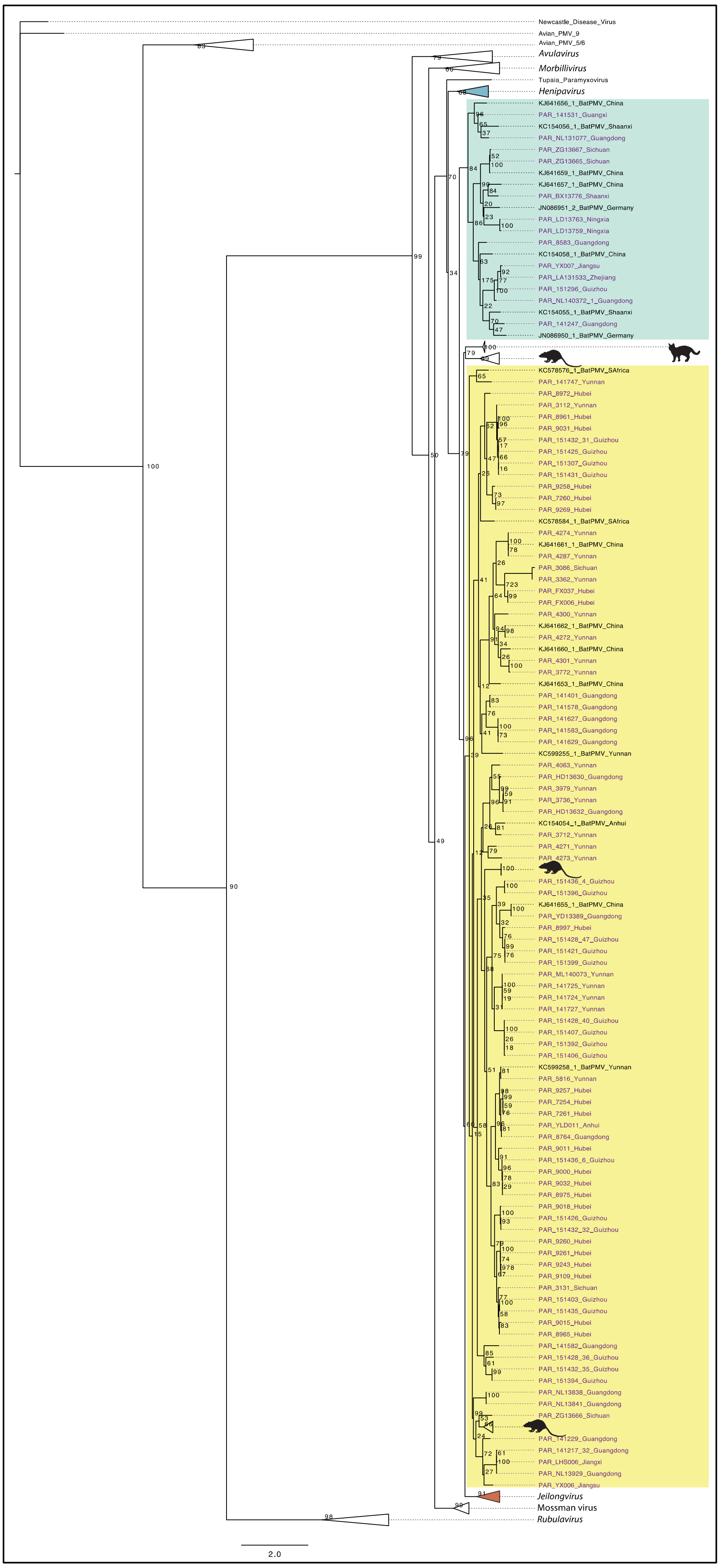


Figure 16. Paramyxovirus Maximum Likelihood phylogenetic tree. Sequences identified in this study are in purple; reference strains are in black. Scale bar indicates 0.8 nucleotide substitutions per site. The tree was constructed by Maximum Likelihood method using PhyML with Smart Model Selection with bootstrap values calculated from 1,000 trees and rooted on Newcastle disease virus. As with Figure 15, *Jeilongvirus* genus is indicated in red, *Henipavirus* genus in blue. Rodents and cat indicate respective reference sequences within the clades. See Figure 17 for more discussion.

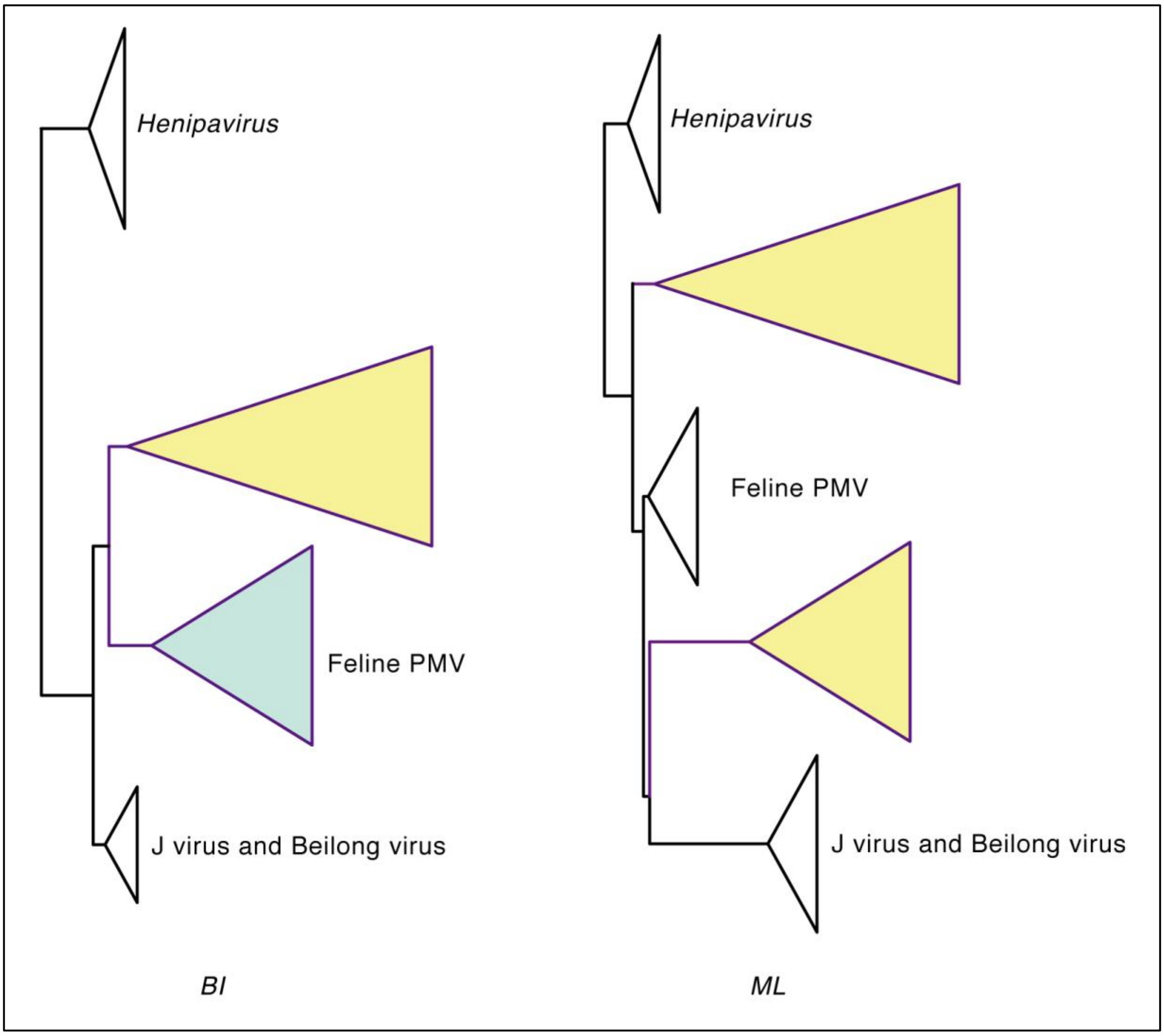


Figure 17. Comparison of Paramyxovirus Bayesian inference and Maximum Likelihood phylogenetic trees. Paramyxoviruses from this study are grouped into clades (yellow triangles). The Baysian Inference (BI) tree has only one clade whereas the Maximum Likelihood (ML) tree has two, distinct clades. Feline Paramyxovirus reference sequences (from cats in Hong Kong (Woo *et al.* 2012b)) fall within one of the two clades in the Maximum Likelihood tree. Support values and other details are shown in Figure 15 and Figure 16.

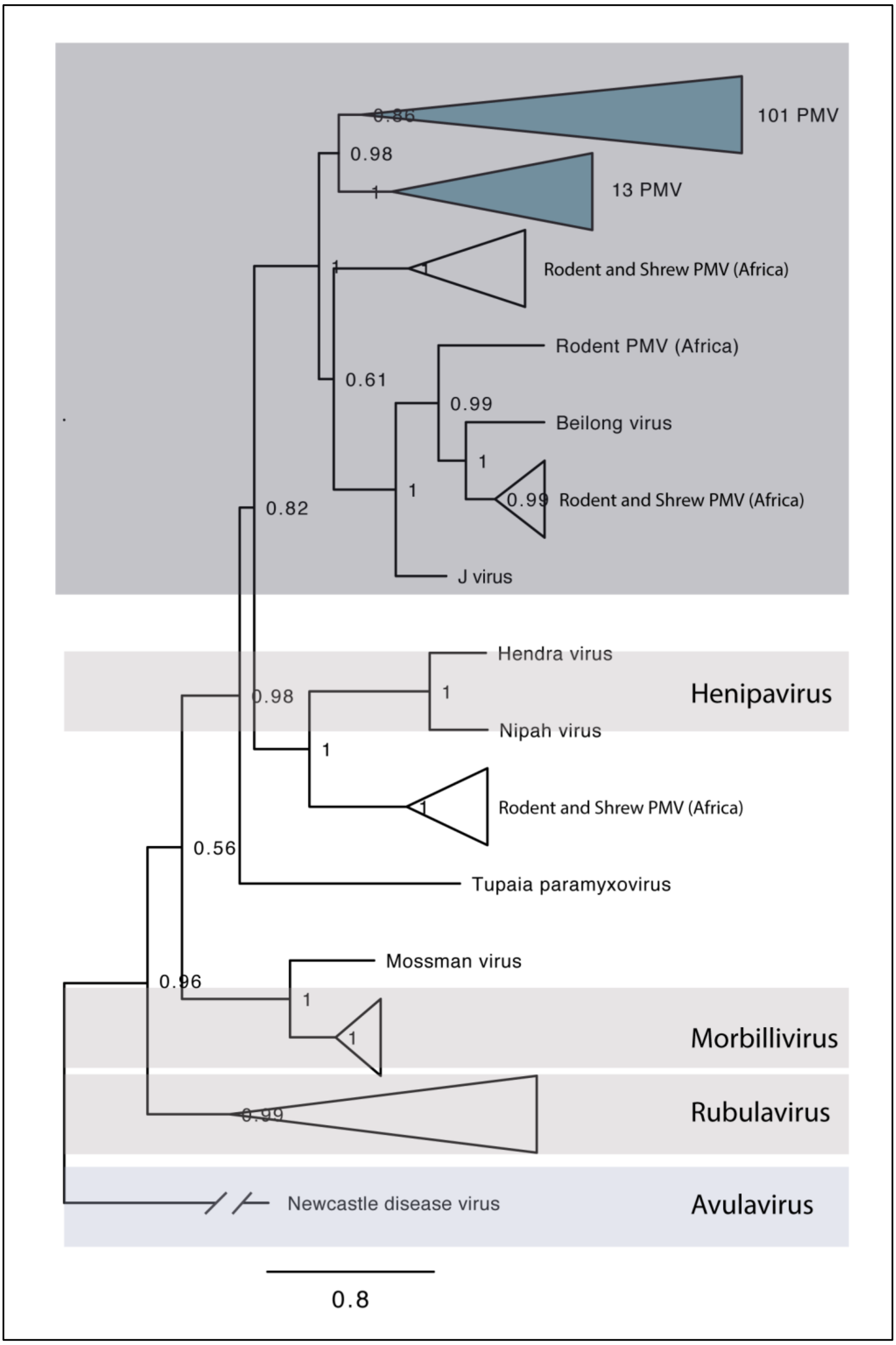


Figure 18. Summary phylogenetic analysis of tree of bat Paramyxoviruses detected in this study. Tree is based upon the partial RdRp gene sequences. Tree was constructed using MrBayes 3.2.6 under assumption of GTR model, using 3,000,000 trees sampled every 100 steps. Node support values are indicated. The 114 Paramyxoviruses identified in this study are grouped within the two triangles at the top labelled *101 PMV* and *13 PMV* respectively. The upper most grey retangle indicates the proposed, novel Jeilong virus genus. Other genera (Henipavirus, Morbillivirus, Rubulavirus, and Avulavirus) are listed in the rectangles below.

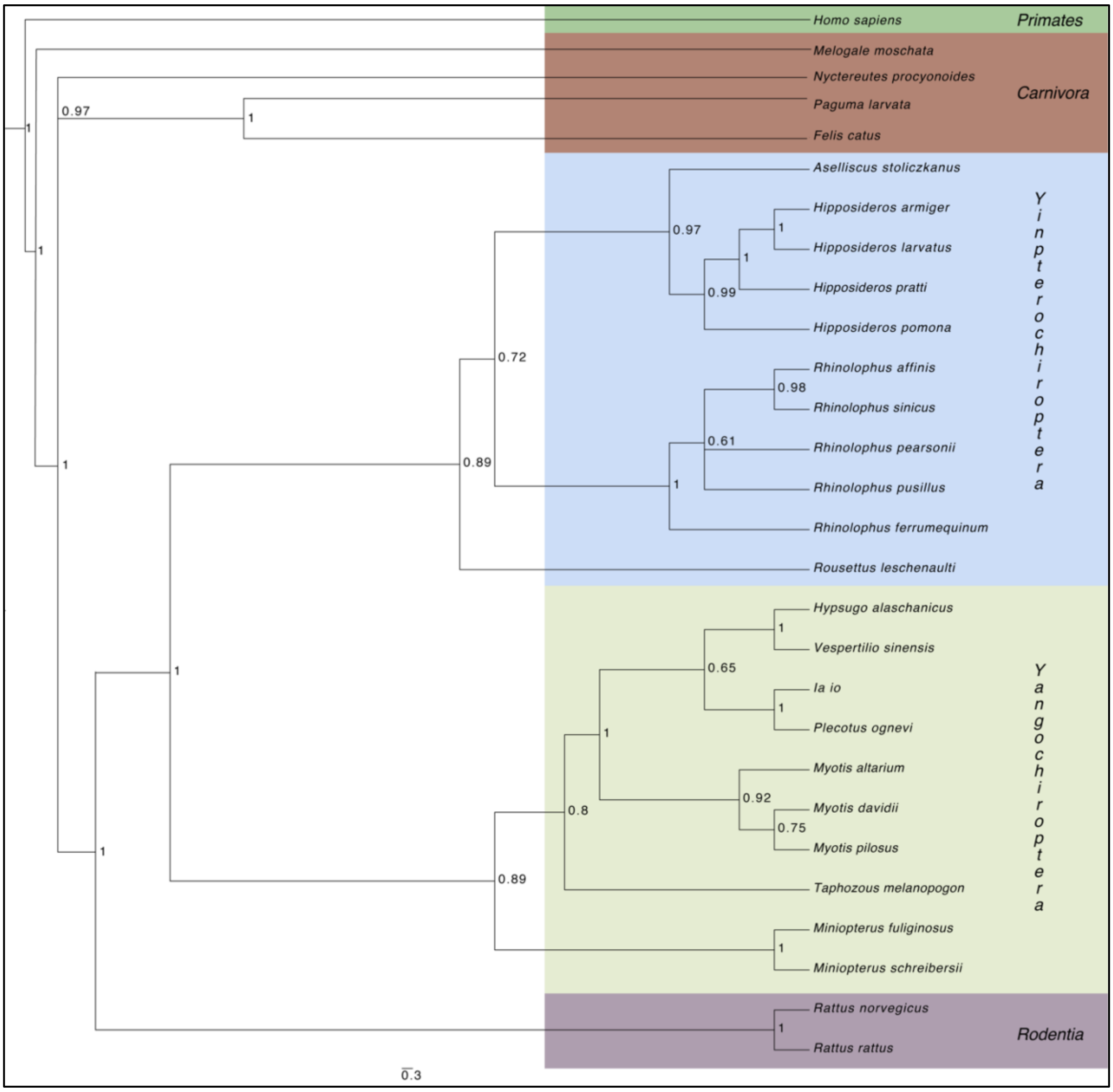


Figure 19. Bayesian inference chytochrome b phylogenetic tree of bat and other species. Tree shows bat species (Chiroptera suborders *Yinpterochiroptera* and *Yangochiroptera*) from which Paramyxovirus samples were collected in this study along with Carnivora including domestic cats (*Felix catus*), civets (*Paguma* larvata), raccoon dogs (*Nyctereutes procyonoides*), and ferret badgers (*Melogale moschata*), Rodentia (*Rattus rattus* and *Rattus norvegicus*), and humans (*Homo sapiens*), since Paramyxoviruses have been discovered in all of these orders. Humans are the outgroup. Tree was constructed using MrBayes 3.2.6 under assumption of GTR model, using 3,000,000 trees sampled every 100 steps. Node support values are indicated. Scale bar indicates 0.3 nucleotide substitutions per site.

3.9 Discussion

While several studies have detected Paramyxoviruses from bats (Li *et al.* 2008; Tong *et al.* 2008; Lau *et al.* 2010b; Yuan *et al.* 2014) and rodents (Li *et al.* 2006; Wu *et al.* 2014b) in China, this is the first study to present such a broad sampling both spatially and temporally – including both wild and farmed animals. The detection of known and potentially novel Paramyxoviruses in 19 species of bats from five families that are natural reservoirs for Paramyxoviruses confirms the hypothesis that diverse Paramyxoviruses are circulating widely in multiple bat populations in China.

Although the Coronavirus sequences discovered in bats in this study were not novel, they were diverse further providing evidence of these viruses circulating within wild bat populations.

As host species, rodents and bats may be persistently and asymptomatically infected by viruses (Schountz 2014; Plowright *et al.* 2015). This also means that viruses would be expected to be shed continually and would be present in samples assayed for viruses by RT-PCR. Although no evidence of Coronaviruses or Paramyxoviruses was found in either farmed or wild populations of rodents in this study, the RT-PCR assay protocol used had been sufficiently sensitive to detect Coronaviruses and Paramyxoviruses in wild rodents collected elsewhere (Funk *et al.* 2009; Woo *et al.* 2011; Sasaki *et al.* 2014; Wilkinson *et al.* 2014; Wang *et al.* 2015). Additionally, out of 871 bats sampled in Hainan province, none tested positive for Paramyxoviruses. No other studies to date have reported Paramyxoviruses in bats in Hainan. Given the detection rates of these viruses elsewhere in China, this suggests that viral detection may be low in rodents and also in bats or associated with temporal or seasonal variance of these viruses in their host reservoirs (Plowright *et al.* 2015). A total of 6 bats in Hainan did test positive for Coronaviruses and unlike the Paramyxoviruses sampled in this study, the Coronaviruses appear to clearly correspond by species to geographic location and host (Figure 12 and Figure 13). No bat species in this study was found to be coinfected with Coronavirus and Paramyxovirus at the time of sampling. Detection of Coronaviruses in Chinese bats from other studies is higher than the 1.2% reported here and ranges from 6.5% (Tang *et al.* 2006) and 6.84% to (Lin *et al.* 2017) as high as 9.8% (Gloza-Rausch *et al.* 2008) and 12% (Woo *et al.* 2006b). Although detection of Coronaviruses in bats in China has been published, detection for Paramyxoviruses is not currently known, but detection of Paramyxoviruses in bats and rodents elsewhere has been published between 10% and 38% (Young & Olival 2016). In this study, a conservative estimate of 10% detection rate was used for both viruses. A 10% detection rate in wild populations of bats or rodents required a sampling of a minimum of 30 individuals per species in order to ensure detection of an infected individual with 95% confidence (Wobeser 2013). Minimum species sampling targets were set to this number, although not always attained for rare species, i.e. *Myotis pilosus* (Csorba & Bates 2008; Smith *et al.* 2010a). The overall detection of 4% Paramyxoviruses and 1.2% Coronaviruses in bats sampled in this study was lower than some other surveys, particularly for Paramyxoviruses in bats in Asia, Australia, and Africa regions (Li *et al.* 2008; Breed *et al.* 2010; Baker *et al.* 2012; Yuan *et al.* 2014) although well within the range of those found in bats in other studies in Asia and in the Americas (Yob *et al.* 2001; Segovia *et al.* 2016). Bat species and effort were not equivalent in all provinces (Table 3) and after initial, broad, China-wide surveys sampling was focused exclusively in southern China, so no conclusions may be drawn from the high rates of detection in Ningxia (12%, 2/7) and Hubei (40%, 29/72) provinces. It is interesting to consider whether this was a result of random sampling or some other effect such as stress (migration, scarcity of food) or mixed species roosting. Likely due to uneven sampling efforts there were no discernible correlations between host or viral species at sampling sites along any gradient: north-to-south; east-to-west; rural-to-urban. In an effort to examine whether there may have been any patterns of genetic diversity correlated with geographic location (sampling sites), population differentiation (Fst) yielded an unexpected result of increased genetic diversity of Paramyxoviruses between geographically proximate locations such as between Guangxi and Guangdong provinces (600km) with decreased genetic diversity between more distant provinces such as between Yunnan and Fujian or Zhejiang (2,400km). The expectation would have been that as host species are more distant and separated both they and their viruses would diverge genetically over time. One explanation for high, local genetic diversity may be barriers to conspecific mixing resulting from changes in bat host ecology such as only some species accessing food sources or roosting sites in proximity to urban habitats. This, though, would not account for the decrease in diversity over distance. A likely explanation for this decrease in diversity may be in the relatively unbalanced sample sizes along the latitudinal gradient in China. A total of 79% (2,485) of bats sampled were in the southern China band of provinces while only 21% (661) were sampled from northern provinces. Positive Coronavirus samples were from southern provinces only, so no gradient for these viruses was discernible.

The stochastic effect of sampling within populations may have resulted in the positive Paramyxovirus sequences geographically most distant to each other having lower overall diversity. Given that bats are volant and no sampling site was outside of the known and shared ranges for these bat species, these results may be explained by seasonal or other migrations of bats. Bat species sharing roosts would also shed and share viruses resulting in co-infections or repeat infections, which would result in viral evolution by increasing diversity. If bats with roost-sharing behaviour in south China were to migrate north in April and May, they or more likely other roost-sharing species could have been sampled again in other regions of China in August. As bats sampled were not tagged for capture-release it is unknown if species were sampled repeatedly along any gradient, although this may be unlikely across distances exceeding 2,000km for example from Jiangsu to Yunnan or Hainan to Ningxia. It must be noted that some species of European and American insectivorous bats have been shown to migrate over 1,000km, although usually along corridors corresponding to river valleys or coastlines (Fleming & Eby 2003; Furmankiewicz & Kucharska 2009; Popa-Lisseanu & Voigt 2009; Altringham 2011).

In currently published literature and online resources there is much information about most of the Chinese bat species sampled in this study, but details about these species' migratory behaviour are absent (Nowak 1994; Fleming & Eby 2003; Francis 2008; Smith *et al.* 2010a; IUCN 2016). Only absence or presence is recorded. Most of the bats sampled in this study are insectivorous (excepting the fishing bats: *Myotis* spp.) and have ranges within southeast Asia spanning China and 18 other countries within Asia as well as for one species (*Miniopterus magnater*) Africa and Europe (Table 12). Most also are found in forested, agricultural and urban environments so have opportunity for contact with humans and domestic animals. Only 1 species is currently listed as threatened (*M. magnater*) and is also found across the greatest geographic range. The 4 bat species sampled that had the highest occurance of Paramyxovirus (*Rhinolophus affinis*, *Hipposideros pomona, Hipposideros armiger*, and *Hipposdieros pratti*) are found across all habitat types: urban, agricultural, forested, and degraded forest (indicated in grey in Table 12). Of the other bats from which positive Paramyxovirus samples were collected, 9 are also present in all habitat types.

Of the 25 species of bats from which positive viral samples were discovered, six (*Taphozous melanopogon*, *Hipposideros armiger*, *Hipposideros larvatus*, *Hipposideros pratti*, *Miniopterus schreibersii*, and *Rhinolophus affinis*) are known to roost with one or more other bat species (Willoughby *et al.* 2017). Three other species (*Rhinolophus pearsonii*, *Rhinolophus pusillus*, *Rhinolophus sinicus*) have no recorded roosting behaviour, but when collected for this study observed to be roosting with other species. One species (*Myotis pilosus*) known to have single-species-roosts, was collected in this study from caves with mixed species assemblages. Multiple species roosts provide ample opportunities for continuous shedding of viral particles within and between species and is theorised as one of the drivers of the diversity of Coronaviruses and Paramyxoviruses in bats (Wong *et al.* 2007; Drexler *et al.* 2012).

Table 12. Geographic ranges, IUCN status, and ecology of bat species sampled in this study. Abbreviations as follows: Y = Yes; N = No; LC = Least Concern; UK = Unknown; NT = Near Threatened; Spp. = Species. Bat species indicated by grey bars were hosts to most (63.2%) Paramyxoviruses found in this study. *R. rex*, *M. fuliginosus*, and *T. pachypus* were hosts to most (59%) of the Coronaviruses found in this study. Species indicated with an § were observed and captured from mixed species roosting areas. Horizontal grey bars indicate the five species that were confirmed hosts to both Paramyxoviruses and Coronaviruses. (Nowak 1994; Smith *et al.* 2010a; IUCN 2016).

Species	Family	China	Malaysia	Vietnam	Myanmar	Thailand	Cambodia	Laos	Bangladesh	India	Nepal	Indonesia	Pakistan	Bhutan	Philippines	Sri Lanka	Mongolia	Russia	Korea	Japan	Europe	Africa	Roosts w/ Other	IUCN	Urban	Forest	Agricultural	Degraded Forest	PMV Detected	CoV Detected
T. melanopogon	Emballonuridae	x	x	x	x	x		x															Y	LC		x	x	x	x	
A. stoliczkanus	Hipposideridae	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x							N	LC	x	x	x	x	x	
H. armiger	Hipposideridae	x	x	x	x	x		x		x	x												Y	LC	x	x	x	x	x	x
H. larvatus	Hipposideridae	x	x	x	x	x	x	x	x	x		x											Y	LC	x	x	x	x	x	
H. pomona	Hipposideridae	x	x	x	x	x	x	x	x	x	x												N	LC	x	x	x	x	x	
H. pratti	Hipposideridae	x	x	x	x	x																	Y	LC	x	x	x	x	x	x
H. terasensis	Hipposideridae	x		x																			UK	UK		x		x		
M. schreibersii	Miniopteridae	x															x	x	x	x			Y	LC		x	x	x	x	
M. fuliginosus	Miniopteridae	x		x	x	x		x		x	x												N	LC		x	x	x	x	x
M. magnater	Miniopteridae	x	x	x	x	x		x		x	x	x			x			x	x	x	x	x	N	NT	x	x	x	x		
C. sphinx	Pteropodidae	x	x	x	x	x	x	x	x	x		x	x	x	x	x							N	UK		x	x	x		
R. affinis	Rhinolophidae	x			x							x											Y	LC	x	x	x	x	x	
R. pearsonii§	Rhinolophidae	x				x																	UK	LC		x		x	x	
R. pusillus§	Rhinolophidae	x	x	x	x	x		x		x		x											UK	LC				x	x	
R. rex	Rhinolophidae	x																					UK	LC	x	x	x	x		x
R. sinicus§	Rhinolophidae	x		x				x															UK	NT		x		x	x	x
H. alaschanicus	Vespertilionidae	x															x	x	x				N	LC	x	x	x	x	x	
I. io	Vespertilionidae	x	x	x	x	x	x	x	x	x	x	x		x									N	LC	x	x	x	x	x	x
M. altarium	Vespertilionidae	x	x	x	x	x		x	x	x	x			x									N	LC	x	x	x	x	x	
M. davidii	Vespertilionidae	x	x	x	x	x	x	x		x	x	x								x			N	LC		x		x	x	
M. pilosus§	Vespertilionidae	x		x	x					x	x												N	LC	x	x	x	x	x	x
P. pipistrellus§	Vespertilionidae	x			x					x			x					x			x	x	N	LC	x		x	x		x
P. ognevi	Vespertilionidae	x	x	x	x	x	x	x		x		x			x	x							N	LC	x	x	x	x	x	
T. pachypus	Vespertilionidae	x	x	x	x	x	x	x	x	x		x											N	LC		x	x	x		x
V. sinensis	Vespertilionidae	x															x	x	x	x			N	LC	x	x	x	x	x	

Most bat and rodent samples (76.3%) were collected between July and September; (96.5%) were collected annually in a 7-month window from April to October after which, bats in southern China were no longer in roosts (personal observations) likely having migrated to the southern areas of their home ranges (Wilson & Reeder 2005; Smith *et al.* 2010a) to southeast Asia (Laos PDR, Thailand, Vietnam, Malaysia, and Cambodia). Although other studies have shown relationships between seasonality and environmental stressors (Plowright *et al.* 2015) and about half (55.3%) of positive samples were collected from bats during the month of August. Other studies have had much higher detection rates for Coronaviruses in bats sampled from November to March (Tang *et al.* 2006) suggesting that longitudinal sampling conducted in the southernmost regions of China, may shed further light on temporal trends in Coronavirus and Paramyxovirus detection in bat populations.

Sample results have a bias towards both abundant species and those that are comparatively easier to trap for effort expended. Bat and rodent species that do not roost or forage in urban, semi-urban, and agricultural areas were more difficult to sample with the same frequencies as those that habituated human dominated landscapes. Some bat and rodent species prefer locations relatively inaccessible to field teams or at times when frequent sampling trips were not possible, e.g. due to seasonal flooding. Sampling efforts were designed to be longitudinal, but in practice weather (flooding) or seasonal variation of target species (migration) hindered repeated sampling efforts. Future studies may consider focusing on longitudinal sampling in locations across the southern region of China, especially during the Oct to April period, to test the hypotheses that there may be a seasonal component to viral spillover from host species.

Figure 19 exhibits the phylogenetic relationships of the mammalian species sampled in this study and includes mammalian species with which they would likely come into contact with such as domestic cats (*Felis catus*), civets (*Paguma* larvata), raccoon dogs (*Nyctereutes procyonoides*), ferret badgers (*Melogale moschata*), rats (*Rattus rattus* and *Rattus norvegicus*), and humans (*Homo sapiens*). Phylogenetic proximity of host species such as bats sharing roosting sites does predict potential for sharing of viral species (Young & Olival 2016). Other studies have suggested that there is direct transmission of Paramyxoviruses between rodent and bat species (Wilkinson *et al.* 2014) and the emergence of SARS-CoV is theorised to have originated via spillover in live animal markets, potentially from a bat to another species (Wang *et al.* 2006). The nucleotide identities of feline Paramyxovirus sequences from NCBI GenBank (Woo *et al.* 2012b; Sieg *et al.* 2015) and bat Paramyxovirus sequences from this study had 50%-78% nucleotide identity. Most sequences from sampled bats in this study had between 50%-80% nucleotide identity to known Paramyxoviruses and greater than 93% nucleotide identity to known Coronaviruses. Some Paramyxovirus sequences from Hubei and Jiangsu had less than 60% nucleotide identity to any currently in GenBank. If a threshold may be set at 80% (or less) nucleotide affinity for novel viral Paramyxovirus species (Anthony *et al.* 2017; ICTV 2017a), then 71% (82 out of 114) of the Paramyxovirus sequences in this study may be considered novel, which confirms this study's hypothesis that there is a diversity of Paramyxoviruses circulating in bats in China. Since only the RdRp gene was analyses in this study, further characterisation of the viruses would be required to confirm whether they are truly novel. For the Coronaviruses in this study, although diverse, none were novel and all had already been described elsewhere. The phylogenies detailed in Figure 12 and in Figure 13 include homologous strains from BLAST results in NCBI.

From the phylogenetic analyses, there is a clear grouping of Paramyxoviruses sequences from this study into two, distinct clades. Together they form a 'super-clade', which may include Paramyxoviruses from rodents, shrews, and domestic cats. The Paramyxovirus sequences from this study would likely be within the proposed *Jeilongvirus* genus (Woo *et al.* 2016), which would be paraphyletic with *Henipavirus* genus. Whether some of this study's potentially novel Paramyxoviruses are ancestral to the feline and rodent Paramyxoviruses is not clearly resolved, but the predator-prey interrelationships among these species would suggest viral exchange.

Rodent Paramyxoviruses have recently been identified as having caused morbidity (Wu *et al.* 2014b) and mortality (Wu *et al.* 2014b) in humans in China. This, as well as the results of this study, provide more evidence that there are a diversity of Paramyxoviruses circulating in wild animals (bats and rodents) and commensal species (rats) (Figure 19). Although the novel Paramyxoviruses identified in this study are not closely related to known viruses of human concern such as Hendra virus and Nipah virus or others affecting livestock, the ubiquity and diversity of these Paramyxoviruses raise the consideration that zoonotic transmission may occur frequently and poses a clear risk to human and animal health.

4 Attitudes of Chinese Millennials Towards Wildlife Trade and Disease Risk

4.1 Introduction

Demand for wildlife and wildlife products is a global threat to conservation (Lenzen *et al.* 2012; Moran & Kanemoto 2017). The wildlife trade has also been linked to a series of emerging diseases and is thought to heighten the risk of zoonotic disease spillover due to increased animal-human interaction (Fèvre *et al.* 2006; Smith *et al.* 2009; Johnson *et al.* 2015). For example Ebola viruses, Marburg virus, and henipaviruses are carried by bats in Africa which are hunted and traded widely and there is evidence of spillover of henipaviruses into people in the region (Kamins *et al.* 2011; Pernet *et al.* 2014). Ebola virus is known to cause illness in primates which are part of the bushmeat trade in Africa, and this has led to previous outbreaks in human (Chapman *et al.* 2005) and non-human primates (Formenty *et al.* 1999). In China, the first spillover of SARS-CoV from bats to civets and humans is thought to have occurred in large wildlife markets in Guangdong province, leading to its pandemic emergence (Guan *et al.* 2003).

Legislation and global conservation efforts have successfully reduced wildlife trade in some regions or for some species, but stronger enforcement and international cooperation have been called for (Zimmerman 2003). As a result of the diversity, breadth, and constant shifts in demand and supply, assessing the volume of wildlife trade is difficult (Karesh *et al.* 2005). Some studies have concluded that, after the USA and Europe, which trade wildlife mainly for pets (Smith *et al.* 2009), China is the third highest consumer of wildlife (Wyler & Sheikh 2008; Patel *et al.* 2015). This is driven by high demand in China for use of wildlife or wildlife products in traditional medicine, food, decorative items, and to a lesser extent as pets (Zhang *et al.* 2008).

4.2 Chinese Wildlife Trade in Ivory

Elephant Ivory is a prime example of a wildlife product in high demand and that has devastating consequences on wildlife populations (Clarke & Babic 2016). Chinese demand for ivory has been considered one of the main drivers of the ivory trade (Gao & Clark 2014). It is difficult to ascertain whether recent legislative prohibitions on ivory trade in China have had any effect on the demand, since there exists a relatively unquantifiable illegal market for ivory. Additionally, there are factors external to China such as a recent decrease in the cost of shipping rates and political instability in countries where elephants are found that make ivory trade more appealing to some of the actors involved (Moyle 2014). Despite the legislation, legal, illegal, and indeterminate trade in ivory persists within China (Gao & Clark 2014).

In 2009, the International Fund for Animal Welfare (IFAW) launched a China-based public awareness campaign to make the Chinese people aware that ivory is sourced from dead elephants. The announcement consisted of a poster (Figure 20) that was placed in airports, office buildings, universities, subways, and other public locations. IFAW hoped that the campaign would discourage Chinese people from buying ivory. IFAW felt that if more Chinese people were made aware that ivory for sale in China came from slaughtered elephants, they might then stop buying ivory (Ge 2011).

Figure 20. International Fund for Animal Welfare public service announcement from Shanghai subway. In the advertisement text, the baby elephant excitedly tells his mother three times, “Mama, I grew my tusks!" She does not respond and he says, “Mama, why are you not happy?” The word for “teeth” and “tusks” in Chinese is the same. To the right of the advertisement (not shown) text explains that elephant ivory or “teeth” come from slaughtered wild elephants. Source: IFAW (2009).

Four years later, an online Chinese language survey assessed both the awareness generated by the campaign and the potential impact either the campaign or the survey had on respondents’ future intent to purchase ivory (Li 2013a). The results showed that 75% of those surveyed (n = 1,067) had viewed the campaign in the past years and of these 66% would not buy ivory in the future (Li *et al.* 2013). Of the surveyed population that had not previously seen the campaign, 33% said they would not buy ivory in the future (Li 2013a).

These survey data show a positive correlation between (a) people being made aware of the harm that ivory trade does to elephant populations and (b) their decision to no longer purchase ivory. The theory is that legislation and enforcement alone cannot stop the supply of wildlife products such as ivory as long as there is still a demand, which must also be addressed. This suggests that awareness campaigns can nudge behaviour and complement legislation and enforcement efforts. In line with these findings, recently there have been calls to consider applying a demand-side approach, via education and awareness campaigns rather than reliance solely upon legislation and enforcement, to reduce or halt the demand for ivory and other wildlife products in China (Zhang *et al.* 2008; Rosen & Smith 2010; Zhang & Yin 2014; Challender *et al.* 2015).

4.3 Wildlife Trade Legislation in China

The Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES) is the primary international treaty protecting the movement of endangered species globally (CITES 1973). CITES' signatories regulate global wildlife trade, protect threatened species, and indirectly reduce disease risk (Rosen & Smith 2010). CITES has proven successful at reducing wildlife trade internationally (Fuchs 2008), but local or national involvement is necessary to achieve this (Cooney & Abensperg-Traun 2013; Biggs *et al.* 2017). In China, a number of laws have been enacted to address wildlife trade. In 1989, the Seventh National People's Congress established the Law of the People's Republic of China on the Protection of Wild Animals (PRC 1988). The stated goal of the law was both to protect and preserve wildlife, but also to develop and use wildlife as a natural resource to be exploited for China's economic gain (Article 1). Domestication and breeding of wildlife and development and use of wildlife resources was specifically encouraged (Article 4 and Article 17). Criticism was directed at the law with calls for amendments to revise or remove the promotion of wildlife as an exploitable resource (Cao 2011). Effective 1st January 2017, the law was amended to forbid the purchase and sale of any wild animals and their products (Articles 15, 27, 44, and 48) (PRC 2016b). The language about wildlife as a resource to be exploited was removed. The penalty for illegal sale or use of wildlife is a fine levied by the local Wildlife Protection Department of at least ten times the sale value of the confiscated wildlife, or if there is no value to the confiscated wildlife, then a fine between RMB10,000 and RMB50,000 (£1,200 and £5,800) (Article 44). The amendment further stated that a list of the national key protected wildlife will be formulated by the Central Government Level Department of Wildlife Conservation after scientific assessment and updated every five years (Article 10).

Chinese legislation is very clear in prohibiting wildlife trade, but defining the specific species protected and the enforcement efforts are left entirely up to regional departments. Local or provincial authorities prohibit or permit in "special circumstances" the sale, purchase, scientific research, artificial breeding, public display, hunting, or use of wild animals and their products (Article 25). Without a concrete list of key protected species, it is also up to the regional authorities and departments to enforce legislation against wildlife hunting, use, and farming. Additional language (Article 25) permits hunting (Articles 21-23), breeding, and sale (Articles 26-28) of wildlife with a license from the relevant local or provincial authorities. At the local and regional level, Chinese government agencies that are responsible for oversight and regulation of wildlife trade include the State Forestry Administration, Bureau of Fisheries, Ministry of Commerce, and Ministry of Police. Currently, there is little or no coordination amongst these agencies (Yiming & Wilcove 2005).

The impact of the amended legislation on reducing wildlife trade is likely to be undermined by both a lack of clear definitions of the protected species and the exceptions, with state sponsored license, to all prohibitions to wildlife trade (Ge 2016; Shytov 2017). Local government and enforcement agencies lack clear and consistent information about which wildlife species and products may or may not be legally traded, hunted, farmed, or otherwise consumed (Zhang *et al.* 2008).

4.4 Wildlife Trade and Zoonotic Emergence in China

In addition to being a hotspot for wildlife trade (Shepherd & Nijman 2007), China has received scientific and media attention as a hotspot for emerging zoonotic diseases, and a recent history of important zoonotic disease outbreaks, including highly pathogenic avian influenza (HPAI) A (H5N1) and severe acute respiratory syndrome (SARS) caused by the SARS coronavirus (SARS-CoV) (Li *et al.* 2004; Jones *et al.* 2008; Hotez *et al.* 2015; Wu *et al.* 2017). SARS-CoV originated in large wildlife markets in southern china in late 2002 (Drosten *et al.* 2003). The virus spread rapidly via international travellers, eventually affecting over 8,000 individuals in 32 countries and causing at least 774 reported deaths by mid-2003 (Riley *et al.* 2003; Tsang *et al.* 2003; Anderson *et al.* 2004; Zhao 2007). During the SARS-CoV outbreak, the earliest cases reported were from restaurant workers (He *et al.* 2003). By the end of the outbreak in May 2003, serological assays for antibodies to SARS-CoV or SARS-like coronaviruses (SL-CoV) in humans around Guangzhou City in China yielded the highest prevalence among workers in wildlife animal markets and traders of wildlife (CDC 2003; Guan *et al.* 2003). These data indicate that people regularly handling wild animals may have been infected with other less virulent strains of coronaviruses and this provided strong evidence for animal origins of SARS-CoV (Wang *et al.* 2006) and for its emergence being driven by the live wild animal trade (Bell *et al.* 2004).

Why had SARS-CoV or SL-CoVs not emerged before? It is possible there had been prior spillover events that were undetected, as evidence of SL-CoV found in the market workers suggested (Guan *et al.* 2003).

Few data are available, but consumers of wildlife in China do so mainly because they enjoy the flavour, although some say they also do so for the status or prestige that comes from hosting a dinner for their colleagues with wildlife on the menu (Zhang *et al.* 2008). Research has shown that individuals with higher income and education, usually men, living in the large, eastern, coastal cities (e.g. Guangzhou, Shanghai, Beijing, Shenzhen) are the principal consumers of wildlife in China (Zhang *et al.* 2008; Zhang & Yin 2014). Urban centres have been identified elsewhere as principal drivers of environmental change (Grimm *et al.* 2008). The demand for wildlife in China greatly increased following the rapid growth of the economy especially starting in the 2000s (World Bank 2016b) coinciding with a growing urban population of younger Chinese with more capital to spend (Woo *et al.* 2006a; Wu *et al.* 2017). As defined by Howe and Strauss (2009) for the West and then expanded to China by others (Moore 2005; Wang 2010), Chinese millennials are those born between 1976 and 2004; they are wealthier, especially as they often have no siblings due to China's one child policy (Yepes 2016), optimistic, better educated, and much more technologically savvy than previous generations (Wang 2010). Despite being the current driver of Chinese wildlife trade there is some evidence that the millennial generation , as they and their children become wealthier, are developing long-term interest in improving and conserving their environment as well as public health (Zhang & Shaw 2015; Kang *et al.* 2016; Li *et al.* 2016). A survey of the attitudes towards animal welfare of 1,300 university students in mainland China found that 62.6% opposed eating wildlife (Shuxian *et al.* 2005). No differences were found between students with and without science backgrounds and more than half the students were from big cities. The survey was conducted in 2003 during the time of the SARS-CoV epidemic in China. Only 13% of the students said that the emergence of SARS-CoV had influenced them in their decision to not eat wildlife.

4.5 Demographics of Chinese Internet Users

In 2015, estimates were that over 50% of the Chinese population (710 million people) had access to the internet (CNNIC 2016). The National Bureau of Statistics of China (2017) reported that 49% (668 million) people in China had an active online presence in 2015 and that this was split between men (53%) and women (47%) at rates comparable to the general population – 51.2% to 48.8% respectively (NBSC 2017). In cities (versus rural areas), 67.2% of the population have internet access with a per-city access increasing by size of city (CNNIC 2016); larger cities such as Beijing, Shanghai, and Guangzhou have higher proportions of populace with access to the internet. Over 87% of internet users in China are between the ages of 10 and 49; 54.6% are millennials between the ages of 20 and 39. Almost half of all Chinese internet users (43%) have a monthly income of RMB3,000 (£350) or more (CNNIC 2016); employed urban monthly salaries in China for the upper quintile were RMB5,169 (£600) in 2016 (NBSC 2017). Of internet users in China, 95% access the internet via a mobile phone. All users spend an average of 3 hours 47 minutes online per day (CNNIC 2016).

Estimates of daily social media platform (e.g. QQ, WeChat, Douban, Renren, Weibo) users by account in 2016 range widely and by application from 200 million for Weibo, 400 million for Qzone, and over 500 million for WeChat (CNNIC 2016; NBSC 2017; Statista 2017). With only 51% of its populace online, China lags behind other regions of the world (only in percentage, not in numbers) such as Europe (72% online) and North America (76% online) (World Bank 2016a), but in all regions globally the online and offline demographics are becoming equivalent (Lindhjem & Navrud 2011), particularly for populations such as the millennials that are by definition familiar with technology and frequently use web-based social media and networking applications (Jiang 2018).

4.6 Questioning Chinese Millennials Attitudes Towards Wildlife Trade

The study detailed in this chapter aims to provide data that support policy and behavioural change interventions to reduce the risk of novel emerging infectious disease (EID) events originating in China. One of these could be an awareness campaign around the risk of viral spillover from wildlife, which may produce similar changes in behaviour as resulted from the IFAW campaign. As *per capita* income continues to increase in China, perhaps interest in the environment and conservation may also increase. Subsequently, this would reduce the demand for wildlife and the risk of zoonotic emergence via the wildlife trade. If so, then intervention policies targeting Chinese millennials may be most effective, since presently it is this population that is driving the demand for wildlife trade (Zhang & Yin 2014; Liu *et al.* 2016b; Wu *et al.* 2017).

The research reported here investigates the attitudes of Chinese millennials in regard to the role wildlife trade plays in emerging diseases in China and globally. An online survey was developed to evaluate whether Chinese millennials are: (a) involved in the wildlife trade; (b) are cognisant of the potential risks wildlife trade poses to human health; and (c) know of, or on being informed were motivated to stop any future involvement in wildlife trade. It was expected that most Chinese millennials are involved in wildlife trade in some way, either presently or in their past. Additionally, this demographic would be expected to be aware of the conservation of wildlife, but be less aware of health risks related to wildlife trade or exposure to wildlife.

4.7 Methods

Following a standardised survey methodology for a stratified random sample (Sturgis 2006; Van Selm & Jankowski 2006) an online survey (Table 13) was designed to test hypotheses about Chinese people’s attitudes towards wildlife trade and disease emergence. The survey was piloted via face-to-face interviews first in English and then in Chinese. This iterative process ensured that the final online Chinese language survey would be easily intelligible to a very general audience and take no more than 5 minutes for a respondent to complete. All respondents were informed at the onset that their responses were completely voluntary and confidential. All respondents had the options to stop the survey at any time and to click on a link at the end to share the survey with their contacts and others in their social network. No data on employment, street address, contact information, affiliation, name, or any other identifying details were collected. Since the survey was designed to target a specific population and the data were planned to be aggregated, non-identifiable, and yield generalisable results no Institutional Review Board approval was required to conduct this survey. The Medical Research Council Health Research Authority checklist confirmed this (See Section 7.9).

The design of the survey background was intended to be nonintrusive with a muted watercolour stock image of a green meadow and blue sky without clearly identifiable details. The survey contained several stick-figure and other single-color stock art images chosen to make the survey appear ‘fun and inviting’ (See Sections 4.7 and 7.10). The survey was designed with four sections:

The first section collected demographic information about the respondents’ age, sex, education, economic status, and home city. The second part of the survey was designed to collect information about the respondents’ knowledge of the wildlife origins of infectious diseases such as HIV/AIDS, SARS-CoV, MERS-CoV, avian influenza, and Ebola and whether they were aware that 60% of infectious diseases emerge from human contact with wildlife (Taylor *et al.* 2001; Jones *et al.* 2008).

The third section collected information about the types of contact the respondent had with wildlife. The respondent could select one or more of the following options: wild animal market visit; ownership of wildlife as a pet; slaughter of wild animals; hunting of wild animals; eating wild animals; use of wildlife as medicine; use as a decorative product; or none. A follow-up question collected information about whether these activities had occurred within the past year.

Table 13. *Wildlife and You!* online surveyEnglish language version, below. See Appendix B: Surveys, Survey Protocols, and Consent Forms for the Online, Chinese language version (sojump.com/jq/6232786.aspx).**Wildlife and You! - Online Survey**

People keep talking about the wildlife trade, but what does that have to do with me? Let's find it out together! You will just need five minutes to find out the answers. Responses are completely voluntary and confidential.

Please tell us about yourself!

1. Your Age?
   - Under 15
   - 15-24
   - 25-39
   - 40-59
   - 60 or older
2. Your monthly income?
   - Less than RMB2,000
   - RMB 2,001- RMB 4,000
   - RMB 4,001- RMB 6,000
   - RMB 6,001- RMB 8,000
   - Above RMB 8,000
3. Where do you live?

(Drop-down option to select Province)

4. Highest level of education completed
   - Primary school
   - Secondary school/Polytechnic
   - College/University
   - Graduate School and beyond

Quiz Time: you can see if you get the correct answers after selecting and clicking 'next'!

5. Which of these diseases come from animals?
   - HIV/AIDS
   - SARS
   - MERS
   - Avian Influenza
   - Ebola
   - SARS and Avian Influenza
   - All of them
6. Now you know about some infectious diseases that come from animals, what percentage of *all* infectious diseases do you think comes from contacting wildlife?
   - 20%
   - 40%
   - 60%
   - 80%

Let's learn more about the wildlife trade!

7. Have you ever done any of the activities below? Check 1 or more:
   - Visited a wildlife market
   - Used wildlife products for decoration?
   - Bought wildlife from live animal market?
   - Kept a wildlife pet?
   - Used wildlife as medicine?
   - Eaten wild animals in a restaurant or at home?
   - Hunted wild animals?
   - Slaughtered wild animals?
   - None
8. Did you conduct any of the activities you selected in #7 in the past 12 months?
   - Yes
   - No

Wildlife trade is any sale or exchange of wildlife animal and plant resources by people, there is always a value chain from the capture or harvesting of wildlife to transportation and marketing to consumers. Did you realize how much you are involved in the links in this chain and how many opportunities you have in to come into contact with wildlife?!

Let Us Hear Your Voices and Opinions!

9. Do you think wildlife trade will lead to (check one or more) …
   - … wildlife extinctions?
   - … environmental degradation?
   - … zoonotic disease emergence?
   - I don't know
10. Do you think people should stop hunting, selling or buying wildlife and wildlife products for food, pets, medicine, or handicraft?
    - Yes
    - No
11. If wildlife trade stops, rank which would be the most likely cause?
    - Laws becoming stricter with strict enforcement
    - Non-wildlife products are cheaper and as good
    - Protecting wildlife and the environment
    - Preventing diseases like Ebola and SARS
    - Public opinion against wildlife and wildlife products
12. Which groups do you have the highest confidence and trust in to stop/reduce the wildlife trade in China? (check one or more)
    - Relevant government organisations (forestry, health, agriculture, etc.)
    - Local community and people
    - Non-governmental organisations
    - Business community
    - Academia/Researchers
    - Other

Your responses help us to make policy recommendations to the government and take effective action to protect wildlife and human health. Click here to share this survey. Thank you for promoting conservation and preventing epidemics!

Before the fourth and final section, a few sentences provided a definition of wildlife trade as the "sale or exchange of wild animal or plant resources" and respondents were informed that there is a connection or "chain" from the capture of wild animals to the end-consumer. The respondents were then asked to consider whether they are involved in this 'chain' and how often they come into contact with wild animals along the chain. The final section of the survey had four questions and asked if the respondents felt that wildlife trade:

Question 9:	will lead to diseases, environmental degradation, and extinctions?
Question 10:	or the use of wild animal products should be stopped?
Question 11:	if it were stopped, would be most due to legislation and enforcement; public health and disease prevention; availability (or competition) of cheaper non-wild-sourced products; or societal awareness and pressure against wildlife consumption?
Question 12:	would be most likely reduced or stopped by researchers; businesses; nongovernmental organisations; local community groups; or governmental agencies (departments of forestry, health, agriculture, etc.)?

Questions 9 and 12 permitted respondents to select as many answers as they felt suitable. Question 10 was a yes/no question. Question 11 required respondents to rank their choices.

Consistent with high internet uptake in China, particularly among urban residents, an online survey platform Sojump (https://www.sojump.com) was selected to distribute the finalised online survey. Sojump has been utilised by published online survey studies of behaviour in China (Li *et al.* 2012; Bai *et al.* 2014; Lien & Cao 2014; Wu & Wang 2016), provides survey research services to Chinese Universities (e.g. Peking University, Chinese University Hong Kong) and also provides commercial survey services to businesses such as McKinsey & Company, BMW, and Walmart. Sojump has a database of 2,600,000 individuals across all 31 of the province-level administrative units of China (Figure 21) and can target surveys by city. The target population was middle class Chinese nationals or millennials at Tier 1 and Tier 2 cities. China has an official system of urban classification by GDP, political autonomy, and population (PRC 1982). Although the PRC constitution does not define the cities, only the classification system, Beijing, Shanghai, Guangzhou, and Shenzhen are usually listed at the Tier 1 cities (Burns 2003). There are 30 Tier 2 cities and all other cities are classed as Tier 3 and Tier 4, all of which are ranked depending upon categories such as real estate, retail, public health, or telecommunications (Daemmrich 2013; Chivakul *et al.* 2015; Liu *et al.* 2016a).

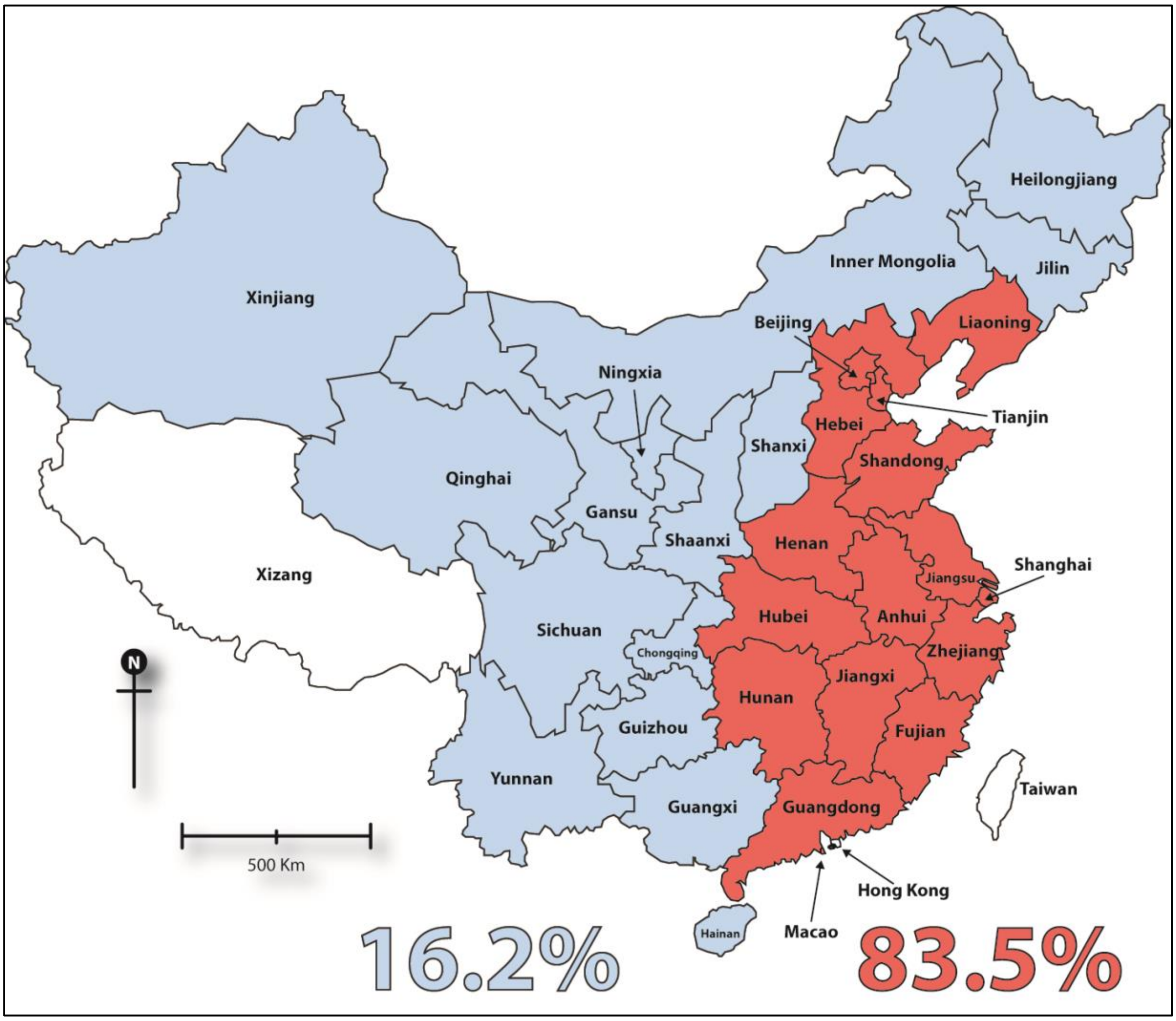


Figure 21. Map of Online Survey Respondants. Chinese Provinceswith provinces shaded in red indicating origin of most (83.5%) respondents and illustrating how they are distributed mostly along the costal and highly urban areas of mainland China. 16.2% of respondents were from the interior and relatively less urban regions of China. Xizang, Hong Kong, and Taiwan were not surveyed and are indicated in white fill, n = 2,238.

Using Sojump's proprietary software a random distribution of survey notifications was sent via direct emails to the specific social media instant messaging platforms. All recipients had the option to respond or not. Only active users across Tier 1 and Tier 2 cities were targeted and these were defined as users who had sent or posted one message in the past 30 days. A target sample size of 1,500 was calculated based upon a conservative estimate of a population of 100 million millennial users of social media in China with a 95% confidence level and a 2.5% margin of error. The online survey was distributed for one month from 15 December 2015 to 15 January 2016 electronically via direct emails from Sojump to both its user base and via the Chinese social media instant messaging platforms QQ and WeChat (im.qq.com, Tencent Holdings Ltd.) as an inserted advertisement. Distribution was irrespective of user preferences including wildlife trade, activism, public health, or conservation. Surveys were sent out to 31 of the province-level administrative units of China. Hong Kong, Xizang, and Taiwan were not surveyed, since they are outside of the Sojump database. Sojump directed the survey to 5,448 online users.

The collected data were statistically analysed by Wizard 1.9.2 and Microsoft Office Excel 15.29.1. Standard statistical analyses were used to summarise the respondents' data. Independent sample *t*-test was used to compare sample means of normally distributed continuous variables. A Pearson Chi square test (DF = 1, Fisher's exact test, two- tailed) was used to analyse the differences between respondents who had some knowledge and experience of wildlife and those who did not. A 95% confidence level ($p = 0.05$) was used. The software IBM SPSS 16.0 (IBM SPSS Inc., Chicago, IL, USA) was used to conduct the analysis.

4.8 Results

During the one month of the survey, 2,106 survey responses were received with a response rate of 38.7%. An additional 132 unsolicited or indirect responses were received in the same time period totalling 2,238 individual respondents from 31 of the provincial level administrative units of China (Figure 21).

The average time to complete the survey was 3 minutes and 52 seconds. There were daily responses during the survey month except for three days: 28, 30, and 31 December 2015. Of respondents, 65.4% were between the ages of 25 and 39 years (Table 14, Figure 22). More than half (1,250; 55.9%) were from only six eastern, coastal province-level administrative units: Zhejiang, Jiangsu, Shandong, Shanghai, Beijing, and Guangdong. Beijing (254, 11.3%) and Guangdong (343,

Table 14. Age classes of online survey respondents, n = 2,238.

Age Class (years)	% (No. Respondents)
≤15	0.5 (10)
15 - 24	15.2 (339)
25 - 39	65.4 (1,463)
40 - 59	18.1 (404)
60 ≥	1.0 (22)

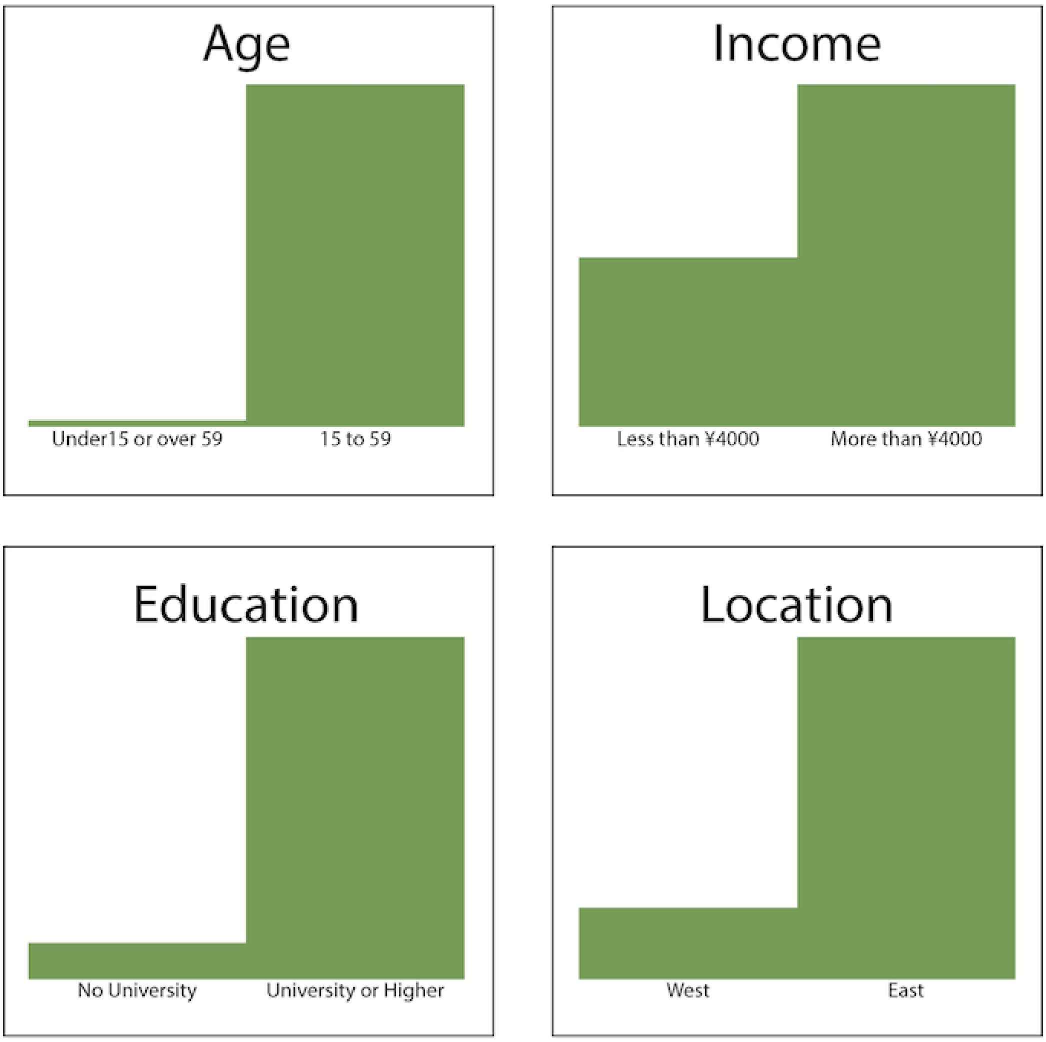


Figure 22. Survey population demographics for education, age, income, and location. See Figure 21 for map further illustrating the east-west split in respondents, n = 2,238.

15.3%) had the highest number of respondents each. Gansu, Xinjiang, Ningxia, Macao, Qinghai, and Hainan had the least (6 or fewer) respondents each (total 0.7% of respondents).

Of the respondents, 83.5% (1,868) were from eastern China; 16.2% (362) were from western China (Figure 21). Most (92%, 2,060) of respondents had completed a university education and 75.7% (1,694) reported earning more than RMB4,000 per month. Despite the east-west split in respondents there were no significant differences in age ($t(30)= 1.029$, $p > .05$) and education ($t(29)= 0.983$, $p> .05$) across this geography and throughout the population. There was a significant difference in income between the east and west groups with higher income being in the east ($t(29)= 2.089$, $p = .046$).

4.9 Awareness of Zoonotic Diseases

Of the 2,238 respondents, 62% (1,395) were aware that SARS-CoV and avian influenza were diseases emerging from wildlife (Figure 23). Fewer respondents were aware of the wildlife origins of Ebola (38.9%, 871), HIV/AIDS (30.6%, 685), and MERS (23.7%, 531); only 14.2% (318) of respondents were aware that all of these zoonotic diseases originated in wildlife. Only 20.1% (450) were aware that at least 60% of all infectious diseases have an animal origin and 71.4% (1,598) thought that 40% or less infectious diseases have an animal origin. When just these data are corrected (no overseas responses nor any >40 yrs or <15 yrs) and compared across the east-west gradient in China there are no statistically significant differences in all of the remaining survey response categories except for MERS-CoV ($t(29) = 2.465$, $p = 0.020$). Up to 28% (522) percent of eastern respondents were aware that MERC-CoV has a wildlife origin, but only 13.6% (49) of western respondents were aware of this same fact.

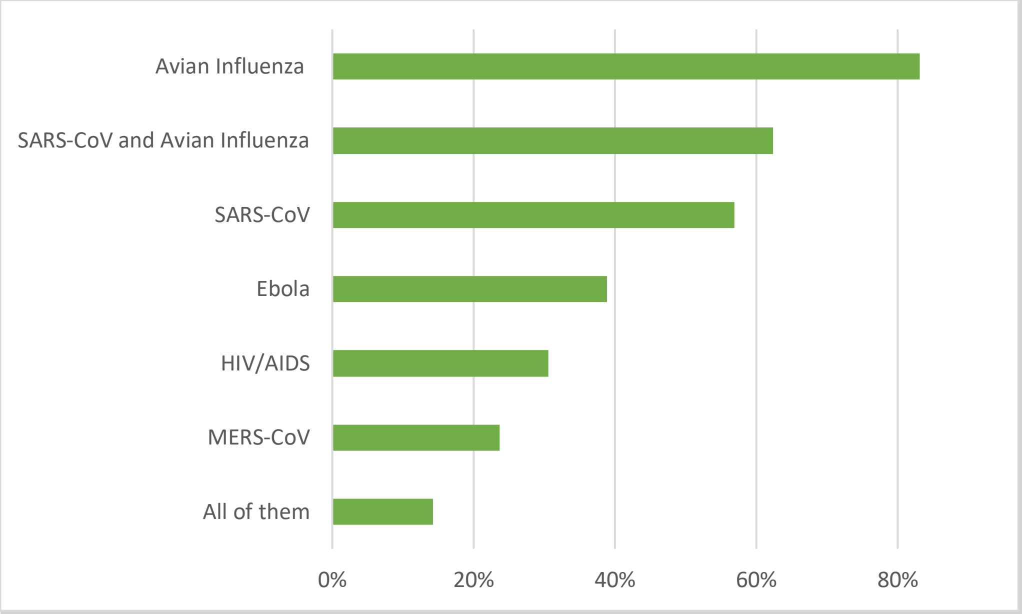


Figure 23. Responses to, *Which of these diseases come from animals?* , n=2,238.

4.10 Involvement in Wildlife Trade

In response to the question about their direct involvement with wildlife, (Figure 24) 72.1% (1,614) of respondents had some interaction with wildlife and more than half (50.2%) claimed they had visited a wild animal market. Only 28.2% (630) claimed to have eaten wildlife and 2.6% (58) had both hunted and slaughtered wildlife. Of all these interactions with wildlife, only 37.8% (846) respondents had engaged in them within the past year. Most respondents (62%, 1,392) had no interactions with wildlife in the past 12-months. Similar numbers of respondents reported having used wildlife for medicinal purposes (391, 17.5%) or having wild animals as a pet (381, 17.0%) and only 5.5% (119) reported having done both, and of these most (74, 62.1%) had done so in the past 12-months.

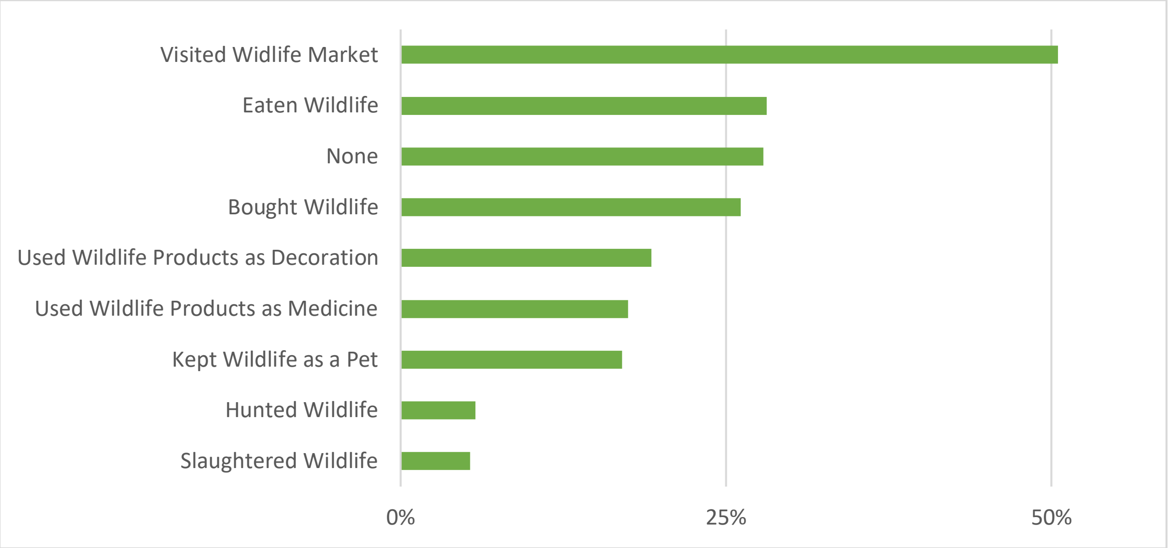


Figure 24. Responses to, *Have you ever…?* , n=2,238.

4.11 Respondents' Attitudes and Knowledge

After reading the brief text defining wildlife trade, respondents were asked what they felt were the impacts of wildlife trade: environmental degradation, species extinctions, or disease emergence. Respondents were able to select as many of these answers as they thought appropriate as well as an "I don't know" option. Most respondents (88.3%, 1,977) believed that wildlife trade would lead to species extinctions, 84.9% (1,902) believed that wildlife trade would lead to environmental degradation, and 74.8% (1,674) understood the link between wildlife and zoonotic disease emergence. A total of 63.2% (1,415) respondents said that they now believed that wildlife trade would lead to all three: extinctions, environmental degradation, and zoonotic disease emergence. The majority (2,155, 96.3%) stated that they believed "people should stop hunting, selling or buying wildlife for food, pets, medicine, or other products".

4.12 Respondents' Thoughts about Stopping Wildlife Trade

Respondents were then asked to rank the most likely reasons that they felt wildlife trade would be stopped (Figure 25). A total of 47.7% (956) felt that legislation and enforcement would be the most effective means of stopping wildlife trade and 29.4% (659) felt that protection of wildlife and the environment would most likely cause wildlife trade to be stopped. Only 13.4% (300) felt that non-wildlife products would mostly likely cause the demand for wildlife to stop. Fewer still, 8.2% (184) and 6.2% (139) respectively felt that disease prevention and public opinion would be most effective reasons for stopping wildlife trade. Respondents were then asked about which group or organisation would be most effective at stopping or reducing wildlife trade (Figure 26). Most respondents (1,950, 87.1%) believed that relevant governmental agencies (departments of forestry, health, and agriculture) would be most effective in reducing or stopping wildlife trade and 1,491 (66.6%) respondents felt that local communities and people would be the most effective groups to stop wildlife trade. Non-governmental organisations (56.1%, 1,256), research (21.8%, 489) and business (37.6%, 842) were considered less effective means.

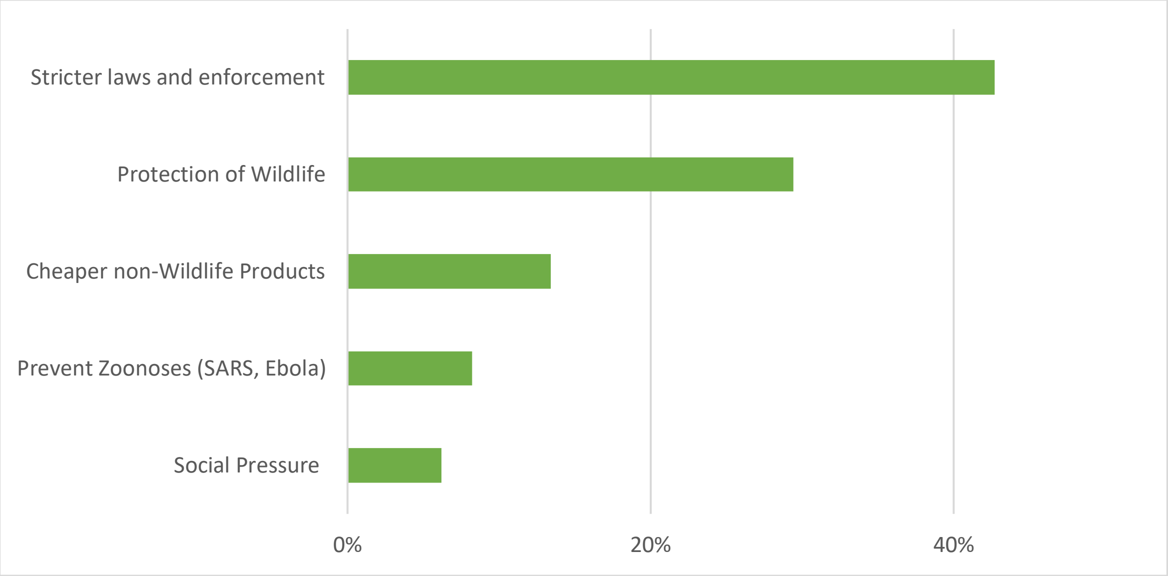


Figure 25. Responses to, *Which could be the most Important reason or motivator to stop wildlife trade*? , n=2,238.

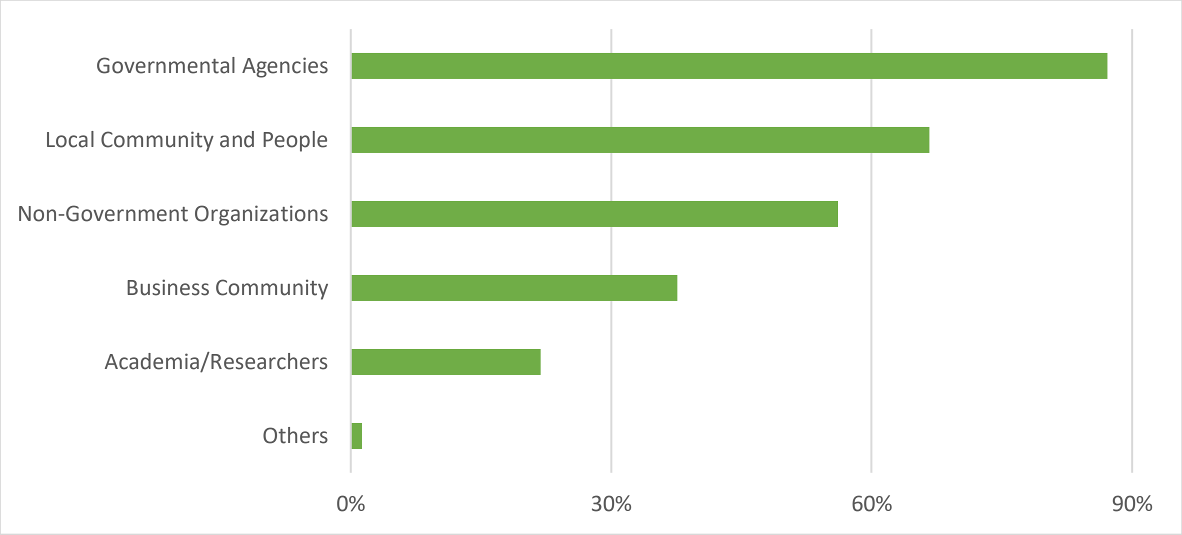


Figure 26. Responses to, *Which group would be most effective in stopping wildlife trade in China*? , n=2,238.

A comparison between the proportion of respondents who (a) claimed to have not had any interactions with wildlife at all and (b) those who had interactions with wildlife in the past 12 months showed no statistically significant difference in response to the question of whether wildlife trade should be stopped (X^2 (N = 1470) = 2.20, p = .138). Over 95% of respondents believed that wildlife trade should be stopped; this was consistent across all strata (Table 15) with the exception of the group of respondents (n = 25) who did not know what the effects of wildlife trade may be. The percentage (68%, 17) in this group to say 'yes' to wildlife trade being stopped was not significantly different from the percentage of respondents saying 'yes' in other categories.

4.13 Discussion

The results from this study suggest that education campaigns around the health-related aspects of wildlife trade may be effective in reducing demand if targeted to Chinese millennials. Firstly, most (86.6%) respondents were aware of the impact of wildlife trade on biodiversity loss and environmental degradation. Secondly, this population is involved in wildlife trade despite understanding of its impact. Finally and importantly, while a majority of the millennials and others in this study realised the link between wildlife and SARS-CoV, they did not yet know about the links to other zoonoses. This suggests that educating this influential group of millennials about the disease impact of wildlife trade, may lead to them reducing their activities further and spreading the information among their own social networks. If this created the appearance of a movement to resist eating wildlife among this visible and influential population of Chinese citizens, it may effectively reduce consumption over the whole of China via viral dissemination and activism (Wang 2002; Moore & Chang 2014).

A total of 2,106 primary respondents filled out the survey sent initially from Sojump. An additional 132 secondary respondents also filled out the survey. These secondary respondents most likely received the survey from primary respondents who opted to pass along the survey to contacts and other members in their social networks. The secondary response rate may not be calculated since there was no way to record how many primary respondents forwarded their surveys. Although indisputably faster and less expensive, online sampling has been criticised as (a) having the potential to be much-reduced (and therefore not representative) from a random selection of an offline population, (b) self-selecting, and (c) without data about non-respondents (Mehta & Sivadas 1995; Kwak & Radler 2002). Most of these issues have been mitigated or negated through technological advances and careful survey design (Cook *et al.* 2000; Evans & Mathur 2005). Although caution

Table 15. Surveyed population in response to, *Should Wildlife Trade be Stopped or Not?* Columns provide number of those surveyed by response to this question (yes, no, total, and percent). Responses are grouped by age class, income, education, and involvement with wildlife as the three questions listed in the table. There was no significant difference between any group ($p > 0.05$), n = 2,238.

	Yes	No	Total Respondents	% Responding Yes
Age Class				
Under 15	10	0	10	100%
15-24	322	17	339	95%
25-39	1415	48	1463	97%
40-59	387	17	404	96%
Over 59	21	1	22	95%
Income (RMB)				
Less than 2000	175	13	188	93%
2001-4000	561	21	582	96%
4001-6000	662	26	688	96%
6000-8000	411	13	424	97%
More than 8000	346	10	356	97%
Education				
Primary School	8	0	8	100%
Secondary School	157	13	170	92%
University	1796	62	1858	97%
Graduate	194	8	202	96%
Involvement with Wildlife				
Visit to Wildlife Market	1093	37	1130	97%
Wildlife Decoration	419	12	431	97%
Bought Wildlife	556	29	585	95%
Kept Wildlife as Pet	366	15	381	96%
Used Wildlife as Medicine	374	17	391	96%
Eaten Wildlife	604	26	630	96%
Hunted Wildlife	124	5	129	96%
Slaughter Wildlife	112	8	120	93%
None	609	15	624	98%
Activities in last 12 Months	814	32	846	96%
What are the Impacts of Wildlife Trade?				
Extinction of Wildlife	1857	45	1902	98%
Environmental Degradation	1924	53	1977	97%
Emerging Diseases	1625	50	1675	97%
Do Not Know	17	8	25	68%
What is the most important reason to stop Wildlife Trade?				
Legislation from Government	927	29	956	97%
Cheaper non-Wildlife Products	286	14	300	95%
Protection of Environment	635	24	659	96%
Prevent Disease Emergence	171	13	184	93%
Social Pressure	136	3	139	98%
Which Group can best stop Wildlife Trade?				
Government	1896	54	1950	97%
Local Community	1445	46	1491	97%
Non-Government Organisations	1215	41	1256	97%
Businesses	816	26	842	97%
Scientists	472	17	489	97%
Others	28	0	28	100%

should be applied to the representative nature of results from online surveys (Lee *et al.* 2015), the principle of random choice can still be maintained, since all active users of an online platform have equal chances of responding (Ilieva *et al.* 2002). For selected populations, online surveys have been demonstrated to yield comparable results to traditional (off-line) results (Krantz *et al.* 1997; Buchanan & Smith 1999; Evans & Mathur 2005; Lindhjem & Navrud 2011).

Online and offline surveys report wide ranges in response rates depending upon type of survey and access the target populations may have to the internet (Kaplowitz *et al.* 2004). This study's response rate (38.7%, 2,106/5,448) was within the range of similar studies (Cook *et al.* 2000; Sills & Song 2002; Kaplowitz *et al.* 2004) providing confidence in the methodology employed here. In two recent, offline studies of attitudes towards wildlife trade, between 31.1% (n = 1,352) (Zhang *et al.* 2008) and 29.6% (n = 315) (Zhang & Yin 2014) of respondents in China said they had consumed wildlife. These percentages are not statistically ($X^2 = 3.50$, p=0.17) different from this study's result of 28.2% of respondents reporting wildlife consumption.

The population targeted in this study was Chinese millennials. The online survey format here was specifically selected to efficiently target Chinese millennials. The population surveyed in this study matched the target group in income, geography, education, and age. By demographic data, the respondents conformed to the definition of millennials in that they were (a) well-educated (92% had university or higher education), wealthy (56.7% reported annual salaries 20% higher than the urban average) and urban dwelling, i.e. the urban middle class (Zhang & Shaw 2015). Since only social media users were targeted, the population was already online, familiar with technology to the extent that installation and use of a web-based application would require, and active users, defined as having posted on their respective accounts or responded to any other survey or campaign within 30-days. Analysis of the data demonstrated that there was no significant variance in responses to questions between any age, geographic cluster, or other grouping and so confidence may be high that the results reported here represent the larger population.

Although the millennials surveyed here may not represent the poorer rural demographic that is more likely to come into contact with wildlife and domestic animals with frequency (Webster *et al.* 2016), they do represent the demographic that is currently driving the demand for wildlife products as food, ornaments, or medicine (Zhang *et al.* 2008; Zhang & Shaw 2015). In other countries, populations putting highest pressure on wildlife (hunters, poachers, and consumers) can sometimes most effectively apply pressure to government and society to conserve the endangered wildlife

(Gibson & Marks 1995; Paulson 2012). Once consumers of a wildlife resource are invested in ensuring its maintenance (as a resource), then often they become conservationists (Leader-Williams 2001; Lindsey *et al.* 2006).

As they, and especially their children, become wealthier or expand the middle class, it seems likely that Chinese millennials will become more invested in improving their environment, conserving wildlife, and importantly in reducing the risk of zoonotic disease emergence (Zhang *et al.* 2008; Zhang & Shaw 2015). The surveyed population in this study confirmed this hypothesis with 96.3% of respondents saying that wildlife trade should be stopped, although not unanimous as to why. One target of behaviour change intervention in China is to convert this population from consumers of wildlife into more savvy protectors of their environment who are also aware of potential public health risks inherent in wildlife trade (Wong 2003). This study presented here strongly suggests that Chinese millennials are already very much aware of conservation or environmental reasons to stop wildlife trade, and there is potential for them to become more aware of the health reasons.

From before and after reading the definition of wildlife trade in the survey, most respondents did not alter their view that protection of wildlife and the environment was the most important reason to stop wildlife trade. As expected, this suggests that (a) the duration of a 5-minute survey was not sufficient to alter perceptions, but it is sufficient to provide an increased level of education around the targeted issue. It would be illustrative to revaluate results after this demographic is exposed to a targeted campaign on wildlife trade and emerging disease risks.

During the survey, respondents did exhibit an increased awareness of some importance of emerging infectious diseases as a reason for stopping wildlife trade. While the population surveyed was mostly aware that SARS-CoV, Ebola, and avian influenza emerge from wildlife, they did not seem to make the connection that wildlife trade may then present very real risks to health. This may be due to most respondents (62.2%) saying they had not had contact with any wildlife in the past 12 months, which would have influenced their valuing disease risk lower than conservation. Actual disease occurrence or outcomes of the surveyed population were not assessed in this study due to the limited response time in the online survey format. Potential future studies could examine whether awareness and attitude regarding disease risks from wildlife trade vary among those having contact with wildlife (including any potential risk factors such as bites or scratches) and any associated infectious diseases.

The emphasis on elephant ivory in anti-wildlife trade messaging in China has helped to shape public awareness of conservation risks. In fact, at the end of 2016, the Chinese government announced a ban on all commercial processing and sale of ivory and ivory products effective by the end of 2017 (PRC 2016a). This legislation has been attributed not only to international pressure, but to education and awareness campaigns within China (Wong & Gettleman 2016). SARS-CoV already has emerged from the local trade in wildlife in China and a similar anti-wildlife trade messaging to promote awareness of health risks is urgently needed and may prove as effective. This study suggests that a dual approach involving both local level and online community education along the lines of IFAW's campaign may work towards mitigating future health and conservation impacts of wildlife trade in China.

5 Behavioural Surveillance and Risk

5.1 Introduction

Determining what causes the emergence of pandemics, and in what context, is the subject of much research and debate (Morse *et al.* 2012). In many cases, this work has involved analysing the causes of specific diseases that emerge, and determining the risk behaviours and environments that are involved in initial spillover of a virus from wildlife (Cleaveland *et al.* 2007; Han *et al.* 2016a). One of the key, high-risk circumstances in which humans and wild animals interact is via wildlife trade and in areas where rural and often poor communities abut wild or forested areas (Cleaveland *et al.* 2007; Grace *et al.* 2012). Different stages of the wildlife trade have been implicated in the emergence of severe acute respiratory syndrome caused by Coronavirus (SARS-CoV), human immunodeficiency virus (HIV), avian influenza (AI), Ebola (EVD), monkeypox, and a series of other high-profile emerging infectious diseases (Hahn *et al.* 2000; Leroy *et al.* 2004; Sejvar *et al.* 2004; Webster 2004; Woo *et al.* 2006a; Ellis *et al.* 2012).

Trade in wildlife originates with wild-sourced animals that are then transported to consumers primarily in urban centres and may include transit through animal markets of all sizes, animal warehouses, animal farms, restaurants, and processing sites or abattoirs (Kruse *et al.* 2004; FAO 2011). In southern China wildlife has long been utilised for food, medicine, pets and as raw material (Zhang *et al.* 2008; Chow *et al.* 2014). Recent globalisation of trade and increasing wealth have resulted in higher demand for wildlife foods, particularly in the wealthier urban centres, both for nutritional purposes and for traditional medicine (Yiming & Wilcove 2005). Large live animal markets selling legal and illegal wildlife such as those in Guangdong Province grew to accommodate this demand (Li *et al.* 1996; Yiming & Dianmo 1998). In these markets and all along the wildlife trade routes, there are many opportunities for zoonotic emergence as wild animals come into frequent contact with each other, domestic animals, and humans (Morse 1995; Karesh *et al.* 2005; Lau *et al.* 2005). Amongst the diverse species traded are bats, rodents, other small mammals such as civets, cats, and dogs, reptiles, amphibians, and nonhuman primates, many of which are reservoirs for zoonotic diseases (McFarlane *et al.* 2012).

The practice of consuming and handling wildlife represents a substantial risk for zoonotic disease spillover, especially given the diversity of wildlife traded as well as the density of the human population in southern China (Cleaveland *et al.* 2007; Yin *et al.* 2016; NBSC 2017). This was highlighted by the emergence of SARS-CoV in 2003, which can be traced back to a series of putative first recorded infections or index cases among restaurant workers and wildlife traders in Guangdong province (Xu *et al.* 2004). The markets from which those infected individuals had purchased wildlife were found to sell SARS-CoV infected civets, and have evidence of prior circulation of SARS-CoV (i.e. antibodies to SARS-CoV) when animals were sampled following the outbreak (Li *et al.* 2005). SARS-CoV has not re-emerged in human populations globally or in China since the last recorded outbreak in early 2004 despite the continued operation of wildlife trade that was implicated in its emergence (Reuters 2004). Wildlife trade routes in southern China have been documented (Yiming & Dianmo 1996; Zhang *et al.* 2008). However, the behaviours and motivations of those involved in the wildlife trade have not been explored in much detail, and understanding these may be key to preventing high-risk behaviour in the future.

5.2 Southern China Rural Communities

Yunnan, Guangxi, and Guangdong provinces were targeted in this study because they have diverse wildlife populations, a large rural population, numerous live animal markets, and wildlife found to harbour viruses with pathogenic potential for humans (Field 2009). These three provinces along with bordering Vietnam, Laos, and Myanmar are known hotspots for faunal diversity (Myers *et al.* 2000) and have long been integral to trading routes to southern China, and especially to Guangdong Province (Yiming & Dianmo 1998). This region is also home to protected forests and other areas where wildlife has been traditionally hunted and captured (Yiming & Wilcove 2005).

Rural communities in south China primarily cultivate rice, and manage orchards, swine, and poultry on small farms of around 0.7 hectares (Huang *et al.* 2012). These farms are usually located in rural and poorly developed areas proximate to natural protected forests, parks, or wetlands. The region is densely populated, and like much of China, urban centres are spread throughout, with farmers often relying on swill from urban restaurants to feed their animals (Wang *et al.* 2016). Rodents are ubiquitous in the rural communities due to the abundance of crops and domestic animal feed (Singleton 2003). This mixed landscape brings humans and domestic animals in close contact among dense populations, and via the wildlife trade, creates an interface of potential high-risk for zoonotic emergence. This study will examine the hypothesis that the populations in these rural communities have direct and frequent contact with their own animals as well as with commensal species and wildlife, and that these associations result in zoonotic spillover.

The Chinese Ministry of Health provides annual national reports of morbidity and mortality of communicable and noncommunicable diseases, and these provide some trends on disease incidence (MOH 2017). For example, the annual number of morbidities due to viral haemorrhagic fever and diseases of unknown aetiology has fluctuated over recent years, with an overall decline from 2003 to

2016 (Figure 27). This may reflect improved diagnostics beginning in 2008 with national healthcare reform (Yip *et al.* 2012), so that the trends are difficult to accurately assess. However, these data show that there are around 10,000 cases of viral haemorrhagic fever and disease of unknown aetiology in China each year. Some of these cases may represent novel emerging diseases such as bat SARS-like Coronavirus (SL-CoV) or a member of several viral families known to harbour viruses that cause haemorrhagic fever such as the Filoviridae (Ebola and Marburg), Flaviviridae (Dengue, Yellow fever, Kyasanur forest disease virus), Bunyaviridae (Rift Valley fever virus), or Arenaviridae (Lassa fever virus) (Hammon *et al.* 1960; Kuhn *et al.* 2016). Diverse viral species from all of these families have been found in bat and rodent reservoirs in China and likely many more remain as yet undiscovered (Wang *et al.* 2009; Li 2013b; He *et al.* 2015; Li *et al.* 2015). This speculative hypothesis that there may be regular viral spillover yielding diseases of unknown aetiology in rural populations in China could be tested by taking samples from people and testing them for evidence of infection by wildlife viruses, e.g. using serology specific to wildlife viruses. In the absence of serological data, conducting behavioural surveillance of individuals with known exposure to wildlife and self-reporting incidence of diseases of unknown aetiology may provide a measure of risk of contact with wildlife and therefore a proxy of spillover risk. Correlation of exposure data with the prevalence of self-reported, (a) undiagnosed severe acute respiratory infections (SARI), (b) influenza-like illnesses (ILI), and (c) encephalitis symptoms may provide a mechanistic understanding of the potential for zoonotic disease emergence in the region. The goal of the current study was to conduct human behavioural surveillance to provide a baseline understanding of the risk of spillover in these communities, which could then be used to identify both the potential for and the drivers of the emergence of novel zoonoses.

Figure 27. Morbidities from viral haemorrhagic fever and disease of unknown aetiology in China. Source: Ministry of Health, People's Republic of China (MOH). Data range is from 2003 to 2016. The increase in morbidities from 2009 to 2012 may have been due to the emergence of the avian influenza H1N1 A virus and associated cases of fever with haemorrhage (Fugate *et al.* 2010), which were most likely reported at that time as diseases of unknown aetiology due to difficulties in accurately diagnosing H1N1 (Hussain *et al.* 2012).

Guangdong Wildlife Markets

Taiping Market and Foshan Market in Guangdong Province in the greater Guangzhou City area (Figure 28) were selected for this study due to their reported size and centrality in the wildlife trade in China (PGGM 2006; Hu & Chen 2007). Towards the end of the SARS outbreak in late July 2003, the Guangdong Provincial Government passed a new health regulation requiring that people stop wild animal consumption to prevent zoonotic diseases (China Daily 2003). This was the first such regulation at a provincial level in policy around consumption of wild animals in China, but it did not address the wild animal markets (Luo 2003). By the end of the SARS epidemic in late 2003 and early January 2004 and as soon as SARS-like Coronaviruses (SL-CoV) had been detected in civets (*Paguma lavarta*) in a Foshan market, thousands of these and other market animals were culled, banned from all markets, and civet farms shut down (Watts 2004; Cheng *et al.* 2007). Within a week and before the end of January of 2004, all the wildlife markets in Guangdong province were closed by the Provincial Government working with the Forestry Department (Guan *et al.* 2003; Luo 2003; Zhong 2004; Zhao 2007). By early March, Guangdong Provincial Forestry Department officials were reported to be discussing plans to reopen wild animal markets (Reuters 2004). In May 2004, when the World Health Organization announced that for over three weeks there had been no cases of human or human transmitted SARS, wildlife markets were already open and operating again in Guangdong Province (WHO 2004; Zhong 2004).

Figure 28. Guangdong Province. The cities of Foshan, Taiping, Guangzhou, Hong Kong, and Shenzhen are indicated. The two markets observed in this study were located in Foshan and Taiping cities respectively.

In November 2006 about four years after the initial outbreak of SARS in China, it was announced on the official Guangzhou City website that the largest wildlife wholesale market in Guangdong Province, and possibly in China, was being relocated from Guangzhou City to Taiping Town in the suburbs of Chonghua City about 60km to the northeast (PGGM 2006). The reason for the move was due to the human population density of Guangzhou City and the potential health risks in the wake of SARS (PGGM 2006). The Taiping City Wildlife Market that opened in 2006 is the same market observed in this study, and was set up jointly by the Guangzhou City Forestry Department, Conghua City Forestry Bureau, and the Taiping Township Forestry Station with an investment of RMB30 million (approximately £2.5 million at 2017 RMB to GBP exchange rates) (PGGM 2006). The purpose of the new market was to permit the wholesale selling of licensed wildlife, and it would be under strict inspection and checks (PGGM 2006). No verifiable reports exist, but claims are made that the Taiping City Wildlife Market is the largest in China and if not, it is a major centre for illegal wildlife trade (Hu & Chen 2007; Anon 2012; Hancock 2014). Starting in 2012 through to the present, there have been many publicised raids followed by repeated closing and reopening of the Taiping Market throughout which period the market continued to sell wildlife species (Xie 2012; GDPPP 2014; GDFN 2016). The Foshan Market about 20km to the southwest of Guangzhou expanded in size around the same time as the official closing of the Guangzhou City wildlife market (Hu & Chen 2007). This market also experienced multiple closures starting in 2007, with some raids confiscating almost 100,000 illegally traded wild animals (Hu & Chen 2007; Huang 2007; Tan 2014).

5.4 Interdisciplinary Disease Surveillance

A growing number of studies has investigated the connection between humans and non-human animals and how this relationship affects health (Daszak *et al.* 2001; Daszak *et al.* 2007; Johnson *et al.* 2015). As the rate of emerging zoonotic diseases increases, a holistic or one health understanding of this interface will become more important to preventing disease emergence (Karesh & Cook 2005; Jones *et al.* 2008). Examining the factors motivating the consumption and use of wild animals requires first an understanding of how humans relate to animals in their shared environment (Hurn 2012; Liu *et al.* 2014). By combining anthropological and ecological methods to evaluate the diverse ways in which human and animals interact (Frake 1962; Abel 1998), effective solutions to the problem of emerging zoonoses may be discovered (Daszak *et al.* 2000; Wood *et al.* 2012).

In this study, both qualitative and quantitative surveys were conducted. The goal of qualitative research is to present an account of activities people conduct in their natural settings and what these actions mean to them (Britten *et al.* 1995; Erikson 2017). A qualitative approach is used in this study to understand the social and environmental context in which infectious disease spillover may occur, i.e. the reason 'why' risk behaviour occurs. The two distinct qualitative methodologies were utilised in this study: observational research and ethnographic or one-on-one interviews. The goal of quantitative research is to empirically investigate data employing measurable evidence (Williams 2007; Firmin 2008) . A quantitative approach was used to identify the types of contact, degree of contact, frequency of contact, and other parameters, i.e. 'what' risk behaviours occurred.

5.5 Ethnographic Approach

Southern China has a history of over 100 years of ethnographic study primarily due to its cultural diversity (Osgood 1963; Guldin 1994; Wang 2005). An ethnographic approach is holistic and integrates different data collection methods such as participant observation, unstructured or semi-structured interviews, and questionnaires to systematically describe perceptions of a community of interest (Bernard 2006). Observations including drawings, notes, photographs, and audio recordings are used to add context to data collection and analyses, particularly in sample site selection (Gray 2009). Drawings of markets are invaluable tools to aid recollection and provide supplemental details during analyses. Ethnographic semi-structured interviews are a series of pre-scripted and open-ended questions to be asked of a participant permitting discussion around a topic, and from which additional information may be gleaned (Bernard 2006). By integrating ethnographic interviews, observations, and quantitative data research the motivations that drive consumption of wildlife and identify the highest risk activities or interactions may be revealed.

In the live animal markets of southern China, the high degree of contact between people and a diversity of animal species due to a high volume of handling, butchering, and other activities may result in conditions that allow viruses like SARS-CoV to emerge and spread. Additionally, contact and exposure to wildlife in rural communities is thought to be frequent and to put this population at risk of infection. To test these assumptions, this study reports on results of surveys of two live animal markets in Guangdong province and the behaviour and attitudes of humans in rural communities with exposure to wildlife. Behaviour and potential for zoonotic spillover to humans with high occupational exposure to bats and other wildlife are also evaluated.

5.6 Research Question, Hypotheses, and General Approach

The general methodology and design employed in this study is visualised in Figure 29. As explored in Chapter 4 on Wildlife Trade, the demand and large-scale consumption of wildlife comes from urban centres, but there is also likely high exposure to wildlife, and among wildlife, domestic animals and people at the interface with wild regions and in rural areas (Webster *et al.* 2016). To better understand the risks of zoonotic emergence, research in this chapter explores the following four areas:

- the types of wildlife exposures experienced by people living and working in an environment known for wildlife trade

- the socioeconomic drivers of the local wildlife trade, as well as the classification and value placed upon different wild animals
- the potential risk factors for zoonotic disease transmission associated with exposure to wild animals particularly bats, rodents, and nonhuman primates
- reported incidences of unusual illnesses.

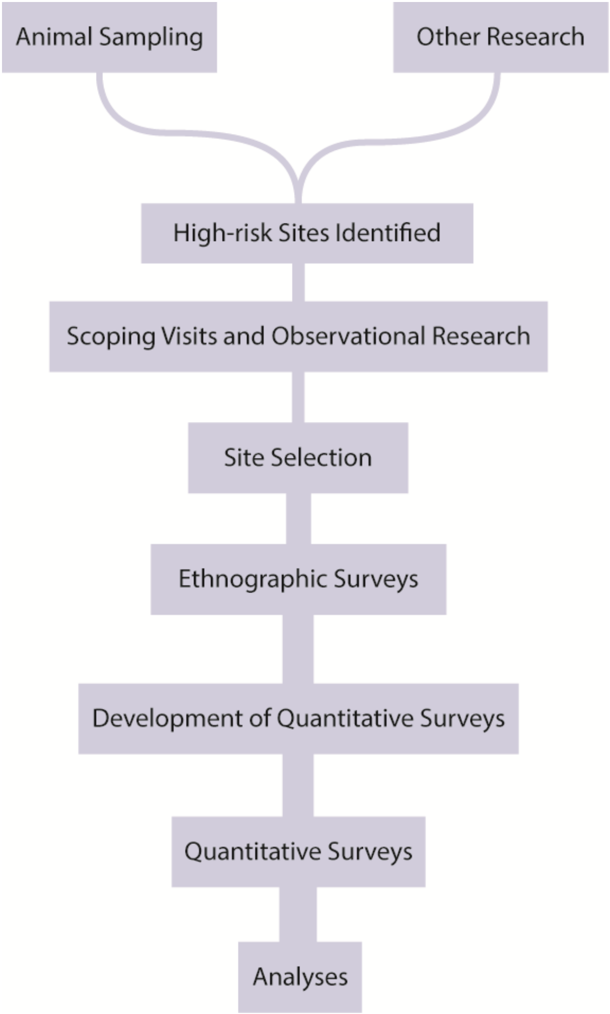


Figure 29. Diagram of methodology and design of field components of this study. Original research from this thesis (See Chapter 3) as well as other published studies and personal observations yielded a pool of sites designated as high-risk for zoonotic viral spillover. Scoping visits were conducted along with observational surveys and based upon criteria detailed in Section 5.9, study sites were selected. Ethnographic surveys were then conducted, transcribed, translated, and coded. Following analyses of the coded surveys, a quantitative survey or structured questionnaire was developed and conducted at a subset of the selected sites. The final step is analyses of the observations and questionnaires.

It is expected that rural Chinese farmers and residents would have the highest exposure to wildlife and given this constant exposure would also be most likely to contract illnesses of unknown aetiology, and likely from pathogens of wild or domestic animals. By combining (a) observational surveys, (b) ethnographic interviews, and (c) structured interviews, the research in this chapter aims to 1) identify biological, behavioural, and ecological factors influencing the risk of viral spillover and

2) determine potential targets for intervention based on high-risk human behaviours and practices that amplify disease transmission. By addressing these aims, the research conducted here will build upon the current understanding of the drivers of zoonotic disease emergence and host-pathogen dynamics.

5.7 Methods

Qualitative and quantitative methodologies were conducted with individuals living in the provinces of Guangdong, Guangxi, and Yunnan in rural southern and southwestern China. The methods consisted of (a) observational surveys, (b) one-on-one ethnographic interviews, and (c) behavioural surveillance consisting of structured surveys. Eligible sites were identified by the presence of virus-positive non-human animal samples (primarily bats and rats) surveyed during the study. The proxy for risk of spillover was the likelihood of human, domestic animal, and wildlife interaction determined following scoping visits and observational surveys.

Community sites were selected based on the following eligibility criteria and following confirmatory scoping visits and initial observational surveys:

- research from this thesis (Chapters 3) and other work (Zhang *et al.* 2009a; Ge *et al.* 2012; Ge *et al.* 2013; He *et al.* 2014) had confirmed large bat populations in caves and other natural or manmade roosting sites
- bats and other animals including humans found to be positive for Paramyxoviruses or Coronaviruses in this study (Chapter 3) or in other studies (Li *et al.* 2005; Li *et al.* 2010; Ge *et al.* 2012; Yuan *et al.* 2014)
- regular contact observed among wildlife, domestic animals, and humans in previous research (Chapter 3)
- wild animal farming, consumption, and trade was known or observed to occur (Li *et al.* 1996; Yiming & Dianmo 1996; Li & Wang 1999)

If interactions were observed among humans, domestic animals, and wildlife at a site, then it was scored highest and considered a ‘high-risk site’, and added to a pool of potential field sites for this study. Accessibility was also a determining factor as some sites were prohibitively distant from other research conducted for this thesis. The terrain, poor road conditions, and adverse weather patterns such as rain storms and flash flooding make long distance travel prohibitive and can isolate certain regions particularly in southern Yunnan and Guangxi provinces (Davies 2014; Meixian *et al.* 2014; Anon 2015; Luan 2016). Duplicate conditions or sites were eliminated and a final list of ‘high-risk’ sites was determined consisting of 18 rural communities (towns or villages) in Guangdong (4), Guangxi (5), and Yunnan (9) Provinces in southern China (Figure 30, Table 16).

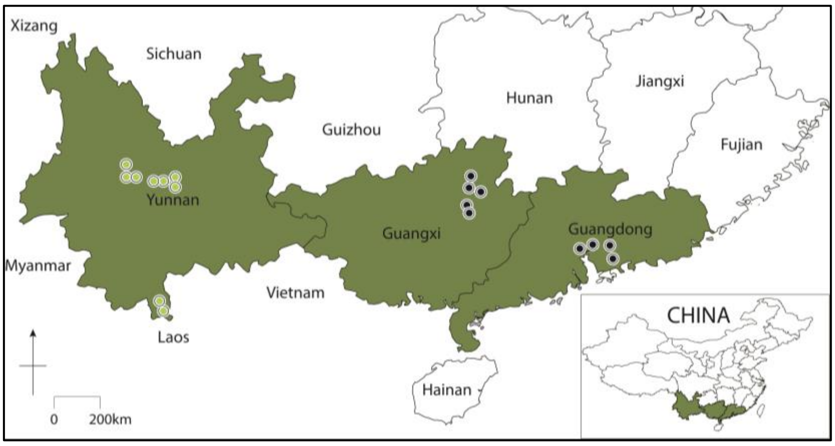


Figure 30. Southern China field sitesindicated by concentric circles. Field sites were in Yunnan, Guangxi, and Guangdong Provinces. Behavioural, observational, and quantitative surveys were conducted at all sites in Yunnan Province (indicated by green circles). Only behavioural and observational surveys were conducted at other sites in Guangxi and Guangdong (indicated by black circles).

Figure 31. Farmland at Zijiadeng villageLat. 25.499607, Long. 100.529432) in Xiangyun county in Yunnan Province. This photo details highly managed or human dominated landscape of terraced rice paddies, houses (white and beige structures) and farms (brick red with black roofs), and small tree plantings. In the back left, some strip mining is visible (grey patch on hills) and orchards are planted on the hills to centre and left immediately above the rice paddies. The vegetation on the hilltops is secondary forest. Some hills in the mid foreground (centre of photo) have evidence of anthropogenic or natural erosion (bare earth patches). The hills on the far right are pine (*Pinus yunnanensis*) plantations. The blue roof in the foreground is the cover of a cement walled shed housing civets (*Paguma larvata*) and Malayan porcupine (*Hystrix brachyura*) at the compound of one of the wildlife farmers interviewed for this study.

Community sites in each province were in a mixed-use rural agricultural landscape containing secondary forested patches abutting croplands that surround villages and towns (Figure 31). Population data were not available for each site, but estimates from local Centres for Disease Control and Prevention were approximately 1,500 individuals within the sprawling village or township with approximately 200-250 individuals per each community site, which was a clustering of several farm houses surrounded by agricultural fields (personal observations). Villages or town areas included the following:

- small live animal markets (5-30 animals) where wildlife was also sold
- farms that bred domestic and wildlife species for consumption and trade
- hunting areas; restaurants butchering and serving wildlife
- facilities where wildlife and domestic animals were housed before being transported to larger markets
- caves where people collected guano
- caves used by tourists
- residential areas with bat roosts

5.8 Targeted Demographic for Human Surveillance

Participants were recruited primarily through local contacts that were cultivated during previous research for this thesis. Local contacts included biologists and researchers from provincial universities or institutes, personnel from local Centres for Disease Control, and wildlife farmers. These contacts facilitated introductions and provided referrals to local residents and community leaders. Individuals who were 18 years of age or older and who were able to provide informed consent were eligible to participate. All participants received a token gift of a bottle of cooking oil valued at US$10 in appreciation of their time.

For the ethnographic interviews and to achieve representation of participants with exposure to wildlife, purposive sampling was employed. In this method, participants were selected because they met predetermined criteria relevant to addressing the research question (Saumure & Given 2008). For the behavioural surveillance, an adaptive cluster sampling method was utilised (Thompson 1990) following identification (by observational surveys and ethnographic interviews) of clusters or communities of highly exposed individuals. Both ethnographic and behavioural surveys were conducted on humans selected based upon the following criteria:

live in or near (4km or less) a bat cave
hunt or farm wildlife
work in markets that sell wildlife
work in or eat at restaurants selling wildlife
work in a nature reserve or other protected wildlife habitat

5.9 Field Sampling Methodology, Biosafety, Security, and Approvals

All field team members were trained in correct use of personal protective equipment (PPE) as well as hygiene and safety to minimise potential exposure or injury. All team members were required to pass the Collaborative Institutional Training Initiative (CITI) Research Team Member training modules in Human Subjects Research, Healthcare Ethics Committee, and Biosafety and Security (about.citiprogram.org). Field team members received a 2-day long interactive training on qualitative and quantitative approaches to human behavioural surveys including ‘mock’ or trial ethnographic interviews and recording sessions.

The methodology and human sample collection protocols in this study (See Chapter 2. Methods and Appendix 7.7 and Appendix 7.8) were approved by the USA-based Hummingbird Institutional Review Board and the Institutional Review Boards of two Chinese institutions: Wuhan University School of Public Health and the Yunnan Institute for Endemic Disease Control and Prevention. Three types of field sampling methodology were conducted for this research: observational, ethnographic interviews, and quantitative surveys.

5.9.1 Observational Methods

Observational surveys were conducted at all identified high-risk locations as part of scoping visits (Figure 29). Once field sites were determined (Figure 30) additional observational surveys were conducted concurrent with ethnographic and quantitative surveys. Site observations were general, open ended, and conducted to (a) identify appropriate field sites and (b) provide supplemental data for the quantitative results. An Observational Checklist (See Appendix 7.5) modified from Gray (2009) was developed to determine if (a) there were evidence of human-animal interaction and (b) observed potential for spillover and transmission of zoonotic diseases from wildlife.

Observations were recorded on paper and digitally (audio) on site whenever possible. If timing or sensitivity of residents to recording activities (e.g. note taking or photographing) did not permit on-site recording, then observations were made immediately upon exiting the location. For site scoping visits two or more team members recorded observations. After recording the information, the observations were pooled by visit. Since all sites were selected based upon the interaction of wild animals, domestic animals, and humans, the following guidelines were developed for observational research conducted in the field:

- Assess human population (customer and vendor in markets or restaurants; inhabitants or area residents elsewhere): age, sex, ethnicity, and relationships.
- Assess facilities and condition, e.g. toilets; hand washing areas; waste disposal method and locations; and drainage. Are there signs of health department inspections or notices about health and safety or regulations? Are permits or licenses displayed?
- Observe if people use any personal protective equipment (PPE), e.g. masks or gloves.
- Observe ventilation conditions and infrastructure including number of floors, exits, and how people, animals, and vehicles move through the site.
- Is there any evidence of butchering activity? E.g. feathers, offal, etc.
- Assess conditions of animals. Estimate the number of cages or holding areas; number of animals in each cage or holding area; and total number of animals in the market. Observe how cages and animals are arrayed, e.g. stacked, spaced, in shade/sun, etc.
- Vehicles: note arrivals and departures. Check license plates to see if vendors or customers are local or from another province.
- Map: sketch an overview of the site including all buildings, roads, activities, and other features

To be as unobtrusive and inconspicuous as possible while maintaining accuracy, animal count estimates in markets and warehouses were always made by the same two members of the field team, recorded immediately upon leaving the market, compared, averaged, and then summarised.

Observational surveys were conducted in two settings: (a) villages and towns and (b) at two of the largest known and operating wildlife and animal markets in China (Zhang & Jiang 2010; Anon 2012). After sites were selected, observational surveys at towns and villages were conducted opportunistically and concurrently with ethnographic and quantitative surveys. Observational surveys at markets were conducted twice per year at six-month intervals each at Taiping Market and Foshan Market. These markets were located in the greater Guangzhou City area. Southwest of Guangzhou City (Lat. 22.640484, Long. 112.258051) in Foshan, Foshan Market is predominantly a seafood market, a parking lot, and a section (approximately 0.5 hectares) of 80-100 covered and connected two-story stalls selling wildlife and domestic animals. Taiping Market is located (Lat. 23.548852, Long. 113.586605) northeast of Guangzhou City in Conghua City and is currently the largest known live animal and wildlife market in China. It consists of up to 160 covered and connected two-story stalls, a parking lot, and two restaurants sprawled across approximately 2 hectares (Figure 32) (PGGM 2006). Above the stalls in each market are living quarters where vendors sleep, wash clothing, care for children, and conduct other activities that are also carried out on the ground-level stalls amongst the caged animals.

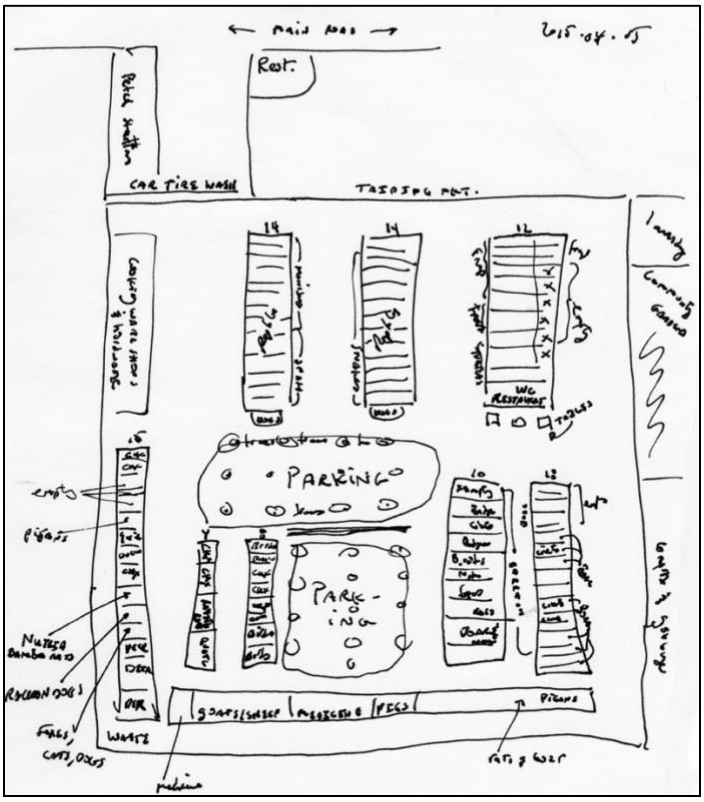


Figure 32. An observational survey diagram of Taiping market in Guangdong Province of China. Initial site survey visit on 05 April 2015. This type of observation supplements other quantitative data following site visits. Two field team members make observational drawings such as the one here and then compare notes for consistency. Cf. Figure 8 with more legible annotations.

Ethnographic Survey Methods

In-depth ethnographic surveys were conducted at 15 field sites in Guangdong, Guangxi, and Yunnan Provinces (Table 16). All sites were selected as per the observational survey site-selection criteria. Surveys were conducted by 3 trained interviewers from regional Centres for Disease Control and Prevention and were designed to last no more than 60 minutes. Interviews were audio-recorded,

Table 16. Field sites. Table presents observational (Obs.), ethnographic (Ethno.), or quantitative (Qnt.) surveys that were conducted in Guangdong, Guangxi, and Yunnan Provinces of China along with corresponding latitude and longitude coordinates.

	Obs.	Ethno.	Qnt.	Latitude	Longitude
Guangdong Province	21	27	0		
Conghua City					
Taiping Market	6	0	0	23.548852	113.586605
Dongxing Village	3	8	0	23.733328	113.827509
Foshan City					
Foshan Market	6	0	0	22.640484	112.258051
Huidong City					
Lianghua Town	2	15	0	23.093938	114.812583
Zengcheng City					
Xiaolou Town	2	3	0	23.369907	113.806924
Zijing City					
Wenquan Village	2	1	0	23.440845	115.165036
Guangxi Province	12	25	0		
Lipu City					
Licheng Town	3	5	0	24.486367	110.392084
Fu Village	3	1	0	24.512104	110.334425
Xin'an City					
Gaozhai Village	2	9	0	25.367259	110.364042
Lengshuitang Village	2	3	0	25.289080	110.379661
Yanqian Village	2	7	0	24.534267	110.515207
Yunnan Province	22	35	685		
Anning City					
Wenquan Town	2	2	130	24.914012	102.478181
Jinning County					
Xiyang Town	2	0	137	24.457291	102.322778
Lufeng City					
Zhong Village	3	5	80	24.986802	102.108419
Xiang Qing Town	3	1	66	25.074788	102.086933
Xiangyun County					
Banqiao Village	3	4	27	25.705578	100.153770
Midian Village	2	10	50	25.680951	100.753154
Zijiadeng Village	2	13	58	25.499607	100.529432
Xishuangbanna County					
Mengla Town	3	0	65	21.486666	101.570706
Jinne Town	2	0	72	21.695760	100.050267
TOTAL	55	87	685		

transcribed, and then translated into English. A total of 10% of the interviews were selected for secondary transcription and translation for quality control. Analysis of themes provided a framework with which to code and analyse data from the ethnographic surveys (Braun & Clarke 2006).

Five core themes were used (Table 17) to form the basis for analysis of the ethnographic interview data. The five themes were: (1) human movement and travel, (2) socioeconomics and daily life, (3) biosecurity in human environments, (4) unusual illness, death, and medical care, and (5) human-animal contact. Individual interviews and field notes were studied to ensure familiarity with the data and to confirm narrative consistency within individual interviews prior to coding. A coding keyword guide (See Section 7.15) permitted consistent coverage of the themes that were the focus of the interviews. Qualitative data were re-examined to develop additional theoretical categories. Central to this analysis was an assessment of the participants' perceptions (including observed changes over time) and participation in the wildlife trade. The data were coded for factors associated with wildlife consumption, the socioeconomic drivers of the local wildlife trade, conservation and legal efforts, the prevalence and types of wildlife observed, and wildlife exposures that could transmit disease to humans. All coding and qualitative data analyses were performed using Quirkos (2017), Microsoft Word, and Excel (2016).

Table 17. Core themes for ethnographic interviewers. These were used as general guides in semi-structured interviews.

Checklist for Core Themes for Ethnographic Interviews

- ☐ Human movement
    - ☐ Home
    - ☐ Work
    - ☐ Travel
    - ☐ Observed environment
- ☐ Socioeconomics
    - ☐ Daily routine
    - ☐ Animal responsibilities
    - ☐ Education
    - ☐ Economics
- ☐ Biosecurity in human environments
    - ☐ Water and food
    - ☐ Sanitation
    - ☐ Hygiene
- ☐ Illness, medical care/treatment and death
    - ☐ Household illness
    - ☐ Illness from animals
    - ☐ Medical care/treatment
    - ☐ Death
- ☐ Human-animal contact
    - ☐ Indirect contact
    - ☐ Direct contact
    - ☐ Animal products/rituals
    - ☐ Animal health
    - ☐ Perceptions/knowledge

5.9.3 Behavioural Surveillance Methods

A structured behavioural questionnaire (See Section 7.20) was designed to measure exposure or behavioural risk to examine the potential mechanisms of zoonotic viral spillover and build on the data acquired via the ethnographic interviews conducted in this study. The questionnaire assessed animal exposures and incidences of illness of unknown aetiology over the respondent's lifetime and during the prior 12 months including contact with animals, travel, health, disease, and hygiene. Standardised syndromic case definitions were used to design questions about experience of illnesses of unknown aetiology, e.g. severe acute respiratory infections (SARI), influenza-like illness (ILI), febrile symptoms, and encephalitis. Each respondent was assessed for undiagnosed illness symptoms in the past year, over his or her lifetime, as well as in respective families. The questionnaire examined the respondent's contact with animals, type of exposure, and the species involved. These data were then evaluated to determine whether there were any correlations between species and types of contact and reported symptoms. The full questionnaire was designed to take no more than 30 minutes. Quiet and private locations were identified before the interviews, which were conducted without other individuals present. To be representative of the larger population, the target sample size was estimated at 132 (95% confidence level ±5) given the local population estimate of a maximum of 250 individuals in each cluster of potential respondents.

5.10 Results

5.10.1 Observational Results

From 11 March 2014 to 2 October 2016, a total of 55 observational surveys were conducted in 20 sites that included 2 large animal markets with wildlife and 18 rural farming towns or villages in Guangdong, Guangxi, and Yunnan Provinces (Table 16). From 24 March to 10 December 2015, 87 ethnographic interviews were conducted in 16 towns or villages with an average of 29 ethnographic interviews in each of these same three provinces. During the months of April, May, and July of 2016, behavioural surveys consisting of 685 quantitative questionnaires were conducted in only Yunnan Province in a total of 9 towns or villages.

5.10.1.1 Observational Survey Results

Initial site selection scoping visits and observational surveys were carried out at 27 towns, villages, and markets across Guangdong, Yunnan, and Guangxi Provinces. Seven locations were considered unsuitable due to limited evidence of contact between humans, wildlife, and domestic animals or site accessibility. A total of 20 locations were selected for this study based upon evidence of human-animal interaction and observed potential for zoonotic pathogen spillover (See Section 5.9). For all 20 sites, at least one additional, post-site-selection observational survey was conducted concurrently with either ethnographic interviews or quantitative behavioural surveillance.

5.10.1.1.1 Town and Village Observational Survey Results

Written observations and details recorded in the Observation Checklist were used to confirm the suitability of each field site based upon evidence of interactions between humans, domestic animals, and wild animals:

> *We entered the community through a gate post. At the top of a rise, we exited the car and went down a flight of rough brick steps to a gated compound. Behind and below were rice paddies and fields of broad beans and rape. Above was a walled area of newly turned and mounded earth. Entering through the gate, I could smell civets immediately. Along the cement path were caged areas each about 2 x 3 meters and about 3 or more meters high. A lot of room, since only the first three contained different dogs. Most were barking. I was not entirely certain if these were guard dogs or for food, but if for food they were not very healthy looking and of different variety than those that I usually see sold for eating. The first and third cage had adult dogs - one each. The second cage held two young dogs that were barking and whining. The last two cages past the dogs were open and empty. Straw and dirt or dried faeces were on the floors some from chickens…. Perpendicular to the aforementioned cages was a rectangular cement house or enclosed area. The owner opened the door, which was locked. Inside the smell of civets and porcupines was quite strong. There were metal stacked cages of civets - about 10 or fewer. Porcupines were in cement enclosures -one or two each - and about the same number. A red bucket of dried corn was on the ledge as food for the porcupines.*
>
> – observation at a wildlife farmer’s compound in Xiangqing Town, Lufeng, Yunnan (26 March 2014)

Other observations addressed issues around hygiene and facilities, and permitted rapid assessment of conditions of animals in farms and markets including estimates of the number of cages or holding areas, number of animals in each cage or holding area, and total number of animals in the market or farm such as in the following observation at a nutria (*Myocastor coypus*) farm:

> *We visited another wildlife farm where nutria were raised. The area was not far from town but was much more rural. The facility was a modern looking house with a cement wall about 10 feet high surrounding it. We were told this was to protect against thieves. An older woman was burning weeds out front. There were sheets and clothes drying on a line as we entered the compound through solid metal gate-doors. There were four rows of pens, with pens on both sides of a row and 10 to 12 pens per side. The first row and one side of the next was empty. The rest of the rows all had nutria. They had orange teeth, webbed feet and adults probably weighed 5-7kg. The farmer told us that they reproduce rapidly. I saw 10 pens with only females and 5 to 8 babies each. The farmer told us that nutria are oilier than bamboo rats and he did not like the taste of them as much. The pens were spotless. The animals were being fed something that looked like lettuce. There were about 150 nutria at this farm – possibly more, since counting the juveniles and babies was difficult to do with our cursory inspection. A system of drains connected all the pens with gates at each one to create a swimming pool for the nutria in the front half of each pen. Some of the animals were in the water. They all seemed healthy and clean. The pens were in excellent shape and had roofs over each row, but the farm was entirely outside. There was a toilet room at one end that was clean. There were no large dogs. Two puppies and one cat were wandering freely about the place.*
>
> – observation at a wildlife farmer’s compound in Fu Village, Lipu, Guangxi (01 November 2014)

Other observations noted presence and shared use of the environment by humans, bats, and domestic animals:

> *I saw bats at night-time exiting caves about 200 meters from houses in the village. Upon entering the next day, I saw bats roosting inside the cave. A stream flowed out of the cave in which I saw local residents washing vegetables and clothing, slaughtering chickens, and bathing. Many children were playing in the stream and nearby the cave. Dogs wandered about. Villagers told me that they eat bats and rats whenever they can catch them.*

– observation at Lengshuitang Village, Xin'an, Guangxi (1 July 2014)

As mentioned briefly above, surveys also captured details about human consumption of wildlife as in the following observation at a restaurant:

> *I watched the chef quickly butcher the bamboo rat, put it in a gutter, and run water from a hose over it for a few minutes. He rinsed his hands with the water then rubbed his face. The kitchen was very dark, but well organised with several prep and cooking stations. Orders were stuck to the exhaust fan by grease. One of the prep cooks butchered two sparrows and threw all of the parts into a pot of water to boil. The walls were brown with grease and did not have the usual government approval ratings that were posted in all officially inspected restaurants. All cooking elements were fuelled by external gas canisters. There were both a refrigerator and a freezer in the kitchen. The menu had one page that exclusively listed wildlife including bamboo rat, cat, dog, badger, porcupine, and civet. When asked about whether that was legal or not, Wei Shangzheng pointed out that the restaurant owner would always say that all the animals were farmed even if they were not, since only farmable animals were listed on the menu.*
>
> – observation at Lipu Restaurant, Lipu, Guangxi (21 September 2014)

5.10.1.1.2 Market Observational Survey Results

Two large animal markets in Guangdong Province at Foshan City and Taiping Town were selected and surveyed a total of 6 times each by the same two observers employing the same methodology: once per market every 6 months from 11 March 2014 to 2 October 2016. Foshan Market was the smaller of the two and characterised as more compact and with more species mixing by observational surveys.

> *The market is very large but primarily focused on seafood. There is also a section where they sell poultry and mammals. There were approximately 60 stalls in this section and about 25 appeared to be open for business on the day we visited. It wasn't clear why some were closed. The shops selling the animals were smaller than at Taiping Market. So too was the market. Also, instead of vendors focusing on one species or one type of animal, there was more variety per each vendor. For example, several vendors have stacked cages of chickens, ducks, wild boar, cats, civets, and bamboo rats. People were friendly here and not guarded like at Taiping Market.*
>
> – observation at Foshan Market, Guangzhou (2 November 2014)

Further observations were made about hygiene and species mixing and human interactions:

> *All of the animals are mixed together in each stall. There was blood and faeces everywhere. Some of the animals looked quite sick, with the exception of the goats. All goats were in their own stalls with no other animals, but all of the goat stalls were spread throughout the market. The goats all had ear tags and appeared healthy and alert. Species were mixed in all of the other stalls. Shops seemed to specialize in having as large a variety as possible. Turtles and snakes were mixed in with poultry, boars, pigs, civets, nutria, bamboo rats, regular rats (that looked particularly ill). The stalls were bigger than in Taiping market probably 4x5m and packed with cages. There were no signs of running water, though there were lots of puddles. There were 2 stories of living space above the stalls instead of one. It was a well-constructed market with cement walls and new-looking blue plastic roofs. There were 6 civets in the market. One in a stall with chickens, ducks, pigs, cats and snakes. Its fur looked matted and dirty. Another stall on a side row had at 5 civets in a cage, halfway back into the stall. There were 20 or 30 shoppers here. We saw one goat slaughtered and thrown into a basket on the back of a motorcycle.*
>
> – observation at Foshan Market, Guangzhou (27 March 2015)

Taiping Market was the larger and characterised with a greater diversity of vertebrate species, but with vendors that were very sensitive to presence of strangers.

> *Only one man was actively cleaning out his stall that housed pigs and poultry. Everyone else was prepping lunch, eating or doing general cleaning. No one handling animals was wearing gloves or masks. Offal from a snake or another reptile was in the lane. There were many more men than women, though mostly women were cleaning the stalls. Most people wore open sandals. Some wore sneakers. There were no old people visible and few young*

> *children though it seemed that most of the people selling at the market lived above or behind their stalls. One end of the lane of stalls had upstairs housing with ceramic tiles and fans and air conditioners plugged into the windows. People's sleeping, living, food prep and slaughter areas were adjacent and without any barriers. There appeared to be 3-to-8 people per stall. People looked tired and thin, but no one appeared ill. Most men were smoking. One of the waiters at the restaurant nearby where we had lunch told us that the market had been shut down for two months following our last visit due to an article published in the paper regarding the unlicensed illegal wild animal trade there.*
>
> – observation at Taiping Market, Guangzhou (15 October 2015)

Vendors at the Taiping Market were overheard saying that they:

> *… assumed we were looking for diseases in the animals. The presence of westerners definitely is a red flag for them as is the presence of non-local Chinese. Unless you speak the local dialect, vendors here are not willing to speak with you.*
>
> – observation at Taiping Market, Guangzhou (11 November 2014)

Observational surveys at Foshan and Taiping markets recorded a combined total of 3,315 (979 and 2,336 respectively) individual, non-domestic wild or wild-farmed mammals of 21 species over the survey period (Figure 33) and 1,727 domestic animals (dogs, cats, goats, and sheep) were also recorded over the same time period. Reptiles, birds, fish, and other taxa were not recorded, but it was noted that all these animals were present in both markets during each observational period. Aquatic animals were mostly to be found in the Foshan Market. An average of 1,105 individual wild or wild-farmed animals were counted within each calendar year at both markets. Foshan Market averaged 326 wild animals per year and Taiping Market averaged 799 per year. Taiping Market primarily sold terrestrial animals and had more individuals per species and more species diversity than Foshan Market. Over the first year of the observation period, counts at both markets decreased by an average of 32.8% (Table 18). In the following year, counts at both markets increased by an average of 59.5%.

Table 18. Percentage decrease or increase in annual count of wild animals at Foshan and Taiping Markets. These markets are located in Guangdong Province, China. Negative values indicate a percentage decrease. Only two years of data were recorded, so the first column shows the change from 2014-2015 and the second column the following year from 2015-2016.

	2015	2016
Foshan Market	-33.7	34.6
Taiping Market	-31.8	84.3
Average	-32.8	59.5

Of all counted species at the markets only two (Table 31) *Arctonyx collaris* (hog badger) and *Rusa unicolor* (sambar deer) are listed on the International Union for Conservation of Nature's Red List of Threatened Species as "vulnerable" (IUCN 2016). Only one, *Cervus elaphus* (red deer), is listed as a Class II threatened species on the China Endangered Species List (PRC 2006).

As of current published reports (Table 31), at least 143 different viruses from 26 viral families (Table 19) have been reported in 18 (71.4%) of the wild animal species regularly observed in Foshan and Taiping markets. Most (80.9%) of these viruses have been reported in only three species observed in the two markets: *Sus scrofa* (wild boar), *Rattus norvegicus* (brown rat), and *Cervus elaphus* (red deer). SL-CoVs have been reported in three species observed in these markets: *Paguma larvata* (masked palm civet), *Nyctereutes procyonoides* (raccoon dog), and *Melogale moschata* (ferret badger). Of the viruses that may be found in the animals in these markets, 60 (42.0%) have also been reported in humans (Table 32). These include the following viral families and specific zoonotic viruses within them: Coronaviridae (SL-CoV), Paramyxoviridae (Menangle virus, Mumps virus, and Nipah virus), and Bunyaviridae (Rift Valley fever virus and Hantavirus). No bats were observed in any market during this period.

Table 19. Families of 26 viruses from species of animals observed in markets in Guangdong Province China. List is in order of number of viruses reported per viral family. Viral Families reported in prior publications as detailed further in Table 31. Two Guangdong markets were observed: Foshan and Taiping.

Viral Family	No. Viruses Reported in Species Observed in Markets
Bunyaviridae	17
Reoviridae	15
Flaviviridae	13
Herpesviridae	11
Paramyxoviridae	10
Poxviridae	8
Caliciviridae	7
Coronaviridae	7
Parvoviridae	7
Picornaviridae	7
Togaviridae	7
Adenoviridae	6
Retroviridae	6
Orthomyxoviridae	3
Papillomaviridae	3
Rhabdoviridae	3
Arenaviridae	2
Astroviridae	2
Circoviridae	2
Anelloviridae	1
Arteriviridae	1
Asfarviridae	1
Bornaviridae	1
Filoviridae	1
Hepeviridae	1
Pneumoviridae	1
Total	143

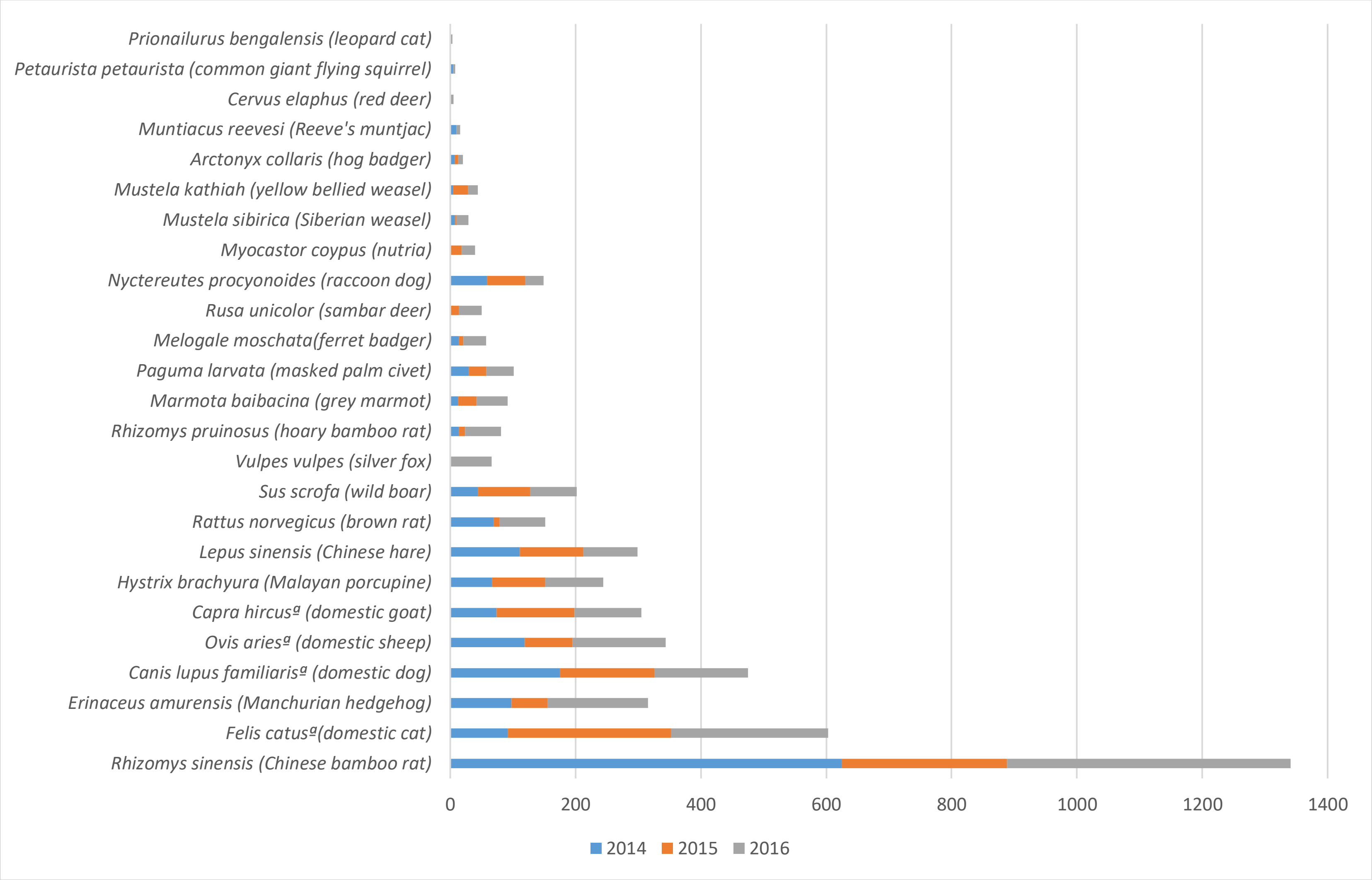


Figure 33. Numbers of wild animals observed in Foshan and Taiping live animal markets. These animals were observed in the greater Guangzhou area in Guangdong province China between March 2014 and October 2016. Four species with a superscript ª are domestic animals. All other species are wild animals and except for *Prionailurus bengalensis* (Leopard cat) known to be both hunted and captive bred in China. 2015 animal counts are lower than 2014 and 2016 counts as indicated by the shorter red bars. 2016 counts at both markets were higher than either preceding year.

5.10.1.2 *Ethnographic Results*

A total of 35 (40.2%) of the 87 ethnographic surveys were conducted in Yunnan Province at six different sites; 25 (28.7%) in Guangxi Province at five different sites; and 27 (31.0%) in Guangdong Province at five different sites (Table 16). Interviews averaged 36 minutes (SD = 16 minutes) with the longest interview lasting 1 hour and 30 minutes and the shortest interview lasting only 13 minutes. Most (64.4%, 56/87) of respondents were between the ages of 36 and 55 (Figure 34) with an average age of 48. The respondents consisted of 28 women (32.2%, 28/87) and 59 men (67.8%, 59/87).

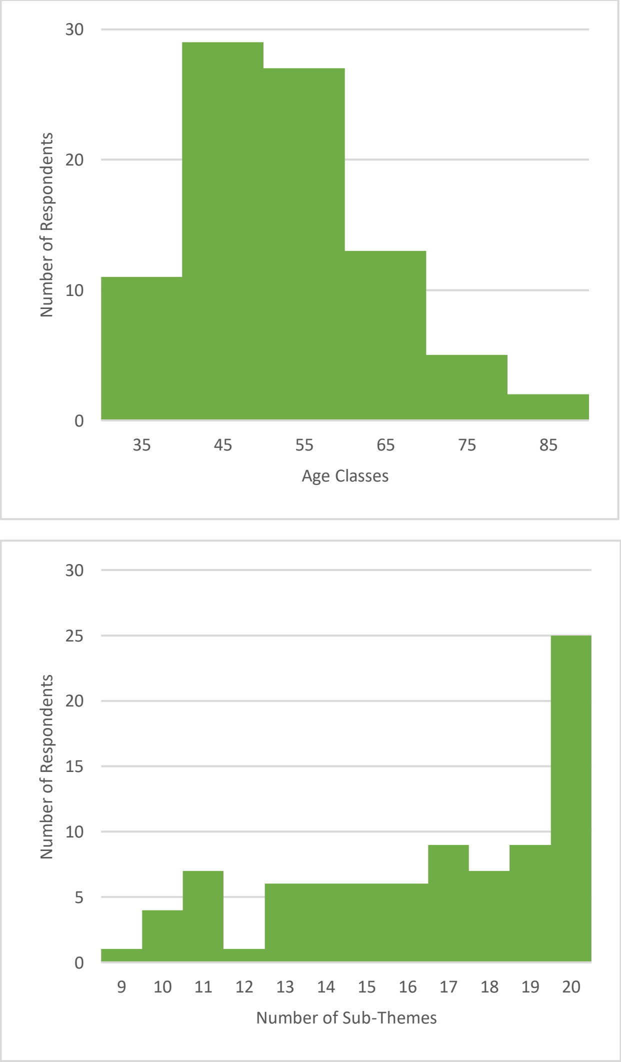


Figure 34. Histograms of ages and sub-themes of ethnographic survey respondents. Respontents (n=87) were from Guangdong, Guangxi, and Yunnan provinces. (Upper) Age classes of respondents. (Lower) Number out of 20 possible sub-themes (x-axis) such as work, travel, education, animal health, etc. (see Table 17) that respondents (y-axis) spoke about in ethnographic surveys.

Educational levels varied among participants, but the majority (78.2%, 68/87) reported having a primary school education. Only three participants (doctor, accountant, and wildlife researcher) claimed to have progressed beyond a 4-year university level of education. Participants (42.5%, 37/87) primarily identified as farmers (Figure 35). Five individuals identified as wildlife farmers stated that they raise animals such as bamboo rats (*Rhizomys sinensis*), civets (*Paguma larvata*), porcupine (*Hystrix brachyura*), or nutria (*Myocastor coypus*). Of the participants, 12 (13.8%, 12/87) identified as field personnel at Nature Reserves: 9 from the Nonggang Nature Reserve in Guangxi Province and 3 from the Gutian Nature Reserve in Guangdong Province; and 4 (4.6%, 4/87) were employees of the Yunnan Endemic Center for Disease Control and Prevention in Dali.

The open ended semi-structured ethnographic interviews were guided by core themes and sub-themes (Table 17). Approximately one quarter of the respondents (28.7%, 25/87) spoke on all sub-themes; 62 respondents (71.2%, 62/87) addressed at least 15 (75%, 15/20) of the 20 sub-themes (Figure 34). The sub-themes least addressed were death (59.8%, 52/87) and travel (69%, 60/87).

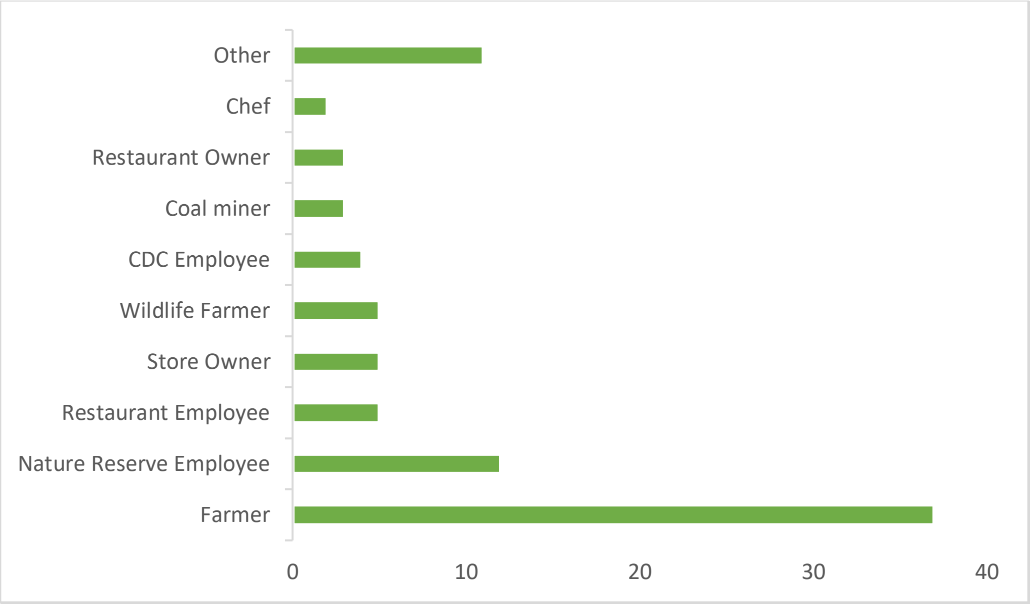


Figure 35. Occupations of ethnographic survey participants in Yunnan, Guangxi, and Guangdong provinces. The topmost horizontal grey bar labelled “Other” includes 11 individuals one each of whom reported the following occupations: Police, Accountant, Butcher, Cleaner, Construction Worker, Doctor, Driver, Hotel Employee, Hotel Owner, Handyman, and Wildlife Scientist.

The majority (94.3%, 82/87) of participants stated that they had some exposure to wild animals in the past year. Only the policeman, three of the store owners, and one restaurant worker said they had no contact with wildlife. When asked to list wildlife species 49.3% (34/69) said they eat wildlife and most often reported consuming snakes, frogs, wild boar, and birds. Of those who reported eating wildlife, 67.6% (23/34) were over the age of 50 and there was a positive correlation between age and consumption of wildlife (*p* = 0.003). Men more than women reported eating wildlife (*p* = 0.022). Several spoke about what they said were infrequently seen animals such as monkeys, wild boar, deer, and bears. Two mentioned pangolin that they claimed were from Vietnam, Laos, or Myanmar. Of those who discussed it (58), 34.5% (20/58) said they had eaten bats.

Most (77%, 45/58) said that wildlife was more plentiful in the past than it was now and 26.4% (15/58) said they hunted wildlife either in the past or from time to time. Several (11.5%, 7/58) claimed to be aware that hunting wildlife without a license is illegal. While 34.5% (30/87) of participants said they had been bitten by either a dog, a rat, or a bat at some point in their lives, 32.2% (13/87) specifically stated that they had been bitten by bats.

5.10.1.2.1 Wildlife consumption

Of respondents who reported eating wildlife (49.3%, 34/69,) 18 either were not directly asked or declined to address the issue. Respondents were cautious in answering the question and it was not always clear if the wildlife were consumed recently or in the past. Among those who reported eating wildlife, several (31.8%, 21/66) stated that it was not out of necessity for subsistence, but because wildlife was ‘tastier’ and ‘more delicious’ than domestic animals or simply that they enjoyed it. Snakes were most frequently reported as consumed for both the flavour as well as for purported health benefits. In the following transcribed and translated quotations both “Interviewer” and “Respondent” are abbreviated as “I” and “R” respectively.

I: Do people eat bat?
R: Yes, lots of people eat bat. Especially in the summer, when they’re easy to find.
I: How do you cook bat?
R: Skin it and cook it. Some people make soup with bat, but I don’t like bat soup.
I: Do you think bat meat is delicious?
R: Yes, of course…better than pork.
– Male farmer, age 60, Lengshuitang Village, Xin’an, Guangxi

I: Do you ever see bats?
R: Yes, bats usually roost in the caves. Local people go to the mine tunnel to catch bats. Bats sometimes live in old houses.
I: Do people here eat bats?
R: Yes, some people eat bats.
–Male Nonggang Nature Reserve employee, age 50, Gaozhai Village, Xin’an, Guangxi

R: People kill rat to eat.
I: How do you eat rat?
R: You don’t know? We cook rat meat with hot pepper. It is more delicious than pork, duck and chicken.
I: When is a good season to eat rat?
R: If you come here in the winter, we can easily catch rats in the field. Winter is a good season to catch and eat rat.
I: Do you make money by catching rats?
R: Nobody catches rats to make money; they catch rats to eat. Nobody could make money that way.
– Male farmer, age 65, Wenquan Village, Zijing, Guangdong

R: Two bats flew into the room, so we caught them and ate them.
I: How did you kill the bats?
R: Put them into boiling water.
I: Did you eat bat skin?
R: Yes.
– Female wildlife farmer, age 60, Lengshuitang Village, Xin’an, Guangxi

I: Who do you sell bamboo rats to?
R: Middlemen or restaurants.
I: Who is your main customer?

R: Middlemen. We sell animals to them. They sell to restaurants.
I: Do local restaurants buy bamboo rats directly from you?
R: Local restaurants still buy my farmed bamboo rats from middlemen. Traditionally, Chinese people love wildlife. They think it is more delicious. People think that the animals they buy from a middleman is wild. Animals you buy from farmers are definitely raised. Consumers cannot distinguish wild animals from raised animals...many people sell raised animals as wild one. It is a kind of marketing strategy.
– Male wildlife farmer, age 50, Lengshuitang Village, Xin'an, Guangxi

I: Can you buy wild animals?
R: Yes. We can, because we know people who hunt in the mountains.
I: What kind of animals?
R: Wild rabbit, pheasant, muntjac, and other things.
I: Is there a hunting season?
R: No.
– Male wildlife farmer, age 38, Midian Village, Xiangyun, Yunnan

I: Does anyone hunt wildlife?
R: Hunting animals is illegal.
I: If people hunted and sold wild animals, they would do this quietly?
R: Maybe they would sell quietly and in a village—not in a market—but in someone's home.
–Male Gutian Nature Reserve employee, age 40, Lianghua Town, Huidong, Guangdong

R: Because the village is small, everyone knows each other. Everyone knows who likes to hunt. We all know as soon as the hunter catches wild boar and then we can buy some from the hunter.
–Male wildlife researcher, age 47, Wenquan Town, Anning, Yunnan

5.10.1.2.2 High-risk interactions with animals

About 1-in-3 (34.5%, 30/87) of respondents reported being bitten by dogs, rodents, bats, and snakes. All respondents were aware of the importance of going to their local clinic to get an injection following any bite from dogs or wild animals. Despite this, when describing their personal experiences, most participants stated that they had not gone to the clinic and that they had been lucky the bite had not resulted in rabies infection. When asked to name some zoonotic diseases, participants mentioned Rabies and some mentioned Plague. Many explicitly were unaware that diseases could be transmitted from wild animals to humans:

I: Have you ever heard of anyone being infected with a wild animal disease?
R: No.
I: What do people do if a dog or a snake bites them?
R: Dog bites are common. People even get bitten by their own dogs. If that happens, they will get a shot for themselves. Vaccines for pets cost about RMB2,000, so they usually just kill their dog and eat it.
–Male Nonggang Nature Reserve worker, age 50, Gaozhai Village, Xin'an, Guangxi,

I: Do you know of any animal illnesses that infect humans?
R: Dog? I don't know.
I: Has anybody gotten sick because of a rat or dog bite?
R: No. I only know that sometimes people want to go get vaccinated.
I: Do you know how animal diseases can infect humans?
R: No.
–Female restaurant cook, age 55, Licheng Town, Lipu, Guangxi

I: Have you ever heard of anyone who was infected or died of a disease caused by an animal?
R: I never heard of that happening.

> *–Male farmer, age 60, Lianghua Town, Huidong, Guangdong*

> *I: Do you know if people can get sick because of an animal infection?*
> *R: It seems no.*
> *I: Do you know how animal diseases can infect people?*
> *R: I have no idea.*
> *–Female small shop owner, age 48, Banqiao Village, Xiangyun, Yunnan*

Several respondents stated that they would no longer eat bats and birds because of the public information around SARS and avian influenza.

> *R: People don't eat bats anymore since CCTV reported that bats are natural reservoir of some kinds of diseases. Also fewer people eat birds for the same reason.*
> *–Male Nonggang Nature Reserve worker, age 45, Gaozhai Village, Xin'an, Guangxi*

The most commonly reported exposures to wildlife (31%, 27/87) were via rodent bites and hunting. All participants who reported hunting used slingshots or traps to capture wildlife, although not recently. The most commonly reported wildlife that respondents had hunted included rodents, deer, wild boar, and a variety of birds including silver pheasants (*Lophura nycthemera*). Few participants would describe hunting in any detail.

> *I: How do you catch bats? With a gun?*
> *R: No. We hunt them with homemade brooms made of bamboo, at the narrow site in the cave when the bats fly out.*
> *–Male Nonggang Nature Reserve worker, age 45, Gaozhai Village, Xin'an, Guangxi*

> *I: What do they do with a slingshot?*
> *R: Hunt birds and rats.*
> *I: Why do they hunt birds and rats?*
> *R: To eat.*
> *I: Where do they go to hunt?*
> *R: Fields or trash collection sites. Rats are active there in the evenings.*
> *–Male farmer, age 60, Yanqian Village, Xin'an, Guangxi*

Other participants reported that rodents would access food stores and sharing water sources with animals.

> *I: Do rats get into your food?*
> *R: Yes, frequently.*
> *I: How do you deal with that?*
> *R: Clean the food and eat it.*
> *–Female farmer, age 80, Yanqian Village, Xin'an, Guangxi*

> *I: Is there a possibility that wild animals or livestock also drink this water?*
> *R: Definitely! We see footprints of wild animals near the water source.*
> *–Male Nonggang Nature Reserve employee, age 30, Fu Village, Lipu, Guangxi*

5.10.1.2.3 Self-reported illnesses of unknown aetiology

Respondents reported annual colds and fevers. Many (42.5%) said they went to local clinics or health centres for treatment, but a few (25.3%) also reported self-treatment with medications acquired from nearby pharmacies. Hospitals were only visited with severe symptoms. Several farmers reported deaths of chickens and ducks. Several respondents reported that at some time in the past their rabbits, dogs, or chicken had all mysteriously died. Of those who discussed it, most felt that vaccination of livestock or pets was prohibitively expensive and it was easier to slaughter and consume or bury any sick animals.

> *I: Do your chickens get sick? If yes, how do you deal with it?*
> *R: I don't have many chickens, so I don't have big problems. But I think if my chickens got sick, I would kill them before they die, and eat them.*
> *I: Don't you worry about getting sick after you eat a sick chicken?*
> *R: No, I'll kill them and eat them before they die.*
> *I: If your chickens died before you could kill them, what would you do then?*
> *R: It would not happen. I am sure I would kill them before they die.*
> *–Male farmer, age 60, Lianghua, Huidong, Guangdong*

> *I: Have you ever had a sudden death of animals?*
> *R: Yes. Rabbits in 2014.*
> *I: What was the reason?*
> *R: We did not find any reason for it.*
> *–Female farmer, age 80, Yanqian Village, Xin'an, Guangxi*

Participants reported disposing of sick or dead animals by dumping them into rubbish sites, burying them, or giving them to their dogs or other animals. Some mentioned that butchered meat from sick or dead animals could be in the markets and ways to avoid this.

> *I: How do you deal with sick animals?*
> *R: My family does not eat sick animals. We bury them, but other people do not do this. For example, some use dead pigs to make bacon. They cut the pork into pieces, salt the meat, and hang it out to dry.*
> *–Male coal plant worker, age 23, Banqiao Village, Xiangyun, Yunnan*

> *I: Have you ever had problems to buying animals in the market?*
> *R: Yes. Sometimes vendors sell old or sick ducks or chickens. If you cannot tell, you might buy animals that have been dead for a long time. We usually go to the same vendor to buy our meat to prevent this.*
> *I: Why would they sell dead animals?*
> *R: If they can sell dead animals, they will make a profit.*
> *–Female farmer, age 60, Lengshuitang Village, Xin'an, Guangxi*

Among the participants who reported illnesses (19.5%, 17/87), few (12.6% 11/87) had illnesses that were unidentified by health professionals or by respondents themselves. Western medicine was the most (92.0%, 80/87) reported treatment for illnesses. Some participants (16.1%, 14/87) also said they supplemented this with Chinese traditional medicine. Illnesses of unknown aetiology were reported by few participants and only two connected this to contact with wild animals.

> *I: Do you know anyone who got sick from an animal bite?*
> *R: Once a bamboo rat bit someone on her hand. She was in hospital for a week.*
> *I: Did she recover?*
> *R: She recovered.*
> *–Male wildlife farmer, age 50, Fu Village, Lipu, Guangxi*

5.10.1.2.4 Respondent perceptions of a decline in local wildlife

Although 85 out of 87 participants reported having some contact with wildlife (bats, birds, rodents, and primates) in the past year, 77% (67/87) noted a decrease in wildlife over time, which was attributed to many factors, but most frequently to development and construction that reduced forested areas. Some reported that the local government built new roads and buildings to increase local tourism.

> *I: Has the village changed a lot since your parents' time?*
> *R: Definitely, built more roads, more beautiful houses, more tourists. The population also increased.*
> *I: Did the area of forest and bamboo groves also increase?*
> *R: Not really.*
> *– Male Nonggang Nature Reserve Employee, age 50, Gaozhai Village, Xin'an, Guangxi*

Participants did not mention wildlife trade or the hunting and sale of wildlife as a cause of observed wildlife depletion. They attributed their own reduction in wildlife hunting and consumption to increased enforcement of laws protecting wildlife. Four Nature Reserve employees each related a story of a hunter who had killed a monkey and was caught.

> *I: Do people still hunt?*
> *R: Several young people catch frogs and pheasants here. Nobody dares to catch monkeys because of a guy who was caught and sentenced.*
> *I: How long was the sentence?*
> *R: Three and a half years.*
> *– Male Nonggang Nature Reserve employee, age 42, Gaozhai Village, Xin'an, Guangxi*

Only 6 out of 23 (26.0%) participants who admitted to having hunted in the past also reported recently hunting.

5.10.1.2.5 Part of the Wildlife Trade - Farming Wild Animals in South China

Seeing neighbours or local businessmen engaged in farming wild animals, some respondents turned to wildlife farming as a way to supplement their income:

> *I: Do you have a license to raise bamboo rats for breeding?*
> *R: No, but I have a wildlife business license and one for raising wildlife.*
> *I: Do you need any proof to sell bamboo rats for breeding to others?*
> *R: Yes, the licenses.*

–Male wildlife farmer, age 42, Xiangqing Town, Lufeng, Yunnan

I: How did your son and his wife learn how to raise and sell nutria?
R: They learned from a book and also online.
– Male wildlife farmer, age 70, Lengshuitang Village, Xin'an, Guangxi

The entire process from acquiring licenses to methods to raise and sell animals is readily available online with whole websites dedicated to elaborate instructions with pictures, chat areas, and daily updated blogs (www.yangzhushu.com, www.nczfj.com/zhushu/20108009.html). Some farmers reported on difficulties in wildlife farming.

R: Somebody raised bamboo rats in this village. Unfortunately, all of his bamboo rats died.
I: Do you know the reason?
R: It was not clear, but I think raising wild animals means that you have to learn how. It takes time. It is not an easy thing.
–Female farmer, age 40, Dongxing Village, Conghua, Guangzhou

The five wildlife farmers interviewed in this study were influential and respected entrepreneurs in their communities. They were involved in farming, real estate, and other activities aside from wildlife farming. They reported initially acquiring their wild animals from the wild. With a license from the Forestry Department it is legal to capture wildlife for the purpose of artificial breeding (PRC 2016b). All five wildlife farmers reported that their customers were principally other wildlife farmers or people intending to start their own wildlife farms and middle men (see Section 5.11.2.6).

I: Where did you get 'seed animals' when you started your farm?
R: The government allowed us to catch some wild animals after our permit was approved.
I: Did you catch wild bamboo rats by yourself?
R: No. We hired local people to catch them for us.
– Male wildlife farmer, age 50, Lengshuitang Village, Xin'an, Guangxi

I: How do you sell your nutria?
R: We post information online.
I: Does your farm have all of the required certifications?
R: Yes.
I: What kind of people buy nutria?
R: Mainly farmers.
I: Why do they buy them?
R: To start a new farm.
– Male wildlife farmer, age 70, Lengshuitang Village, Xin'an, Guangxi

One farmer in Guangxi was particularly forthcoming about his business, local renown, and had even published a book on bamboo rat (*Rhizomys sinensis*) husbandry (Figure 36). He had appeared on television and in newspapers. Locally, at least, he was something of a celebrity which may have promoted not only his own wild animal sales (to other local residents interested in starting their own farms), but also the industry as a whole:

> *I: When did you decide to start raising bamboo rats, porcupines, and other species?*
> *R: I started to raise bamboo rats in 2003. In 2009, I began to raise porcupine and civets. In 2013, I began raising nutria.*
> *I: What made you start raising these animals?*
> *R: I started to raise bamboo rats because I felt it was a good opportunity to make money. I saw there was a high demand for this, and little supply from the wild.*
> *I: How is raising these animals different from domestic animals?*
> *R: Raising these animals is unique and I had to find my own way to raise them by trial and error as there were no experts in raising these animals. Making sales is the biggest challenge. At the beginning, I was afraid not make a living, but I persevered to raise these animals and to try to make good sales and am now successful!*
> *I: Is it popular to eat these wild animals?*
> *R: Many people eat bamboo rat, civet cat, porcupine, and nutria all the time. Here we like to taste fresh killed meat, so supplying fresh meat always has a demand.*
> *I: Do you like raising these animals?*
> *R: Of course, I like them or I would not do this! I also make good money. I deal with these animals every day. I enjoy studying the problems and successes I encounter in the breeding process. I wrote a book about all these experiences to help other people who want to raise Bamboo rats and other wild animals.*
> *– Male wildlife farmer, age 50, Lengshuitang Village, Xin'an, Guangxi*

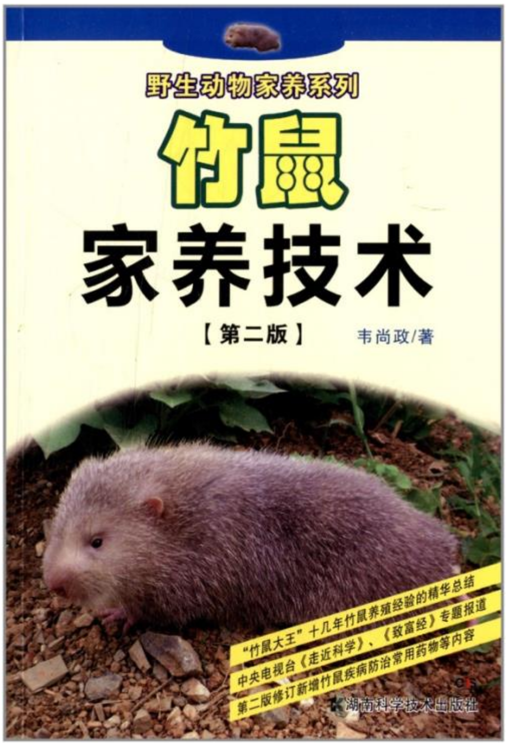


Figure 36. A Wildlife Domestication Series book on bamboo rat (*Rhizomys sinensis*) husbandry. *Bamboo Rat: Domestication Techniques*, (2nd edition) is self-published by a bamboo rat farmer also known as "Bamboo Rat King" (printed on the book cover) from Guangxi and also one of this study's ethnographic interview participants. Participant gave permission to reproduce the book cover image and display his name.

From respondents' statements, wildlife farmers vend their animals to middlemen who sell the same animals as farm raised wildlife or sometimes deliberately as wild-caught wildlife to restaurants, markets, hotels, and even other middlemen or farmers. Selling wildlife openly in markets in rural regions has become rare according to respondents:

> *I: Have you seen people selling wild animals at market?*
> *R: No, they will be in trouble if they sell wild animals at markets. The Department of Forest and Forest Police will arrest them.*
> *– Male Nonggang Nature Reserve employee, age 45, Gaozhai Village, Xin'an, Guangxi*

> *R: I heard about people selling wild animals, but have not seen that myself*
> *– Female physician, age 41, Banqiao Village, Xiangyun, Yunnan*

> *I: Did you see someone selling wild animals on this trip?*
> *R: Yes, but I am not sure if it was wild.*
> *I: Who do they sell to?*
> *R: I do not know…I often see people selling animals at the roadside.*
> *– Male driver, age 35, Zijiadeng Village, Xiangyun, Yunnan*

5.10.1.2.6 Middlemen and their Key Role in Transport of Wildlife to Markets

Several farmers reported seeing non-local middlemen in the area and said they were there to buy or hunt rodents, cats, wild boars, and eagles. Respondents stated that these middlemen stockpile, transport, and sell farmed-raised or wild animals to big, wholesale markets like Taiping and Foshan in Guangdong Province.

> *I: Do you know any middlemen who buy or sell wild animals?*
> *R: I used to know a guy who sold wild boar at the market. He used a gun to hunt.*
> *I: What other kinds of wild animals do the middlemen sell?*
> *R: They sell silver pheasant secretly. They also sell bear, deer, boar, snake and frog. They sell egret during migration season. Very few wild animals are traded in the market. Instead, buyers will contact middlemen directly. Most wild animals sold at the markets really are farmed by farmers.*
> *–Male Nonggang Nature Reserve Employee, age 50, Gaozhai Village, Xin'an, Guangxi*

> *R: If a middleman comes to town, local people will go to the field to catch birds.*
> *–Male wildlife farmer, age 50*

> *I: What are the kinds of wild animals that middlemen buy from local people?*
> *R: Flying squirrel, frog, snake, boar.*
> *–Male Nonggang Nature Reserve Employee, age 38, Gaozhai Village, Xin'an, Guangxi*

5.10.2 Quantitative Behavioural Surveillance Results

Of the 685 participants in Yunnan province who completed the behavioural questionnaire (Table 20), 402 (58.7%) were women and 283 (41.3%) were men with a mean age of 49 (range: 18-99) Most (81.5%) were over 35 years old. Almost three quarters (72.8%, 499) of the respondents only had a primary level of education or less. About half (43.2%, 296) of the participants had a monthly family income of less than RMB3,000 (£350). Most (98.2%, 673) had resided in their respective villages and towns over 5 years and were living with family (98.0%, 671).

Table 20. Demographic data on Yunnan Province residents living in proximity to bats, n = 685.

	No.	(%)
Sex		
Female	402	58.7%
Male	283	41.3%
Age (in years)		
Under 35	127	18.5%
Over 35	558	81.5%
Education		
None	220	32.1%
Primary	279	40.7%
Secondary	184	26.9%
Post-Secondary	2	0.3%
Income		
<3000 RMB	296	43.2%
3000-10,000 RMB	250	36.5%
>10,000 RMB	139	20.3%
Length of Time in Current Home		
1 month	2	0.3%
1-5 yrs	10	1.5%
Over 5 yrs	673	98.2%
Other Sociodemographic Data		
Lives with family	671	98.0%
Children < 5 yrs old in household	199	29.1%
No Travel (in past 12 months)	405	59.1%

Only 29.1% (199/685) of participants reported having children under the age of 5 in their households and most (59.1%, 405/685) had not travelled outside their villages and towns in the past year. All reported multiple activities to earn their livelihoods, but most (99.1%, 679/685) reported having worked as farmers. Few participants 5.3% (36/685) also worked as migrant labourers and 1.8% (12/685) on construction. Five respondents also earned money as wildlife farmers or worked with buying or selling wildlife. Two were community healers or barefoot doctors (Zhang & Unschuld 2008).

In the 12-months prior to the survey, most respondents reported exposure to swine, poultry, rodents, dogs, cats, and birds (Table 21). Only 21.2% (145/685) reported any exposure to bats and 8.5% (58/685) to non-human primates. Respondents reported on general and intimate (repeated, prolonged) exposures to animals (Table 22) with most reporting raising live animals (92.3%, 632/685), having animals in their houses (90.1%, 617/685), preparing slaughtered animals (77.4%, 530/685), and handling live animals (63.9%, 438/685).

Table 21 Animal exposures in the past 12 months among 685 Yunnan residents. Residents were observed living in close proximity to bats and other wildlife.

Taxa	No.	%
Swine	582	85.0
Poultry	562	82.0
Rodents/Shrews	524	76.5
Dogs	454	66.3
Birds	448	65.4
Cats	350	51.1
Bats	145	21.2
Cattle/Buffalo	135	19.7
Carnivores	131	19.1
Goats/Sheep	111	16.2
Ungulates	69	10.1
Non-human primates	58	8.5

Table 22. Reported general and intimate exposures to animals. 685 Yunnan residents were surveyed for their general and intimate exposures to animals over the past 12 months.

	No.	%
General Exposures		
Raise live animals	632	92.3
Animals come inside dwelling	617	90.1
Handle live animals	438	63.9
Live with pet	384	56.1
Purchase live animals at market	175	25.5
Share water source with animals	95	13.9
Seen faeces in or near food	56	8.2
Eaten food damaged by animal	43	6.3
Intimate Exposures		
Cook or handle recently slaughtered animals	530	77.4
Slaughter animals	368	53.7
Eat raw, undercooked meat, organs, or blood	69	10.1
Scratched or bitten by animal	39	5.7
Hunt or trap animals	34	5.0
Collect dead animals to eat, share, or sell	14	2.0
Eat known sick animal	7	1.0

Different human types of human and animal contact were evaluated (Table 23) with the most frequently reported interactions being raising, handling, and slaughtering poultry, swine, and dogs or having dogs, cats, and birds as pets. Rodents, cats, dogs, birds, and bats were often reported in houses. Hunting and being bitten were among the least frequently reported human and animal interactions.

Table 23. Type of human-animal contact by taxa for respondents surveyed. Values with more than 40% of respondents (274) reporting contact with specific taxa are shaded in grey, n = 685.

	Pets	Handled	Raised	In House	Cooked/ handled	Eaten raw/ undercooked	Eaten Sick	Found dead collected	Scratched/ Bitten	Slaughtered	Hunted/ Trapped
Rodents/Shrews	0	21	1	505	31	2	0	1	0	18	10
Bats	0	5	0	125	8	0	0	0	13	5	11
NH-Primates	4	19	2	5	4	0	0	9	0	22	5
Birds	108	14	2	296	31	3	29	0	0	17	30
Carnivores	2	7	1	39	12	5	0	0	4	72	26
Ungulates	0	1	4	17	2	0	0	10	10	32	5
Poultry	2	297	466	88	369	4	5	53	5	306	6
Goats/Sheep	0	8	25	2	2	30	9	1	57	6	0
Swine		189	421	38	272	151	1	69	1	128	1
Cattle/Buffalo	1	7	62	10	19	16	49	1	0	7	1
Dogs	278	23	174	183	17	68	0	0	14	10	3
Cats	133	4	93	213	50	0	0	0	6	8	0

Severe acute respiratory infection (SARI) symptoms were reported by 61 (8.9%) respondents, influenza-like illnesses (ILI) symptoms were reported by 151 (22.0%) respondents; and encephalitis symptoms were reported by 77 (11.2%) respondents. When asked about the occurrence of the same types of symptoms only in the past 12-months, respondents also reported more incidences of ILI symptoms than they did SARI or encephalitis symptoms (Table 24). Additionally, 42.0% (63/148) of respondents who reported any of these symptoms in the past 12 months also reported the same symptoms in their family members (Table 24 and Table 25).

Table 24. Severe acute respiratory infection (SARI), influenza like illness (ILI), and encephalitis symptoms. Self reported symptoms reported by respondents for themselves, family members, and for both from over the past 12-month period. Percentages are of all respondents, n = 865.

Symptoms	Past 12 months	%	In Family Member	%	In Both	
SARI	37	5.4%	22	3.2%	16	2.3%
ILI	66	9.6%	92	13.4%	31	4.5%
Encephalitis	45	6.6%	26	3.8%	16	2.3%

Chi-squared tests of independence conducted to examine the relationships between (a) self-reporting symptoms of severe acute respiratory infection (SARI), influenza-like illness (ILI), and encephalitis compared with (b) respondents' responses to survey categories (Table 25) indicated that there were significant correlations between 8 survey categories and SARI symptoms; 11 survey categories and ILI symptoms; and 14 survey categories and encephalitis symptoms. Of the respondents who reported recent (past 12-months) SARI, ILI, and encephalitis symptoms, at least 60% in each category reported raising animals, having pets and wild animals in their houses, and handling or cooking slaughtered animals. There was no significant correlation between age and symptoms in any category.

Respondents were asked to say what they thought may have been the source of their SARI, ILI, or encephalitis symptoms. None reported any kind of animal exposure as a potential source of infection; 8.1% (12/450) thought bad food or water may have caused the symptoms; and 2.0% (3/148) each reported contact with sick people and spirits as possible sources. Most (87.2%, 129/148) said they had no idea what may have caused their symptoms.

Few respondents (25.5%, 175/685) reported purchasing animals from a live animal market in the past year. Almost half (46.9%, 321/685) of respondents were worried about disease or disease outbreaks in animals at live animal markets and about the same number (47.6%, 326/685) of people believed that animals can spread disease. When asked about changes in their behaviour at live animal markets in the last 12 months (Table 26), participants reported washing their hands (33.1%, 58/176) and buying live animals less often (33.1%, 58/176), only buying farmed wildlife (44.6%, 78/176), and sometimes buying meat at the supermarket (23.4%). Only 25.1% (44/176) stated that they no longer shopped at live animal markets. For the 39 participants who reported slaughtering animals and also having been scratched or bitten in the past year, when asked about medical measures taken post exposure, 38 (97.4%, 38/39) respondents said they had visited a clinic.

Table 25. Correlations of diseases of unknown aetiology and behaviour. Chi-squared tests of independence were performed to examine the relationships between (a) self-reporting symptoms of severe acute respiratory infection (SARI), influenza-like illness (ILI), and encephalitis compared with (b) respondents' responses to survey categories including demographic details, exposure to animals and types of exposure, and activities (n = 685). Significant relationships are shown in this table with *p*-values less than 0.05 and shaded in grey. Survey categories compared with significant illness symptoms with *p*-values greater than 0.05 for all three types of symptoms are not shown. An alpha level of .05 was used for all tests.

	SARI					ILI					Encephalitis				
	Positive		Negative		*p*-value	Positive		Negative		*p*-value	Positive		Negative		*p*-value
	37		648			66		619			45		640		
Demographics	No.	(%)	No.	(%)		No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Household member with same syndrome	7	18.9	15	2.3	<0.001	27	40.9	65	10.5	<0.001	10	22.2	16	2.5	<0.001
Income <RMB3000	22	59.5	271	41.8	0.039	29	43.9	267	43.1	0.890	21	46.7	275	43.0	0.622
Animal Exposures															
Faeces in or near food	7	18.9	49	7.6	0.014	6	9.1	50	8.1	0.772	8	17.8	48	7.5	0.015
Shared water source	8	21.6	87	13.4	0.159	8	12.1	87	14.1	0.669	12	26.7	83	13.0	0.010
Pets	24	64.9	360	55.6	0.262	47	71.2	337	54.4	0.009	27	60.0	357	55.8	0.574
Consume raw or undercooked	5	13.5	64	9.9	0.473	8	12.1	61	9.9	0.558	9	20.0	60	9.4	0.022
Buy animals at animal market	9	24.3	166	25.6	0.865	12	18.2	163	26.3	0.151	3	6.7	172	26.9	0.002
Hunt	2	5.4	32	4.9	0.897	5	7.6	29	4.7	0.302	6	13.3	28	4.4	0.007
Taxa															
Bats	14	37.8	131	20.2	0.011	21	31.8	124	20.0	0.025	22	48.9	123	19.2	<0.001
Poultry	36	97.3	526	81.2	0.012	63	95.5	499	80.6	0.003	44	97.8	518	80.9	0.004
Rodents and shrews	33	89.2	491	75.8	0.059	61	92.4	463	74.8	0.001	42	93.3	482	75.3	0.006
Carnivores	3	8.1	128	19.8	0.08	9	13.6	122	19.7	0.235	2	4.4	129	20.2	0.010
Dogs	23	62.2	431	66.5	0.595	53	80.3	401	64.8	0.011	28	62.2	426	66.6	0.562
Activity															
Handled live animals	25	67.6	302	46.6	0.013	40	60.6	287	46.4	0.027	31	68.9	296	46.3	0.003
Raised live animals	33	89.2	456	70.4	0.013	57	86.4	432	69.8	0.004	43	95.6	446	69.7	<0.001
Animals in house	34	91.9	501	77.3	0.036	58	87.9	477	77.1	0.041	43	95.6	492	76.9	0.003
Cooked and handled slaughtered animals	27	73.0	404	62.3	0.189	52	78.8	379	61.2	0.005	31	68.9	400	62.5	0.384
Found dead and collected animals	4	10.8	106	16.4	0.373	5	7.6	105	17.0	0.049	1	2.2	109	17.0	0.009

Table 26. Behavioural changes. Percentages of respondents who purchased animals at markets that sell live domestic and wild animals and their behavioural changes leading to increased biosecurity, n = 175.

Behaviour	No.	%
Wash hands	58	33.1%
Buy live animals less often	58	33.1%
Buy only farmed wildlife	78	44.6%
Sometimes shop for meat at supermarket	41	23.4%
Wear gloves	5	2.9%
Wear a mask	4	2.3%
No longer go to wild animal markets	44	25.1%

5.11 Discussion

This research highlights the application of qualitative ethnographic and quantitative approaches to identifying risks of zoonotic disease transmission from wildlife to humans in rural southern China. Observational surveys were an efficient method to identify field sites, aided in development of both the ethnographic and behavioural survey questionnaires, and provided valuable secondary resources for data analysis.

5.11.1 Observational Discussion

Other than bamboo rats (*Rhizomys* sp.), nutria (*Myocastor coypus*), porcupines (*Hystrix brachyura*), hog badgers (*Arctonyx collaris*) and civet cats (*Paguma larvata*), wildlife was not found in live animal markets at any sites observed in either Yunnan or Guangxi. Even these species were infrequently observed and none since from February 2015 to present. Prior research visits from 2007 to 2013 to the same or similar communities noted in addition to the aforementioned species diverse rodents including squirrels, deer, several species of bats, and wild boar in the animal markets. When questioned, residents in Yunnan and Guangxi attributed the change to enforcement of the new wildlife protection laws and scarcity of wildlife in the surrounding area.

In July of 2015, researchers in this study were informed of the sale of 24 unidentified bats in a wild and domestic animal market in the Cultural Square of Yunfu City in Luoding County in western Guangdong Province (Lat. 22.768611, Long. 111.570000), about 50km from the border of Guangxi Province. When researchers from this study conducted surveillance of Luoding County markets from August to October of 2015, neither bats nor other wildlife were reported in markets. This anecdote serves to support the observations, informal interviews, and ethnographic survey reports in this study that, since 2003 and the emergence of the SARS pandemic, wildlife in rural Chinese markets has become less frequently displayed or non-existent.

Upon scoping visits, observation surveys in markets by non-local Chinese field team members were met with apparent suspicion and photography was generally not permitted by market vendors and personnel. Both Taiping and Foshan live animal wildlife markets have repeatedly been closed down since 2007 (Hu & Chen 2007; Xie 2012; Tan 2014; GDFN 2016), yet as per this study's observational surveys both still remain open and doing business. One of the Taiping Market vendors stated:

> *Local politicians and police chiefs like to come with reporters and TV crews to shut down the market. It stays closed for 2 or 3 days and then reopens. None of them care about it beyond their own publicity. This is why we do not like you taking notes and pictures.*
>
> – Market worker at Taiping Market, Conghua, Guangdong (21 September 2014)

Other vendors and local area restaurant workers corroborated the market closures. Generally, the Taiping market vendors were the most suspicious and unwilling to speak with strangers. This may have been due to the Taiping market being renowned as the largest legal and illegal wildlife market in China. Only a section of Foshan market was allocated to the sale of wild animals and was set back from the main area. Some venders in the Foshan market said they had been closed for a while after SARS, but none commented on any recent closures.

Observational surveys in Taiping and Foshan markets in Guangdong province were opportunistic in that they were not conducted over 24-hour periods nor over a series of days. A fine scale observation of either market would illustrate market dynamics in ways that this study was not designed to encompass, but that would build upon the research detailed here and greatly expand current knowledge of wildlife trade and market dynamics.

The animal counts in the two markets observed in this study do support an overall decrease in animals from 2014 to 2015 and a subsequent increase after 2015. Reports of a 2015 China-wide government enforcement crackdown primarily targeting ivory and international trade seems to have had some short-lived local effects in several Chinese provinces resulting in temporary closures of animal markets (CWCA 2015; TRAFFIC 2015). It may be that several market closures in 2015 reduced the volume of animals that vendors were willing to risk keeping in stock that year or more simply that fewer vendors had fewer animals on display. The animal count numbers in 2016 were higher with an 84% increase in animals over the observation period in 2016 at Taiping market. It would be informative to evaluate whether the actual volume of animals sold (in other words not the wildlife on display) in 2015 was subsequently diminished, remained the same, or increased to match the trend in counts reported here from 2016. The observational surveys reported here of these two markets further support the hypothesis that current regulation efforts do little to reduce the overall supply and demand of wildlife in these southern China markets.

One convincing theory is that the centre for illegal wildlife shifts repeatedly in response to each raid or market shutdown (Zhang & Jiang 2010). Once a market is raided and closed temporarily, the supply is shunted to another market, so the bulk of the illegal wildlife shift from the market in Guangzhou to Foshan and then to Taiping. Legislation and enforcement may have resulted in the supply of illegal wildlife moving underground, but the demand, as per the numbers of animals counted in this study, appears to increase.

5.11.2 Ethnographic Discussion

By using qualitative methods, this research illustrates both changes in drivers of wildlife trade and high-risk activities of rural populations in contact with wildlife. The sample size (n =87) was small and the results not generalisable due to purposive rather than random sampling, but the data here do provide a completely novel report of attitudes and behaviour around wildlife consumption and trade in the southern China region. Additionally, these qualitative data provided a framework for the development of the quantitative questionnaire in this study. By coding and examining these 87 ethnographic surveys, an awareness of the types of questions, the vocabulary necessary, and how to ask them was generated so as to best generate quantitative data on human exposure to wildlife and high-risk behaviour.

5.11.2.1 Participants’ Reporting of Wildlife Consumption

Older male respondents were the primary consumers of wildlife, although younger and other demographic strata of respondents such as gender, education, or income were not evenly represented in this survey. Although (49.3%) of respondents did report having consumed or hunted wildlife either recently or in the past and 59.8% stated that they were aware of the legal protection of wildlife, it is possible that some were not comfortable speaking openly to strangers and the consumption rate may be higher. All of those stating they consumed wildlife primarily consumed frogs, snakes, and birds, which they or their family members trapped. Since they had Forestry Department issued licenses to raise wild animals, the five wildlife farmers all openly admitted eating bamboo rats and other farmed species. Of the individuals who did not identify as farmers (nature reserve employees, CDC employees, hotel employees, chefs, wildlife researcher, and doctor), most either stated they did not consume wildlife although some admitted to having tried wild species in the past or when they were young. Other wildlife such as pangolin, civets, bears, eagles, and monkeys were not only considered rare, but also too expensive for respondents to consume. Several of the Nonggang Nature Reserve employees stated that area residents who hunt and trap animals have ‘a hard life’ and catch little wildlife. Local stories of those who were incarcerated and fined for violating the laws protecting wildlife may have had an impact on reducing hunting by local residents in these communities. Scarcity of wildlife, enforcement of wildlife protection laws (PRC 2016b), strict gun regulations, and required hunting licenses (PRC 1996) may make it more economical for rural residents to slaughter their own poultry and pigs rather than hunt pheasant and wild boar.

Five respondents identified themselves as ethnically Han Chinese and one as Muslim. All six said they never had hunted or tasted wildlife, but that the indigenous ethnic minorities did hunt and consume wild animals. From this survey, some divides arise, although they are not testable. There was an apparent dichotomy between those who did and did not identify as local, ethnic minorities. The local people openly acknowledged consumption of wildlife in the past as well as in recent times, although usually frogs, snakes, and birds. The individuals identifying as Han, Muslim, and with university level or above educations all stated they would not consume wildlife although some admitted to having done so in the past or tried it at a restaurant at a meal hosted by someone else. Respondents report that wildlife is generally expensive to buy both directly and in restaurants. Those who might afford purchasing wildlife such as the educated and relatively wealthier individuals surveyed in these communities claim to eschew consumption of wildlife and are aware of the legislation prohibiting it, risks (SARS and avian influenza at least), and conservation implications (species extinction and habitat loss). This is in contrast to studies of the wealthier, younger urban males in Guangdong, Shenzhen, and other Tier 1 and Tier 2 Chinese cities who have recently been reported to be the primary consumers of wild animals and products in China (Zhang *et al.* 2008; Zhang & Yin 2014). These survey results emphasise that there are different types of consumers, attitudes, and motivating factors in and around the wildlife trade in China.

5.11.2.2 High-risk interactions with animals

These ethnographic data reported here provide some evidence of high-risk interactions through which zoonotic disease spillover could occur in rural communities in the three provinces surveyed. All participants who discussed rabies stated that they were aware that it could be contracted from dogs and cats, although one erroneously mentioned snakes as well. Many had some knowledge about bats and birds as the sources of SARS and avian influenza respectively, but contrastingly all were unconcerned about presence of rodents, bats, or birds or even the excrement of these same animals within their houses. This suggests that the older (average of 48 years) population surveyed did not perceive wildlife with which they had frequent, daily contact as being a potential source of any type of zoonotic emergence. As discussed above, the more educated, urban, and wealthier respondents in this survey all stated that they have no or little contact with wild animals. Some reported consuming wild boar, bamboo rats, and other species. The Nature Reserve employees, wildlife researcher, Yunnan CDC employees, and wildlife farmers all had occupational contact and exposure to wild animals and most were aware of or had been trained in necessary safe handing techniques and precautions.

Direct contact with rodents was reported by most participants with exposures including bites, scratches, capture, slaughtering, butchering, and farming. Respondents also reported indirect contact with rodents such as faeces in or around food. Indirect exposures to animals have been implicated in zoonotic disease outbreaks including Mojiang virus, Hantavirus, and Nipah virus (Chua 2003; Zhang *et al.* 2009c; Wu *et al.* 2014b). Respondents did not express concern about raw food contaminated with rodent faeces nor any awareness that this could be an avenue of zoonotic spillover.

Few respondents (14.9%) reported being bitten by bats and only when actively hunting or trapping them and even then, at some unspecified time in the past. None went to clinics after this exposure. Most reported being aware of bats in caves nearby. All individuals surveyed for this study do live in regions where multiple bat species feed and roost. Ebola, SL-CoVs, and other viruses of importance to human health have been identified in bats from the provinces of the respondents of this study. (Lau *et al.* 2010b; Yuan *et al.* 2012; Ge *et al.* 2013).

5.11.2.3 Self-reported illnesses of unknown aetiology

Unexpectedly few participants (12.6%) reported illnesses of unknown causes although potentially from zoonotic diseases. Illnesses were generally described by respondents as transitory fevers and colds and medical care was not sought unless the illness were debilitating or chronic. Given the impact that SARS had in China, there are surprisingly few serological or molecular assays of humans for SARS-CoVs and SL-CoVs after 2003 (CDC 2003). Those that have been conducted mostly focused on Middle East respiratory syndrome coronavirus in the Middle East and Africa (MERS-CoV) (Zaki *et al.* 2012; Liljander *et al.* 2016). It may well be that the illnesses reported as fevers and colds by the respondents in this study were not all caused by known viruses. Additionally, vaccination and veterinary care were described as unnecessary and expensive by respondents, which could increase risk of exposure to domestic animal borne or amplified diseases such as leptospirosis, hepatitis, brucellosis, and others (Zheng *et al.* 2006; Zhang *et al.* 2010; Bao *et al.* 2011).

5.11.2.4 *Participants' perceptions of a decline in local wildlife*

Given that most participants (54.0%) were over the age of 55 and reported to be local residents for most of their lives, they would have had opportunity to witness changes in their surroundings. Most (77.0%) did report witnessing a decrease in wildlife over time. Respondents stated that local government had invested in roads and local infrastructure with the intention of increasing tourism. According to participants, this reduced forested area. Hunting and selling of wildlife was also not mentioned by any participant as a cause of observed local wildlife depletion.

Some (30%) remarked that wildlife was no longer available in local live animal markets. Fewer (12%) mentioned wild animals still being vended along roadsides or to visiting tourists, political leaders, and businessmen. Published reports from the past 10 years (Zhang *et al.* 2008; Zhang & Yin 2014) and corroborating observations made by this study's researchers over the same period also have marked a reduction in markets selling wild animals. Although not tested in this study, perhaps national public awareness campaigns by nongovernmental organisations such as TRAFFIC China, IFAW, AITA, and others have contributed to an awareness of wildlife conservation in these communities (Liou 2007; Li 2013a; AITA 2015).

5.11.2.5 *Farming wild animals*

Despite recently amendments expanding and strengthening China's legislation protecting wildlife (PRC 2016b) and increased enforcement of these laws (TRAFFIC 2015), wildlife trade is still an avenue for profit due to the sustained and increasing demand for legally and illegally sourced wild animals from Chinese cities (Zhang & Yin 2014). One strategy for rural residents to capitalise on this urban demand and avoid penalties is to farm wildlife. Over the past 20 years, wildlife farms have expanded across southern China in response to active encouragement by local and national government policies (PRC 1988; Ma 1992) and to growing demand for wildlife from cities (Zhang & Yin 2014). Wildlife farmers in this study reported raising bamboo rats (*Rhizomys sinensis*), nutria (*Myocastor coypus*), porcupines (*Hystrix brachyura*), civets (*Paguma larvata*), peacocks (*Pavo cristatus*), pheasants (*Phasianus colchicus*), and other animals.

The sale of Forestry Department licensed farmed wildlife is legal anywhere in China (PRC 2016b), but accurately distinguishing it from wild caught wildlife is not easy. As required by law (PRC 1988, 2016b), licences are posted clearly in wild animal farms and in markets vending these species (personal observations). Recent investigations by Forestry Departments Officials in the Foshan Market and surrounding area reported that all vendors had current and valid licenses (GMAFFB 2017). Yet without expensive and time consuming procedures including genetic testing, how can anyone, official, vendor, middleman, farmer, or buyer, know whether the animals are actually farm-raised or from the wild?

Some of the respondents in this study (12%) stated that wild caught animals are larger and tastier and others stated the opposite (8%). There is no clear consensus on differences in size or flavour of wild animals versus farmed wild animals even among the wildlife farmers interviewed. All five wildlife farmers openly stated that their founding stock were from the wild and that they would from time to time replenish their stock with wild species. Until recently, this was explicitly supported for licensed wildlife farmers by the Chinese Wildlife Law (PRC 1988). When questioned further about where and from whom they sourced their wild species, the farmers became vague either due to unwillingness to provide these details and risk any fines (for harvesting wild animals) or possibly because they did not know. It may be that their wild animals were purchased from other farmers, provided by local residents, or bought from middlemen all of whom could be motivated to present a farmed animal as wild or vice versa depending upon the situation. Empirically, there is variation in both farmed and wild-sourced wildlife and it would not be possible to distinguish by sight or flavour without some sort of inbreeding traits resulting from domestication as reported in *Canis familiaris* and even in *Vulpes vulpes* elsewhere (Trut *et al.* 2009).

The inability to accurately distinguish the origin of animals in the wildlife trade is one of the arguments made by conservation organisations for stopping all wildlife farming and trade (Downes 2015), but also economic and other assessments raise serious concerns as to whether farming has any net conservation benefits (Abebe 2003; Bulte & Damania 2005; Tensen 2016). Some of these analyses state that the only way effective conservation would benefit would be through highly regulated wildlife farming including (a) caps on numbers of farmers, (b) pricing controls, and (c) highly trained officials to monitor the system (Bulte & Damania 2005). Due to economic incentives, the farming of wild animals in China is unlikely to stop any time soon, so through education addressing the demand side of the supply-demand equation may be a more effective solution to unite both conservation and public health goals.

5.11.2.6 Middlemen transport wildlife to markets

This study does not determine, nor are there published data on, whether middlemen have always operated and to what extent in wildlife trade in China. All wildlife farmers in this study reported vending their animals to middlemen and none said they sell directly to markets. Middlemen were reported by respondents to frequent villages and rural areas to both hunt and buy live wild animals. Some research shows that in other countries middlemen may be locals who are familiar with the social networks and enforcement efforts so as to generally avoid being apprehended (Broad *et al.* 2003; Nijman 2010; McNamara *et al.* 2016). Whether local or from urban centres or as is likely a mixture of both, middlemen in China act as “fences” (Wyatt 2013) or agents who transport illegal products to legal markets. Respondents stated that middlemen conveyed wild animals from both wildlife farms and the wild to consumers in markets, restaurants, and hotels. Depending upon the source of the wild animal and their customers’ preferences, these “fences” also provide either a legal (licensed, farmed wild animals) or illegal provenance for the wild animals they bring to consumers. This may well be one of the reasons why no data exist on numbers of middlemen operating and their activities are largely unknown. Some research suggests that illegal wildlife trade functions precisely because of operators such as “fences” and corrupt officials (Broad *et al.* 2003; Wyatt 2013; Bennett 2015). This may explain how illegal wildlife trade has persisted in China despite legislation and enforcement efforts. From the perspective of public health, it is critical to know more about the operations of the middlemen, since they are key points in the wildlife trade networks where wild, farmed wild, and domestic animals of different species, perhaps in volume, are quartered in close contact and therefore potentially providing the possibility for zoonotic spillover to occur. From personal observations in the field, rural middlemen pile open cages of multiple species onto their vehicles, which permits spillover of pathogens from species to species as they sometimes come into direct and indirect contact with each other.

This study was designed based around the hypotheses that viruses spillover from wild animal reservoirs into rural human populations where population may be relatively lower, but exposure is greatest. The World Health Organization principal case definitions (WHO 2014) for severe acute respiratory infection (SARI), influenza-like illnesses (ILI), and encephalitis symptoms were used as evidence of potential viral infections of zoonotic origins (Miller & Hagan 2017). More than half (54.5%, 373/685) of the rural populations surveyed in this study reported illnesses of unknown aetiology at some time in the past and only 5.54% (21/373) reported going to clinic or doctor when experiencing these or other symptoms. Correlating these illness symptoms with behaviour and exposures provided strong evidence that as expected the highest risk demographic was the rural poor family units. Also, as hypothesised, highest-risk behaviours were those involving wild and domestic animals particularly rodents, bats, and poultry. Those with pets and who hunted or bought live animals at animal markets were at highest risk of these symptoms as well.

As discussed in Chapter 3, some bat species can migrate across distances of 1,000km or more (Altringham 2011), so Coronaviruses circulating in bat reservoirs distributed throughout this region would have opportunity to potentially spillover to humans and other animals given the right conditions. The question remains as to what exactly are these right conditions? How are viruses spilling over from bat reservoirs and into humans? This study was not focused on serological or molecular assays for SL-CoVs, PMVs, and other viruses in the humans surveyed, but that would be a crucial next step and important future work. The high contact rates among humans, wild animals, and domestic animals recorded in this study call for surveys of SL-CoVs and other viruses in human populations in these rural regions of China where humans, domestic animals, and wild animals interface. It is highly likely that humans are being repeatedly infected through activities identified in this study or as yet unidentified involving wildlife or domestic animals.

If antibodies to SL-CoV or other viruses of zoonotic origin were discovered within the human population surveyed in this study, this would build upon the work described here as well as provide the first evidence since 2006 that SL-CoV or other viral spillover is regularly occurring from animal reservoirs to humans exposed to wildlife in China (Woo *et al.* 2005; Wang *et al.* 2006). Identifying the specific high-risk activities resulting in SL-CoV spillover is key to preventing zoonotic emergence and re-emergence.

5.11.3 Summary

This study brings together novel data, both in quantity and geographic breadth, on rural populations in southern China that regularly come in contact with wildlife and therefore are at risk of zoonotic spillover. Additionally, surveys of two large centres of vending wildlife in China have provided some details on the diversity and consistency of wildlife traded in these venues. The findings here are summarised as follows:

- 21 diverse mammalian wildlife species including two listed by IUCN as vulnerable and one on China's endangered species list were observed to be maintained in circulation over three years in two of the largest live animal wildlife markets in south China. An overall increase in the volume of wildlife traded in the two markets was also observed.

- Exposure to wildlife is frequent in the rural areas surveyed, but primarily through environmental exposure and not activities such as hunting and consumption.
- Rural populations of older farmers were not consistent on how to distinguish between wild-sourced and farmed wild animals. These same populations seemed unaware of any activities that may place them, their families, or their domestic animals at risk of zoonotic spillover.
- Rural demand for wildlife is low and wildlife in rural markets was not found in this study. Wildlife including bats and rodents were not observed nor reported in rural markets.
- Farmed and wild-caught wildlife from rural areas are reported to be traded primarily via middlemen and brought to large, urban live animal markets.
- Exposure to bats, poultry, and rats as well as handling, hunting, and raising animals were all positively correlated with symptoms of viral infections of unknown aetiology and potentially of zoonotic pathogens.

Rural farms as well as wildlife farms, trading routes, and large urban live animal wildlife markets bring diverse wildlife species, humans, and domestic animals together in novel ways and frequencies that would not occur in their native habitats. These associations present multiple opportunities for viral spillover and zoonotic emergence. From observations, ethnographic surveys, and quantitative surveys of human behaviour, this study provides clues as to how to prevent zoonotic emergence in rural southern China. By building upon the local population's awareness of the importance of conservation, knowledge of the health risks of zoonotic emergence may be distributed by forestry and public departments, but also by NGOs in the same ways that wildlife conservation campaigns have been conducted. The data reported here calls for future longitudinal surveillance for evidence of human infections of SL-CoVs and other viruses, so that mapping of areas of highest-risk of spillover as well as confirming high-risk behaviours may lead to effective ways to mitigate or prevent future zoonotic epidemics.

6 Discussion and Future Directions

6.1 Concluding Remarks

With its rapid shift from an agricultural to urbanised and industrial society, China has experienced within the span of a few decades what many western countries experienced over the course of a century or more (Yang 2013). This rate of change has brought with it increased health risks particularly from emerging infectious diseases of zoonotic origin (Liu *et al.* 2014). With almost one-fifth of the world's population, epidemics affecting China can swiftly have global consequences (UN 2015). The pandemic spread of SARS-CoV and highly pathogenic avian influenza A (H5N1) provided important lessons in how China's public health issues became matters for global public health. Research on zoonotic disease emergence in China may greatly inform the country's ability to respond to and prevent future pandemics. The identification of the wildlife trade as a key driver of zoonotic emergence in China highlights this pathway as a primary focus for disease surveillance in China (Bell *et al.* 2004; Fèvre *et al.* 2006). The research reported in this thesis on the ecology of host species and their pathogens, human behaviour driving the wildlife trade, and detailed analyses at the interface between wildlife, domestic animals, and humans provides data that may help in predicting and preventing zoonotic emergence in the region and globally.

6.2 Risk of Emergence of Novel Paramyxoviruses and Coronaviruses in Chinese Bats

A number of other researchers have identified novel Paramyxoviruses in bats (Amman *et al.* 2015), rodents (Brooks *et al.* 2014), and other species (Lau *et al.* 2013) and assessed the pathogenic potential of these new viruses (Zeltina *et al.* 2016). The research in this thesis provides the first evidence of a high diversity of Paramyxoviruses circulating in migratory bat populations that are in close contact with humans across a wide geographic range of China. Although the 82 potentially novel sequences identified here are not similar to known pathogenic Paramyxoviruses, they do form two clades (or possibly a super-clade) that is paraphyletic with the *Henipavirus* genus containing viruses known to be lethal such as Nipah virus and Hendra virus (Chua *et al.* 1999; Field *et al.* 2001). This raises the questions of whether the molecular mechanisms of host infection among the novel viral species identified in this study are similar to those in the *Henipavirus* genus, if they are able to infect humans, or if viral evolution would be required in market, farm, or other settings to result in pathogenic viruses? Recent analyses have indicated that zoonotic Paramyxoviruses are likely to be of bat origin (Drexler *et al.* 2012). The large diversity of Paramyxoviruses in Chinese bats as detailed in this thesis supports this hypothesis by further demonstrating that the order Chiroptera (bats) has an unusually diverse array of Paramyxoviruses, i.e. an evolutionary radiation that suggests prolonged coevolution over a significant time period (Drexler *et al.* 2012; Vidgen *et al.* 2015).

The Coronaviruses reported here may not be novel strains, but provide evidence that diverse viruses are circulating in bat populations in China. Several strains are closely associated with Coronaviruses known to be similar to Civet or Human SARS-like Coronaviruses (Ge *et al.* 2013; Hu *et al.* 2015). The results reported here support the hypotheses that bat Coronaviruses may still have the genetic potential to spill over into humans, if the right circumstances were to coincide, as probably occured in the SARS-CoV outbreak when bats, civets, and humans came into repeated contact with each other in live animal markets.

The migratory nature of some of the bat species identified in this study as reservoirs of Coronaviruses and Paramyxoviruses likely adds to their potential to cause outbreaks. Very little has been published about their migratory patterns, but based upon species ranges and personal observations of absence/presence, it appears that most bat species sampled for this study (See Table 12) migrate from southern China to Myanmar, Vietnam, Lao, Thailand, and Cambodia (Nowak 1994; Zhang *et al.* 2009a; Smith *et al.* 2010a). Only three species sampled for this study (*Miniopterus schreibersii*, *Hypsugo alaschanicus*, and *Vespertilio sinensis*) were found exclusively in northern China and have home ranges in northern Asia, Russia, or parts of Europe (Smith *et al.* 2010a). In China and these neighbouring countries hunting and consumption of bats and other wildlife occurs (Mickleburgh *et al.* 2009; Ripple *et al.* 2016), leading to a geographically extended interface among humans and wildlife. Previous studies have demonstrated that anthropogenic change and ecosystem disruption are linked to disease emergence in specific cases (Patz & Olson 2006; McKenzie & Townsend 2007) and in general (Myers & Patz 2009). Anthropogenic changes to land use and natural ecosystems likely influence bat migratory feeding and roosting patterns and may lead to increased risk of disease emergence. Localised climate changes resulting from urban heat islands, deforestation and reforestation, drought, flooding, pollution, and other driverss may also have effects on the duration that bats inhabit a region as well as the availability of food resources. Even rural agricultural pesticide reduction associated with the rise in genetically modified crops (Huang *et al.* 2015; Zhang *et al.* 2016) would be expected to result in the increase in bat food sources such as insects and could alter the human-wildlife interface.

Longitudinal studies of Coronaviruses and Paramyxoviruses with their host species Chinese bats would enhance our understanding of disease risk. The region surveyed in this thesis is vast and contains a high diversity of host species. Surveillance, monitoring, and laboratory assays across this area would be costly and logistically challenging. Following the suggestions from other studies (Morse *et al.* 2012; Levinson *et al.* 2013), the research here has incorporated a more efficient approach by targeting surveillance in regions where humans, bats, and other animals were known to interact. The surveys from this study could be supplemented by continuous or longitudinal sampling efforts in selected communities with evidence of exposure to bats. It would also be informative to investigate whether rodent Paramyxoviruses, which were not identified in the animals sampled in this study, might be identified in these regions using a similar approach. This would test whether there are specific regions, times, and activities with a higher risk for viral spillover as well as provide further insight into how viral pathogens circulate and evolve within animal populations.

As Coronaviruses and Paramyxoviruses of bat, rodent, or other animal origin have been implicated in human diseases in China (Coleman & Frieman 2014; Wu *et al.* 2014b; Yang *et al.* 2014b) and elsewhere (Baker *et al.* 2013; Albariño *et al.* 2014; Ching *et al.* 2015), the findings in this study are a step towards evaluating the risks of zoonotic emergence not only in China, but globally.

6.3 Changing the attitudes of Chinese millennials around wildlife trade and consumption

Although prior studies have examined human attitudes toward conservation in China (Zhang *et al.* 2008; Zhang & Yin 2014; Liu *et al.* 2016b), none have linked conservation with public health from the perspective of emerging zoonotic diseases in the region. The importance of this perspective has been proposed previously as a necessary approach in both predicting and preventing zoonotic spillover (Daszak *et al.* 2000; Daszak *et al.* 2004; Daszak *et al.* 2007). The main driver of wildlife trade in China has been identified as the newly wealthy, urban, and growing middle class (Zhang *et al.* 2008; Zhang & Yin 2014) also known as Chinese millennials (Moore 2005; Fish 2015). There is every expectation that the population in urban centres and millennials in particular will expand dramatically in China in the next three decades (Peng 2011). Unless millennials' attitudes can change, their demand for wildlife may also increase (Lau *et al.* 2010a; Wu *et al.* 2017).

The online survey reported in this study is the first attempt to examine the attitudes and awareness of Chinese millennials to wildlife as an origin of diseases and the consequent implications to human health. The data presented here show that millennials have an awareness of the effects wildlife trade may have upon habitat loss or species extinction, but they less aware of any risks of zoonotic emergence driven by wildlife trade. This suggests that employing the same approach used to promote a conservation message may also be highly effective at conveying information about the health risks of wildlife trade and consumption to Chinese millennials. Today, campaigns launched via social media should reach a wider audience as not only millennials, but more Chinese are networked via popular social media applications. Although social networking is a relatively novel phenomenon and there are few studies evaluating its efficacy in affecting behaviour, there is agreement that social networking sites can increase awareness and positively motivate behaviour around health (Maher *et al.* 2014; Laranjo *et al.* 2015) and even social responsibility (Young *et al.* 2017).

Utilising social networks as a means of distributing public health or public service messages about the health risks of wildlife trade and consumption could yield positive results and begin to effect change around consumption of wildlife in China. Non-governmental organisations such IFAW and AITA detailed in Chapter 4 as well as others could readily launch public awareness campaigns via social media platforms in coordination with public health, centres for disease control, and forestry departments.

Rapidly reducing China's consumption of wildlife and eliminating this as a potential avenue of zoonotic epidemic or pandemic emergence is vital to global health. As occurred with the emergence of SARS-CoV and highly pathogenic avian influenza, relatively localised outbreaks in China swiftly became costly and global public health concerns. Localised study of wildlife trade and zoonotic emergence risk reduction in China may be applied in other countries in Oceania, Asia, Africa, and the Americas in regions where wildlife consumption and trade are also potential drivers of zoonotic emergence.

6.4 Integrated zoonotic disease surveillance to predict and prevent outbreaks in China

During and following the emergence and spread of SARS in 2002-2004, China significantly improved its ability to respond to emerging infectious diseases (Feng *et al.* 2009). A system of mandatory reporting of 'atypical pneumonias' and other illnesses of unknown aetiology was instituted (Ahmad *et al.* 2009) and a centralised, web-based, and connected CDC system was set up (Hipgrave 2011). All of this enabled China to respond to the next emerging diseases such as avian influenza A H5N1 and H7N9 far more rapidly and effectively (Ma *et al.* 2016). Despite these improvements in public health response and communication, drivers of disease emergence such as wildlife trade were not addressed other than via unenforced legislation (See Chapter 5).

It was reported that live, wildlife animal markets were briefly shut down in China during the SARS epidemic, but they reopened shortly thereafter (Reuters 2004). No published reports on this aspect exist, but the survey and observational data presented in Chapter 5 provide the first, strong evidence that rural and urban market consumption of bats is now effectively non-existent in China. If this were further corroborated by broader surveys, then it would provide additional evidence that campaigns implicating bats as the origin of SARS and making wildlife consumption illegal are, at least for bats, having the intended effects in China.

Future work may include further testing the hypothesis that bat consumption has reduced by surveying whether there is a demand in urban centres. If so, and urban participants confirm consuming bats, this would suggest that (a) wild populations of bats are still being accessed and possibly depleted and (b) there is an illegal market for bats. Confirming or rejecting the hypothesis will provide invaluable public health data about high-risk interactions with potential for zoonotic spillover between humans and bats as well as, assist in targeting future surveillance and improving response efforts in the event of any future zoonotic emergence.

The population surveyed for this study provided foundational evidence that knowledge, attitudes, and practices around wildlife trade, and even what is and what is not considered wildlife, in rural China are changing. This has potential conservation benefits, but an awareness of the risk of zoonotic spill-over via contact with wildlife appears to be lacking in these populations. Campaigns and public service announcements about the health risks of wildlife trade particularly around sanitation and biosafety may be employed in the same ways that these were conducted for conservation efforts. Similar local campaigns around avian influenza A (H10N8) and directed at individuals with highest-risk of exposure have been successful in improving poultry market workers' sanitation and biosafety awareness elsewhere in China (Chen *et al.* 2015). Other studies suggest that effective educational campaigns in these rural areas need to account for differences in knowledge, attitudes, and practices correlated with gender and levels of education (Wang *et al.* 2017). By having follow-up questionnaires with the same respondents or in the same communities, larger surveys could test the efficacy of wildlife trade and public health awareness campaigns.

One component repeatedly illustrated in the interviews conducted for this study was the importance of middlemen in wildlife trade. Given the illegal nature of the activities of the middlemen, they are difficult to approach and survey. An indirect approach to these individuals may be via the rural wildlife farmer networks that sell to the middlemen. Further integrated behavioural and quantitative surveillance of the wildlife trade networks in China will improve understanding of how wild animals from remote naturally biodiverse regions are farmed, traded and brought in densely populated urban centres in southern China. Beginning to quantify how these animals are mixed, transported, and handled will highlight how and where zoonotic disease spillover and amplification may occur and provide invaluable information for disease risk prevention measures.

6.5 Future Directions

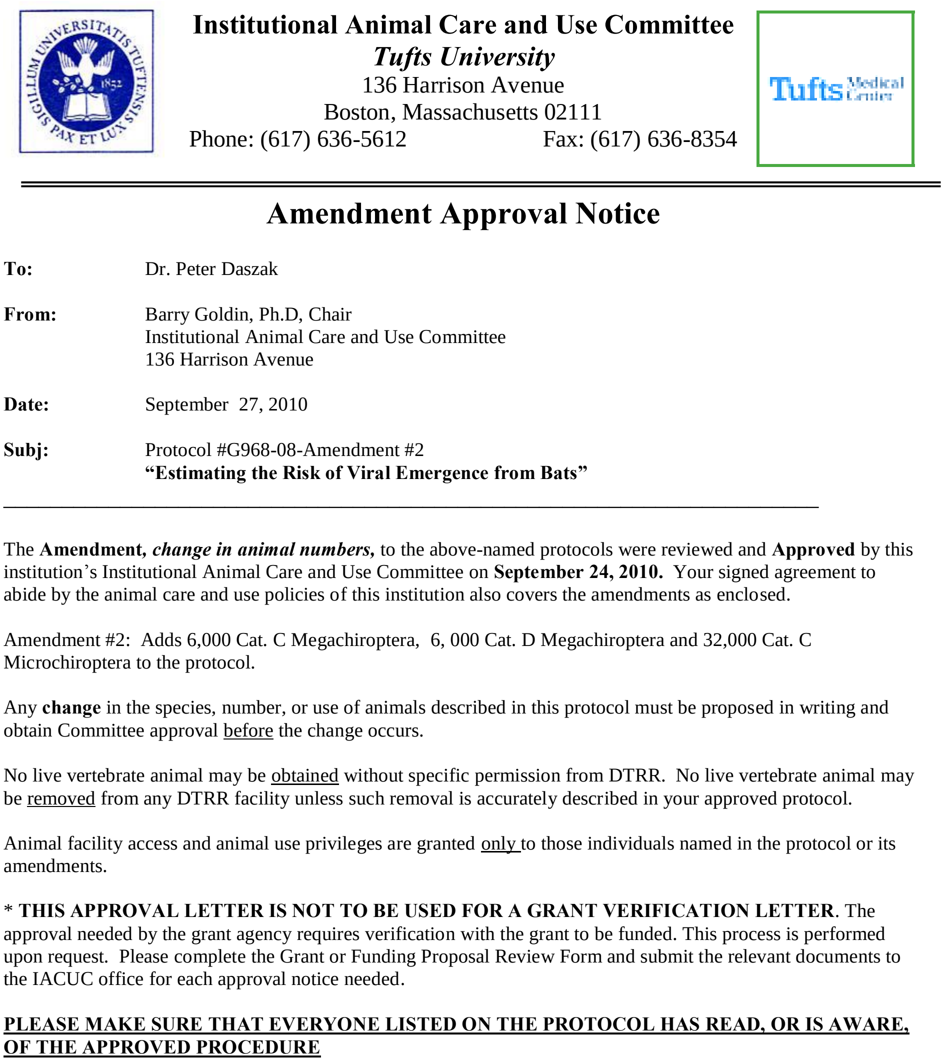
The outbreaks of SARS demonstrated that wildlife trade can have dire consequences for global health as well as the global economy (Lee & McKibbin 2004). In the aftermath of the disease, many countries improved their national public health systems so as to better coordinate a rapid response in the event of the next pandemic (Koplan *et al.* 2013). In 2006, two organisations of the United Nations, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), along with the World Organisation for Animal Health (OIE) coordinated in an effort to prevent pandemics. They established a Global Early Warning System for Major Animal Diseases in order to 'prevent, detect, control, eliminate, or reduce disease risk to humans originating directly or indirectly from domestic or wild animals and their environments', (GLEWS 2017). This "One Health" approach was mirrored in a series of research, international development, and outreach programs for zoonotic disease prediction and prevention (Bogich *et al.* 2012; Morse *et al.* 2012; Mazet *et al.* 2015). The research conducted in this study was funded by one such collaborative initiative and the questions raised in preceding chapters and immediately above will continue to be explored in the near future, including:

- Serological and molecular surveys for CoVs, PMVs, and other viral pathogens in human populations in China that are exposed to wildlife both in rural areas (where individuals with undiagnosed illnesses were identified) and in large urban markets like Taiping and Foshan.
- Using next generation sequencing techniques to conduct rapid and broad pathogen surveillance assays on bat and rodent samples from this region.
- Longitudinal surveillance of bats and their migratory behaviour correlated to agricultural, population, weather, and anthropogenic factors that may influence bat feeding and roosting behaviour.
- Defining and mapping the networks of wildlife trade in southern China starting from wildlife farmers and tracing both to middlemen and to sources of wildlife.
- Full genomic sequencing and characterisation of the potentially novel strains of PMV discovered in this research.
- Longitudinal behavioural surveillance of rural populations to see if public health and conservation public service announcements and campaigns are effecting changes in knowledge, attitudes, and practices around wildlife and high-risk behaviours.

Continued exploration along these lines into the factors affecting zoonotic emergence in China is critically needed to prevent future epidemics and pandemics and understanding the underlying risk of spillover events taking place.

7 APPENDICES

7.1 Tufts University IACUC Field Sampling Protocol Approval Notice



Institutional Animal Care and Use Committee
Tufts University
136 Harrison Avenue
Boston, Massachusetts 02111
Phone: (617) 636-5612 Fax: (617) 636-8354

Amendment Approval Notice

To: Dr. Peter Daszak

From: Barry Goldin, Ph.D, Chair
Institutional Animal Care and Use Committee
136 Harrison Avenue

Date: September 27, 2010

Subj: Protocol #G968-08-Amendment #2
"Estimating the Risk of Viral Emergence from Bats"

The **Amendment, *change in animal numbers*,** to the above-named protocols were reviewed and **Approved** by this institution's Institutional Animal Care and Use Committee on **September 24, 2010.** Your signed agreement to abide by the animal care and use policies of this institution also covers the amendments as enclosed.

Amendment #2: Adds 6,000 Cat. C Megachiroptera, 6, 000 Cat. D Megachiroptera and 32,000 Cat. C Microchiroptera to the protocol.

Any **change** in the species, number, or use of animals described in this protocol must be proposed in writing and obtain Committee approval before the change occurs.

No live vertebrate animal may be obtained without specific permission from DTRR. No live vertebrate animal may be removed from any DTRR facility unless such removal is accurately described in your approved protocol.

Animal facility access and animal use privileges are granted only to those individuals named in the protocol or its amendments.

* **THIS APPROVAL LETTER IS NOT TO BE USED FOR A GRANT VERIFICATION LETTER**. The approval needed by the grant agency requires verification with the grant to be funded. This process is performed upon request. Please complete the Grant or Funding Proposal Review Form and submit the relevant documents to the IACUC office for each approval notice needed.

PLEASE MAKE SURE THAT EVERYONE LISTED ON THE PROTOCOL HAS READ, OR IS AWARE, OF THE APPROVED PROCEDURE

7.2 Bat and Rodent Handling and Sampling

- Live bats should be placed into cotton bags (with draw-string mouths) and kept in a cool dry place until sampling time, not to exceed 6 hours. Rodents will chew through bags, so should be removed from cages or compounds and sampled immediately.
- Animals should be weighed (in grams) in bags using a Pesola hanging scale or a tabl-top scale with or without a container (such as a cup). The container should be tared and both bat and bag should be weighed. Once the animal is removed from the bag for sampling, the bag should be re-weighed and subtracted from previous total.
- The bat should be removed from the bag and urine should be collected and then duplicate oral and rectal swabs should be taken with fine aluminium swabs. Swabs should be cut on the shaft as close as possible to the end-swab without touching it and placed into VTM and in TRIzol. Scissors should be wiped with ethanol or isopropyl alcohol between each sample.
- All sample cryovials should be clearly labelled and stored in a liquid nitrogen dry shipper or dewar and transferred to -80˚C freezer in the Laboratory as soon as possible.
- **Oropharyngeal Swabs**: Using sterile, polyester-tipped swabs with either an aluminium or plastic shaft, rub the swab tip gently but thoroughly against the back of the animal's throat, saturating the swab with saliva. Place duplicate swabs in a cryovials filled with 500µl of VTM and TRIzol respectively.
- **Faeces:** Place duplicate 200 mg samples of fresh faeces in cryovials with 500µl VTM and TRIzol respectively.
- **Rectal Swabs**: Dip sterile swab tips briefly into sterile saline solution before inserting gently into the animal's rectum or (if too small) around the urogenital area. Place duplicate swabs in cryovials filled with 500µl of VTM and TRIzol respectively using a flame-sterilised scissors to cut the shaft of the swab above the tip.
- **Blood:** For animals weighing more than 100gm, use a non-heparinised syringe to collect blood (not to exceed 1% of the circulating blood volume). Make 2 thin smears on glass microscope slides, fix with methanol or ethanol, and store in slide box. Place rest of blood into a serum vacutainer (red-top) tube containing serum-clotting factor. After allowing the blood to clot, either spin tube in a centrifuge or allow tube to stand vertically on ice overnight. Use a sterile pipette tip and pipette gun to draw off serum and place even aliquots into cryovials. The remaining blood clot should be divided between cryovials with 1.0 ml VTM and TRIzol respectively. For animals weighing less than 100gm, use the blood dilution technique as per Smith *et al.* (2010b). Animal is restrained in one hand and the wing or leg is extended. The radial artery or vein is punctured using the tip of a sterile 25G needle and a bleb of blood is allowed to form. Using a pipette or pipette gun draw up 12µl and place it into 108µl phosphate buffered saline (PBS) in a 1.2 ml Eppendorf tube. Continue to draw up blood in 12 µl aliquots and place in the same tube until the maximum amount of blood safely allowed (approx 6µl blood volume per gm of animal mass) is collected. Add an equal number of 108µl aliquots of PBS to maintain a 1:10 dilution. Blood tubes can be centrifuged to separate dilute serum from blood cell pellet. Make sure to note the dilution of blood on data sheet. Collect cell pellet as above.
- **Urine:** collect urine sample in VTM and in TRIzol as detailed for swabs, above.

7.3 Collection Protocol for microbats and small megabats

- Date & Location are recorded using GPS along with temperature and weather conditions including time (or duration) of trapping and any other variables. All notes are written in a notebook and later transferred to an excel file. After sampling, photographs are taken of each note page to ensure against loss of hard-copy.
- Pre-printed bar-scan labels affixed to all vials. Bat samples are coded with the following method:

    - 04.12.09 – XRD
    - BZ – P. hypomelanus
    - Oral XXX

    - From top to bottom, left-to-right:
        - Date in following format: DD.MM.YY
        - Location-specific Name; i.e. XianRenDong = XRDG; HaiKou GongYuan = HKGY
        - Laboratory to which the sample is intended; i.e. CH (for China) or US
        - Genus & Species in format of "G. species"
        - Sample Type – serum, whole blood, feces, oral or anal swab, &tc.
        - Bat-specific number given per site. Usually commencing with '001' up to n with n being the highest-count per sampling effort of bat.

- Bats are caught using mist-net, hand-net, or harp-trap and placed into cloth bags (with draw-string mouths). These cloth bags are then suspended from pole permitting air to circulate between each bat-with-bag. In warm months, someone must monitor captured bats' local environment to ensure adequate ventilation and to prevent excessive humidity.
- Bats are weighed (in grams) while still in bags. Scale with or without container or cup is tared and both bat & bag are weighed. Once bat is removed for biometry, bag is re-weighed and subtracted from previous total.
- Bat is removed from bag, identified (genus, species, age class, and sex) and photographed.
- The following biometrics (in centimetres) are taken:
    - Forearm length ('elbow to wrist')
    - Ear Length (most distal tip of ear to base)
    - Tragus length (top of tragus to base of ear)
    - Body Length (measured ventrally from top of nose to base of tail)
    - Hind Foot Length ('ankle to toe')
    - Tail Length (from base to tip)
    - Tibia ('knee to ankle')

- 2 2mm wing punches are taken from each bat and placed in ethanol in a 0.5ml vial.
- Oral and anal swabs are taken with fine aluminium-shaft swab. If any faeces or urine are clearly excreted directly from the bat as it is being handled, these excreta may be placed into separate vials of 500µl of VTM and 500µl of TRIzol and in 1ml cryovials (with internal O-ring washers). Care must be taken not to sample any loose faeces from bat-bag, unless no other bat has previously been kept therein since last laving of bat-bags. Oral and anal swabs are cut on the shaft as close as possible to the end-swab without touching it and dropped into duplicate tubes containing respectively TRIzol and VTM. The swab-tip and any sample must be completely submerged in the buffer. Shears or cutters are sterilised between bats with ethanol and flame. Sealed, labelled vials with samples are put into dry-shipper filled with liquid N_2 or otherwise stored at -80˚C.
- If bats must be bled with caution to maintain a ratio no greater than 1g of bat weight to 10µl of blood. For bleeding, bats are manually restrained and not sedated. Even with micro-bats, two people are preferred for these manipulations; one to safely manage the bat and the other to take and record samples. The bleed site is prepared with a pre-injection swab of ethanol and a 25 gauge ¾ needle is used to only pierce the saphenous, brachial, or propatagial veins. Which vein used will depend upon sampler's experience and success with specific species. The saphenous vein permits the sampling site to be furthest from the bat's head.
- Blood beads on the skin surface and may be collected using a 100 µl pipette with sterile tip and added directly to PBS in a Eppendorf vial and mixed briefly with the pipette tip. NB, quantity of blood extracted from bat should not exceed a ratio of 1g of bat weight to 10µl of blood. Discard tips between bats. PBS formula: dissolve the following in 800ml distilled H_2O. 8g of NaCl; 0.2g of KCl; 1.44g of Na_2HPO_4; 0.24g of KH_2PO_4; Adjust pH to 7.4; Adjust volume to 1L with additional distilled H_2O; Sterilize by autoclaving
- When the maximum permissible volume is collected, a cotton ball and pressure are applied until a clot has formed and bleeding ceases. If blood flow is difficult to stop, a styptic powder may be used. If excessive bleeding occurs repeatedly and clot is slow to form, puncture should be made more distally along the chosen vein.
- Once all trapped bats are processed and returned to their respective bags, the collected blood in Eppendorf diluted with PBS is then spun in a micro-centrifuge briefly (1-3 mins) and allowed to settle overnight or for a minimum of one hour.

- Once the blood-plus-PBS in the Eppendorf tubes has settled, the serum is removed with a sterile pipette tip and put into duplicate, labelled 700µl vials <u>without buffer</u> for storage and later analysis. Serum vials may be stored on ice for up to 24 hours during transit and then at -80˚C or put directly into N_2 for return to laboratory.
- Remaining blood cells are pipetted into two vials and TRIzol (approximately equivalent to the serum fraction removed) is added to the remaining blood cells to maintain – at most - a 1:10 dilution and provide a haemostatic buffer. To maintain the viability of cellular DNA for further genetic analysis the diluted blood cell fraction is stored with oral/anal samples (above) in a dry-shipper filled with liquid N_2 (in collar) until they may be deposited in a -80˚C freezer.
- Bats are released as close to their site of capture as possible.
- If Avisoft or other sonic-detection device is available, bats' calls upon release are recorded (optional).
- For any euthanised bats, liver, kidney, brain, heart, spleen, reproductive tissue (testes & uterus), and lung will be sampled and frozen directly into vials with TRIzol. Each sample should be mashed briefly in the buffer solution. Salivary gland (not vital, if already swabbing for saliva).

TOTAL NUMBER OF SAMPLES PER BAT: 8

a) 2 x oral swab in approx. 0.5ml of TRIzol and VTM, respectively. Stored in Dry Shipper with N_2 and transferred to -80˚C freezer

b) 2 x anal swab in approx. 0.5ml of TRIzol and VTM, respectively. Stored in Dry Shipper with N_2 and transferred to -80˚C freezer

c) 2 x serum. For non-euthanised bats, whole blood from bats not to exceed 10µl per 1g of bat-weight. Blood to be put into Eppendorf tubes with PBS to dilute to a maximum ratio of 1:10 (minimum ratio of 1:3). Diluted blood is then spun and let sit for at least three hours, but no more than 24 hours. A minimum of 60µl or 200µl of diluted serum pipetted off, put into vials, and stored in Dry Shipper & transferred to -80˚C freezer. NB. MINIMUM WEIGHT FOR BAT WILL BE 6g to have at least 60µl of whole blood.

d) 2 x remaining blood-cells after serum is removed. This remaining blood will be mixed with TRIzol at a ratio equal to or greater than one part blood to 3-parts buffer

7.4 CoV and PMV BLAST Results and Reference Sequences

Table 27. A total of 39 positive Coronavirus sequences from Chinese bats. Sample numbers host species listed. Length of sequences in nucleotide base pairs (bp) is given. NCBI (ncbi.nlm.nih.gov) BLAST results record number, BLAST results host species, percent of nucleotides covered by BLAST results, and pairwise identities are in right-most columns. Discussed in detail in Chapter 4.

Sample Number	Host Species	Length (bp)	BLAST Result	BLAST Result Host Species	Cover	Identities
15612NL131033	*Hipposideros pratti*	387	KP876528	*Rhinolophus affinis*	100.00%	93.30%
15612NL13812	*Rhinolophus rex*	387	KP876528	*Rhinolophus affinis*	100.00%	93.30%
15612NL13814	*Rhinolophus rex*	348	KP895492	*Myotis daubentonii*	100.00%	93.40%
15612NL13815	*Rhinolophus rex*	349	KP895492	*Myotis daubentonii*	100.00%	93.40%
15612NL13817	*Rhinolophus rex*	347	KP895492	*Myotis daubentonii*	100.00%	93.40%
15612NL13813	*Rhinolophus rex*	387	KP876528	*Rhinolophus affinis*	100.00%	93.50%
15612NL13816	*Rhinolophus rex*	354	KP895492	*Myotis daubentonii*	100.00%	93.50%
15612NL13820	*Rhinolophus rex*	339	KP895492	*Myotis daubentonii*	100.00%	93.50%
15610HNCM020	*Miniopterus fuliginosus*	383	DQ648852	Bat CoV	98.97%	94.80%
15610HNYG063	*Miniopterus fuliginosus*	387	KP876519	*Miniopterus schreibersii*	100.00%	96.40%
15610HNLS008	*Miniopterus fuliginosus*	385	KP876519	*Miniopterus schreibersii*	99.48%	96.60%
15611YNMXR003	*Miniopterus fuliginosus*	384	KP876507	*Miniopterus schreibersii*	99.22%	96.60%
15610HNLS007	*Miniopterus fuliginosus*	387	KJ020613	*Miniopterus magnater*	100.00%	97.40%
15612NL13856	*Pipistrellus pipistrellus*	387	DQ648802	Bat CoV	100.00%	97.40%
15612NL13847	*Pipistrellus pipistrellus*	387	KC522076	*Pipistrellus* sp.	100.00%	97.70%
15612NL13849	*Pipistrellus pipistrellus*	387	KY432470	*Pipistrellus abramus*	100.00%	97.70%
15612GXLZ006	*Tylonycteris pachypus*	367	KJ473822	*Tylonycteris pachypus*	100.00%	97.80%
15612NL131015	*Hipposideros armiger*	387	KY009624	*Eptesicus serotinus*	100.00%	97.90%
15612GXLZ009	*Tylonycteris pachypus*	367	KJ473822	*Tylonycteris pachypus*	100.00%	98.10%
15610HNCM030	*Miniopterus fuliginosus*	387	KJ473802	*Miniopterus fuliginosus*	100.00%	98.20%
15612NL13832	*Ia io*	387	KY009624	*Eptesicus serotinus*	100.00%	98.20%
15612NL13845	*Ia io*	387	KY009624	*Eptesicus serotinus*	100.00%	98.20%
15611YNMXR002	*Miniopterus fuliginosus*	387	KP876507	*Miniopterus schreibersii*	100.00%	98.40%
15612GXLZ027	*Tylonycteris pachypus*	367	DQ648803	Bat CoV	100.00%	98.40%
15612HZ13484	*Rhinolophus sinicus*	387	KJ473815	*Tylonycteris pachypus*	100.00%	98.40%
15612NL13973	*Rhinolophus sinicus*	387	KJ473815	*Tylonycteris pachypus*	100.00%	98.70%
15611YNMXR005	*Miniopterus fuliginosus*	362	KP876513	*Miniopterus schreibersii*	93.54%	98.90%
15610HNCM033	*Miniopterus fuliginosus*	387	KJ473802	*Miniopterus fuliginosus*	100.00%	99.00%
15610YNYJ028	*Miniopterus fuliginosus*	387	KP876523	*Miniopterus schreibersii*	100.00%	99.00%
15612NL13953	*Myotis ricketti*	343	DQ648836	Bat CoV	100.00%	99.10%
15612NL13937	*Myotis ricketti*	351	KY009616	*Myotis ricketti*	100.00%	99.40%
15612GXLZ047	*Tylonycteris pachypus*	367	KJ473822	*Tylonycteris pachypus*	100.00%	99.50%
15612GXLZ059	*Tylonycteris pachypus*	387	KJ473822	*Tylonycteris pachypus*	100.00%	99.50%
15612NL13946	*Myotis ricketti*	387	DQ648826	Bat CoV	100.00%	99.50%
15612NL13928	*Myotis ricketti*	359	DQ648844	Bat CoV	100.00%	99.70%
15612NL13945	*Myotis ricketti*	348	DQ648844	Bat CoV	100.00%	99.70%
15611YNSHC014	*Rhinolophus sinicus*	387	KF367457	*Rhinolophus sinicus*	100.00%	100.00%
15612GXLZ001	*Tylonycteris pachypus*	367	KJ473822	*Tylonycteris pachypus*	100.00%	100.00%
15612HZ13488	*Rhinolophus sinicus*	387	KJ473815	*Tylonycteris pachypus*	100.00%	100.00%

Table 28. Reference sequences used in constructing Coronavirus phylogenies. Downloaded from NCBI (ncbi.nlm.nih.gov). Discussed in detail in Chapter 4.

Genus	Species	NCBI Acession Number
Alphacoronavirus	Feline Coronavirus	AB907634
Alphacoronavirus	Canine Coronavirus	AB907633
Alphacoronavirus	Human coronavirus 229E	KT253266
Alphacoronavirus	Human coronavirus NL63	KF530113
Alphacoronavirus	Miniopterus bat coronavirus 1	AY864196
Alphacoronavirus	Miniopterus bat coronavirus HKU8	NC010438
Alphacoronavirus	Porcine epidemic diarrhea virus	NC003436
Alphacoronavirus	Rhinolophus bat coronavirus HKU2	NC009988
Alphacoronavirus	Scotophilus bat coronavirus 512	NC009657
Alphacoronavirus	Transmissible gastroenteritis coronavirus	KX499468
Alphacoronavirus	Chinese Bamboo Rat CoV	EF584902
Betscoronavirus	Bat Hipposideros pratti coronavirus	KF636753
Betacoronavirus	Bat Ia io coronavirus	KY770857
Betacoronavirus	Bat Myotis ricketti coronavirus	KY009625
Betacoronavirus	China Rattus coronavirus HKU24	NC026011
Betacoronavirus	Human coronavirus OC43	KF572933
Betacoronavirus	Human coronavirus HKU1	KY674921
Betacoronavirus	MERS-CoV	KP209313
Betacoronavirus	SARS-CoV	NC006577
Betacoronavirus	Pipistrellus bat coronavirus HKU5	DQ249217
Betacoronavirus	Tylonycteris bat coronavirus HKU4	DQ074652
Betacoronavirus	Rousettus bat coronavirus HKU9	KY010629
Betacoronavirus	London1 novel CoV/2012	KC164505
Betacoronavirus	HCoV-EMC/2012	JX869059
Betacoronavirus	Bat SL-CoV-WIV1	KF367457
Betacoronavirus	Civet SARS CoV SZ3	AY304486
Betacoronavirus	Hipposideros Bat CoV	KJ020601
Gammacoronavirus	Turkey coronavirus	FN811146
Gammacoronavirus	Beluga whale coronavirus SW1	EU111742
Deltacoronavirus	Bulbul coronavirus HKU11	FJ376619

Table 29. A total of 114 positive Paramyxovirus sequences. Sample number and host species are shown from Chinese bats. Length of sequences in nucleotide base pairs (bp) is given. NCBI (ncbi.nlm.nih.gov) BLAST results, record number, BLAST results host species, percent of nucleotides covered by BLAST results, and pairwise identities are in right-most columns. Discussed in detail in Chapter 4.

Sample Number	Host Species	Length (bp)	BLAST Result	BLAST Result Host Species	Cover	Identities
PAR-4274	*R. affinis*	572	GU128081	*R. leschenaulti*	100%	68%
HD13632	*M. schreibersii*	533	KC692406	Bat PMV	99%	71%
PAR1-141731	*A. stoliczkanus*	503	AB844425	Bat PMV	100%	73%
PAR-5816	*H. armiger*	506	AB844425	Bat PMV	100%	73%
141531	*M. davidii*	532	KP159805	Feline PMV	97%	74%
PAR-3712	*M. fuliginosus*	513	KC599261	Bat PMV	99%	74%
ZG13666	*V. sinensis*	533	KC578584	Bat PMV	96%	74%
PAR2-141724	*A. stoliczkanus*	609	KJ641655.1	Bat PMV	99%	75%
PAR2-141725	*A. stoliczkanus*	515	KC578584	Bat PMV	100%	75%
PAR2-141727	*A. stoliczkanus*	511	KC599258	Bat PMV	100%	75%
ML140073	*A. stoliczkanus*	471	KC154054	*M. schreibersii*	96%	75%
PAR2-151431	*H. pratti*	497	KC578575	Bat PMV	73%	75%
LD13759	*P. ognevi*	533	JN086951	Bat PMV	99%	75%
JL035	*R. pusillus*	530	KC578584	Bat PMV	97%	75%
PAR1-141747	*T. melanopogon*	530	KC599258	Bat PMV	98%	75%
GL13561	*T. melanopogon*	520	KC599258	Bat PMV	100%	75%
PAR-3131	*H. armiger*	512	JN086951	Bat PMV	100%	76%
PAR-7260	*H. armiger*	499	KC578569	Bat PMV	100%	76%
PAR-7261	*H. armiger*	305	KC578572	Bat PMV	100%	76%
PAR-141582	*H. pomona*	491	KC578572	Bat PMV	99%	76%
PAR2-151428-36	*H. pratti*	582	KC154054.1	Bat PMV	98%	76%
PAR2-151393	*H. pratti*	535	KC154055.1	*M. schreibersii*	99%	76%
PAR-141229	*M. pilosus*	520	KC599258	Bat PMV	99%	76%
PAR-141247	*M. pilosus*	520	KC599258	Bat PMV	98%	76%
LHS006R	*M. pilosus*	518	KC578572	Bat PMV	96%	76%
LD13763	*P. ognevi*	509	JN086951	Bat PMV	99%	76%
PAR-4271	*R. affinis*	533	KC154054	*M. schreibersii*	97%	76%
PAR1-8583	*R. sinicus*	536	KC599258	Bat PMV	100%	76%
ZG13667	*V. sinensis*	448	JN086951	Bat PMV	98%	76%
BX13776	*H. alaschanicus*	517	JN086951	Bat PMV	100%	77%
PAR-3130	*H. armiger*	499	KC578572	Bat PMV	100%	77%
PAR-141583	*H. pomona*	505	KC578572	Bat PMV	97%	77%
PAR2-141401	*H. pomona*	505	KC599258	Bat PMV	100%	77%
PAR-141578	*H. pomona*	508	KC154054	*M. schreibersii*	99%	77%
PAR-141624	*H. pomona*	497	KC154056	*M. schreibersii*	100%	77%
PAR2-151392	*H. pratti*	536	KC578579.1	Bat PMV	100%	77%
PAR2-151407	*H. pratti*	535	KC578572.1	Bat PMV	99%	77%
PAR2-151432-35	*H. pratti*	466	KC578584.1	Bat PMV	95%	77%
PAR2-151436-4	*H. pratti*	520	KJ641655.1	Bat PMV	100%	77%
PAR2-9016	*H. pratti*	495	KC578584.1	Bat PMV	100%	77%
PAR-3979	*M. magnater*	600	KJ641655.1	Bat PMV	100%	77%
PAR-4063	*M. magnater*	599	KJ641655.1	Bat PMV	99%	77%

Positive Paramyxovirus sequences (ctd).

Sample Number	Host Species	Length (bp)	BLAST Result	BLAST Result Host Species	Cover	Identities
NL13929	*M. pilosus*	540	KC599258	Bat PMV	98%	77%
PAR-4272	*R. affinis*	610	KJ641655.1	Bat PMV	100%	77%
PAR-4273	*R. affinis*	520	KJ641655.1	Bat PMV	99%	77%
PAR-3772	*R. sinicus*	508	KC154054	*M. schreibersii*	99%	77%
PAR1-141735	*A. stoliczkanus*	515	KC599258	Bat PMV	99%	78%
PAR2-8764	*H. armiger*	490	JN086950	Bat PMV	99%	78%
PAR2-9243	*H. armiger*	506	KC578584.1	Bat PMV	98%	78%
PAR-141629	*H. pomona*	611	KJ641657.1	Bat PMV	99%	78%
PAR-141627	*H. pomona*	533	KC154058	*R. ferrumequinum*	99%	78%
PAR2-151394	*H. pratti*	522	KC578584.1	Bat PMV	99%	78%
PAR2-151403	*H. pratti*	525	KJ641655.1	Bat PMV	99%	78%
PAR2-151405	*H. pratti*	536	KJ641655.1	Bat PMV	100%	78%
PAR2-151435	*H. pratti*	608	KJ641655.1	Bat PMV	94%	78%
PAR2-8961	*H. pratti*	505	KJ641655.1	Bat PMV	100%	78%
NL13838	*I. io*	476	KC599258.1	Bat PMV	100%	78%
NL13841	*I. io*	513	KC599258	Bat PMV	99%	78%
YX006	*M. altarium*	337	KC599258	Bat PMV	98%	78%
PAR-3736	*M. fuliginosus*	482	KC599258.1	Bat PMV	94%	78%
YX007	*R. affinis*	490	JN086950	Bat PMV	92%	78%
LA131533	*R. affinis*	527	KC154058	*R. ferrumequinum*	99%	78%
PAR2-151307	*R. sinicus*	535	KJ641655.1	Bat PMV	99%	78%
FX037	*R. sinicus*	534	KC578572	Bat PMV	100%	78%
PAR-3086	*R. sinicus*	529	KC578584	Bat PMV	95%	78%
PAR-3112	*R. sinicus*	522	KC578584	Bat PMV	95%	78%
YD13389	*H. larvatus*	535	KC578572	Bat PMV	99%	79%
PAR-141595	*H. pomona*	531	KC154054	*M. schreibersii*	99%	79%
PAR2-151396	*H. pratti*	535	KJ641655.1	Bat PMV	100%	79%
PAR2-151432-31	*H. pratti*	585	KJ641655.1	Bat PMV	97%	79%
PAR2-8972	*H. pratti*	477	KC599258.1	Bat PMV	100%	79%
PAR-141594	*H. pomona*	519	KC154054	*M. schreibersii*	97%	80%
PAR2-151426	*H. pratti*	557	KC599258.1	Bat PMV	92%	80%
PAR2-151428-47	*H. pratti*	585	KJ641655.1	Bat PMV	97%	80%
PAR2-8962	*H. pratti*	472	KC578584.1	Bat PMV	100%	80%
PAR2-9015	*H. pratti*	502	KC599258.1	Bat PMV	97%	80%
NL131077	*M. davidii*	515	KC578584	Bat PMV	99%	80%
PAR-141217-32	*M. pilosus*	510	KC578584	Bat PMV	99%	80%
HD13630	*M. schreibersii*	520	KC578584	Bat PMV	98%	80%
PAR-4301	*R. affinis*	503	KC154054	*M. schreibersii*	98%	80%
NL140372-1	*R. pearsonii*	514	KC578584	Bat PMV	99%	80%
PAR-3362	*R. sinicus*	490	KC154054	*M. schreibersii*	98%	80%
PAR2-9032	*H. pratti*	505	KC599258.1	Bat PMV	100%	81%
PAR2-9257	*H. armiger*	505	KC599258.1	Bat PMV	99%	82%
PAR2-9258	*H. armiger*	450	KC599258.1	Bat PMV	98%	82%
PAR2-9259	*H. armiger*	501	KC599258.1	Bat PMV	99%	82%

Positive Paramyxovirus sequences (ctd).

Sample Number	Host Species	Length (bp)	BLAST Result	BLAST Result Host Species	Cover	Identities
PAR2-9260	*H. armiger*	498	KC599258.1	Bat PMV	100%	82%
PAR2-9261	*H. armiger*	504	KC599258.1	Bat PMV	98%	82%
PAR2-151421	*H. pratti*	489	KJ641655.1	Bat PMV	100%	82%
PAR2-151425	*H. pratti*	513	KJ641655.1	Bat PMV	100%	82%
PAR2-151428-40	*H. pratti*	585	KJ641655.1	Bat PMV	97%	82%
PAR2-9033	*H. pratti*	505	KC599258.1	Bat PMV	100%	82%
PAR-4300	*R. affinis*	505	KC154054	*M. schreibersii*	98%	82%
PAR2-151296	*R. pearsonii*	525	KJ641655.1	Bat PMV	99%	82%
ZG13665	*V. sinensis*	533	KC599258	Bat PMV	96%	82%
PAR2-151399	*H. pratti*	512	KJ641655.1	Bat PMV	100%	83%
PAR2-151406	*H. pratti*	558	KJ641655.1	Bat PMV	97%	83%
PAR2-151432-32	*H. pratti*	663	KJ641655.1	Bat PMV	100%	83%
PAR2-9011	*H. pratti*	501	KC599258.1	Bat PMV	100%	83%
PAR2-8965	*H. pratti*	504	KC599258.1	Bat PMV	98%	85%
PAR2-8975	*H. pratti*	499	KC599258.1	Bat PMV	99%	85%
PAR2-8997	*H. pratti*	495	KC599258.1	Bat PMV	100%	85%
PAR2-9000	*H. pratti*	497	KC599258.1	Bat PMV	99%	85%
PAR2-9012	*H. pratti*	504	KC599258.1	Bat PMV	98%	85%
PAR2-9018	*H. pratti*	489	KC599258.1	Bat PMV	100%	85%
PAR2-9031	*H. pratti*	409	KC599258.1	Bat PMV	100%	85%
PAR2-151436-6	*H. pratti*	520	KC599258	Bat PMV	99%	86%
PAR2-8998	*H. pratti*	475	KC599258.1	Bat PMV	99%	86%
PAR2-9269	*H. armiger*	507	KC599258.1	Bat PMV	98%	87%
PAR-7254	*H. armiger*	518	KC599258	Bat PMV	99%	91%
PAR2-9109	*H. armiger*	508	KC599258.1	Bat PMV	99%	92%
YLD011	*H. armiger*	403	KC599258	Bat PMV	99%	92%
FX006	*R. sinicus*	508	KC599258	Bat PMV	100%	92%
PAR-4287	*R. affinis*	519	KC599258	Bat PMV	100%	99%

Table 30. Reference Sequences used to construct Paramyxovirus phylogenies. Downloaded from NCBI (ncbi.nlm.nih.gov). Discussed in detail in Chapter 4.

Genus	Species	NCBI Accession Number
Avulavirus	Newcastle Disease Virus	AB854735
Avulavirus	Avian Paramyxovirus 6	AB759118
Avulavirus	Avian PMV 9	EU910942
Avulavirus	Avian PMV 5	GU206351
Ferlavirus	Fer de Lance PMV	AF286043
Henipavirus	Hendra Virus	JN255818
Henipavirus	Nipah Virus	JN255818
Morbillivirus	Canine Distemper Virus	KX371588
Morbillivirus	Phocine Distemper Virus	PVU65446
Morbillivirus	Measles Virus	AY037033
Morbillivirus	Rinderpest Virus	AY954037
Morbillivirus	Cetacean Morbillivirus	NC_005283
Morbillivirus	Peste des Petits Ruminants Virus	AJ849636
Respirovirus	Bovine Parainfluenza Virus 3	AB770484
Respirovirus	Human Parainfluenza Virus 3	AB195610
Respirovirus	Sendai Virus	AB005795
Rubulavirus	Human Parainfluenza Virus 1	AF117818
Rubulavirus	Porcine Rubulavirus	AF416650
Rubulavirus	Mumps Virus	AB904528
Rubulavirus	Human Parainfluenza Virus 2	AB176531
Rubulavirus	Parainfluenza Virus 5	AB853926
Rubulavirus	Mapuera Virus	NC_009489
Rubulavirus	Simian Virus 41	NC_006428
Rubulavirus	Simian Virus 10	HM583801
Rubulavirus	Human Parainfluenza Virus 4a	AB543336
Unclassified	Beilong Virus	KX940964
Unclassified	Tupaia Paramyxovirus	NC_002199
Unclassified	Tuhoko Virus	NC_025348
Unclassified	Tioman Virus	AF298895
Unclassified	Menangle Virus	AF326114
Unclassified	Salem Virus	JQ697837
Unclassified	Mossman Virus	AY286409
Unclassified	J Virus	AY900001

7.5 Observational Survey Checklist

(English language version)

Observer Name: ____

Site Name & Location: ____

Date/Time of day/weekday or weekend ____

Season/weather conditions of the day: ____

I. **Primary Activity (circle one or more)** (1) Land use change (2) Animal production intensification (3) Animal value chain

I. **Environment (circle one)**

1. Urban (densely living population, little green space visible, eg, parks, cultivation or raising animals)
2. Peri-urban (may still be dense population but more green space/scrub, could be used for cultivation or raising animals or fallow)
3. Rural (could be abutting pristine, majority of land does not have dwellings, could be used for agricultural—crops or animals; may be fallow; may be extractive industry)
4. Low disturbance (no obvious signs of human dwellings or established plantations, extractive industry, congregation sites for animal sales or slaughter, very few signs of human activity.)

II. **Type of site (circle one)**

1) Market (formal or informal)
2) Dwelling
3) Extractive industry (eg, mining, logging, palm oil plantation)
4) Farm
5) Natural habitat
6) Restaurant (including street vendors, chop shops, informal or impromptu set-ups)

III. **Observed living animals and humans**

	Yes	**No**	**Total No. Obsv'd**	**No. of species Obsv'd**
Livestock/domestic animals (including cats and dogs)	Y	N	<10, 10-50, >50-1000, >1000	1, 2-5, >5
Wildlife/nondomestic animals (including rodents, non-farmed birds and farmed wildlife	Y	N	<10, 10-50, >50-1000, >1000	1, 2-5, >5-15, >15
Humans	Y	N	1, 2-10, >10-50, >50	N/A

Is there indirect evidence of other animals not seen?

Feces	Y	N	Footprints	Y	N
Eaten fruit or other food	Y	N	Chew marks	Y	N

Which animals can be directly or indirectly observed at this site?

Livestock	**Yes**	**No**
Chickens	Y	N
Ducks	Y	N
Pigs	Y	N
Cows	Y	N
Dogs	Y	N
Cats	Y	N
Goats/Sheep	Y	N
Camels	Y	N

Wildlife	**Yes**	**No**
Rodents (rats/mice, porcupines, squirrels)	Y	N
Bats	Y	N
Nonhuman primates	Y	N
Civets	Y	N
Non-farmed wild birds	Y	N
Farmed wildlife	Y	N

IV. **Animal-human mixing**

Circle yes, no, or not applicable (N/A).	**Yes**	**No**	**N/A**
Does the site have structures to limit animal movement (eg, cages, buildings, stalls, fencing)?	Y	N	N/A
Are different species observed sharing the same space or resources (eg, water, food, physical location, cages, stall, pasture)? **If "Yes",** select all that apply:	Y	N	N/A
a. Wild animal with wild animal	Y	N	N/A
b. Wild animal with livestock/domestic animal	Y	N	N/A
c. Livestock/domestic animal with livestock/domestic animal	Y	N	N/A
Are there any (wildlife or livestock/domestic) animals roaming freely?	Y	N	N/A
Do any animals appear to be sick?	Y	N	N/A
Is there any slaughtering of animals occurring on site?	Y	N	N/A
Is there any butchering of animals occurring on site?	Y	N	N/A
Are there any animals near food preparation or eating areas?	Y	N	N/A
Is there evidence that people live with their animals?	Y	N	N/A
Do any people appear to be sick?	Y	N	N/A
Are there any children at the site?	Y	N	N/A

V. Sanitation and hygiene

Circle yes, no, or not applicable (N/A).	**Yes**	**No**	**N/A**
Does the site have structures to protect against the weather or other animals?	Y	N	N/A
Are the structures permanent?	Y	N	N/A
Is there a dedicated location for animal waste?	Y	N	N/A
Is there a dedicated location for human garbage?	Y	N	N/A

Quality of animal waste and garbage disposal (circle one)

0. None observed
1. Dedicated location, either not maintained or not used
2. Dedicated location, well maintained and used

Are there toilets, latrines or other public facilities/designated areas for human waste?	Y	N	N/A

Quality of human waste disposal (circle one)

0. None observed
1. Dedicated location, either not maintained or not used
2. Dedicated location, well maintained and used

Is there a central source of water?	Y	N	N/A

Quality of water (circle one)

0. Fully unprotected: pond, uncovered well
1. Rainwater harvesting, water trucking, open well
2. Fully protected: water network with taps, covered well

Access to water (circle one)

0. >30 minutes walking
1. 5-30 min
2. 0-5 minutes walking

Add Site Observations here (use additional pages as necessary):

Observational Survey Checklist – Chinese language version

行为风险调查表

调查表的主要目的为以下两方面：

1. 是否有人和动物互动的证据？
2. 人和动物互动这一背景是否潜在的促成了病毒的跨种传播？

观察者： ____

观察点&GPS： ____

观察日期/时间/是否工作日____

季节/调查日天气 ____

I. **通路观察** （选择一个） (1)土地使用变化情况 (2) 动物产品集约化 (3) 动物价值链

I. 环境方面（选择一个）

1. 都市（高密度聚居人群，很少公园等绿地空间用来培植或者饲养的动物。）
2. 城乡结合部（仍然有较多人群聚居但是具有更多绿地和灌木可以用来培植或者饲养动物，或者有较多的弃耕土地。）
3. 郊区（景观较为原始，大部分的地区没有定居居民，土地主要为种植庄稼、饲养动物等农业用途；或者有弃耕田地；或者采摘农业。）
4. 人烟稀少地区（没有明显的定居特征、植被特征、采摘农业特征，动物屠宰和动物买卖特征，极少人类活动。）

II 观察点类型（选择一个）

1) 贸易市场(正规的 非正规的)
2) 聚居点
3) 采伐业 (例如,采矿业，石油勘探或者种植业)
4) 农场
5) 自然栖息地
6) 餐馆 (包括街头摊贩点, 地下贩卖点, 非正规的随机设立摊点)

III 观察到的人和活的动物

	是 否	**观察到的动物总数量**	**观察到的动物种类**
家畜和饲养动物（包括猫，狗）	是 否	<10, 10-50, >50-1000, >1000	1, 2-5, >5
野生动物/非饲养动物（包括啮齿类，非饲养鸟类和饲养野生动物	是 否	<10, 10-50, >50-1000, >1000	1, 2-5, >5-15, >15
人	是 否	1, 2-10, >10-50, >50	不适用

有没有明显的遗漏其它动物数量和种类的特征？

粪便	是	否	足迹	是	否
吃剩的水果或者其他食物	是	否	咀嚼残渣	是	否

在该观察点能够直接或者间接观察到的动物都有哪些？

家畜	**是**	**否**
鸡	是	否
鸭	是	否
猪	是	否
牛	是	否
狗	是	否
猫	是	否
山羊/绵羊	是	否

野生动物	**是**	**否**
啮齿类 (大鼠/小鼠,豪猪,松鼠)	是	否
蝙蝠	是	否
非人灵长类	是	否
果子狸	是	否
非饲养野生鸟类	是	否
饲养的野生动物	是	否

骆驼　是　否

IV 动物和人混居情况

选择是、否或者不适用	**是 否 不适用**
该观察点是否有限制动物活动设施 (笼子，建筑，篱舍，篱笆)?	是 否 不适用
是否有不同种动物（混养，公用饮用水，食物，笼舍，畜栏，牧场）? 如果有，选择所有存在的情况:	是 否 不适用
d. 野生动物和野生动物	是 否 不适用
e. 野生动物和家畜/家养动物	是 否 不适用
f. 家畜/家养动物和家畜/家养动物	是 否 不适用
动物 (野生动物和家畜/家养动物)是否能够自由活动?	是 否 不适用
是否有动物出现病症?	是 否 不适用
在该点是否屠宰过动物?	是 否 不适用
有没有在这里屠宰动物?	是 否 不适用
是否有些动物离厨房很近?	是 否 不适用
是否有人和动物一起生活的证据?	是 否 不适用
是否有人生病?	是 否 不适用
该观察点是否有小孩子活动?	是 否 不适用

V. 卫生设备和卫生状况

选择是、否或者不适用	**是 否 不适用**
该点是否有抵御天气和其它动物的设施?	是 否 不适用
这些是永久性设施吗?	是 否 不适用
该点是否有专门地点收集动物排泄物，食物残渣等废弃物?	是 否 不适用
该点是否有专门地方存放生活垃圾?	是 否 不适用
动物废弃物和生活垃圾存放点状况 (选择 1 个) 3. 未观察到 4. 有固定存放点，既没有维护，也未使用 5. 有固定存放点，维护很好，在使用	
是否有厕所，公共厕所或者其它指定存放生活垃圾的地方?	是 否 不适用
生活垃圾存放点状况 (选择 1 个) 3. 未观察到 4. 有固定存放点，既没有维护，也未使用 5. 有固定存放点，维护很好，在使用	
该地方是否有重要水源?	是 否 不适用
水源状况(选择 1 个) 3. 完全无保护，池塘, 没有顶盖 4. 雨水收集点, 运水车, 完全开放 5. 完全保护: 水利系统	
到水源地时间(选择 1 个) 3. >30 分钟步行 4. 5-30 分钟步行 5. 0-5 分钟步行	
写:	

7.6 Taiping and Foshan Market Species and Associated Viruses

Table 31. List of species observed in Taiping and Foshan markets in the greater Guangzhou area of Guangdong province China from 2014 to 2016. Information on IUCN and China Forestry Department endangered species classification as well as number of known viruses hosted by the species and associated references are also listed. China endangered species are from List of National Key Protected Wild Animals (PRC 2006). Class I lists critically endangered species and Class II lists endangered species (Cao 2015). The Red List of Threatened Species is from International Union for Conservation of Nature with the following terms: LC = Least Concern; NE = Not Evaluated; VU = Vulnerable (IUCN 2016).

Genus species	**Common Name**	**IUCN**	**China Endangered Species List**	**Number of Viruses Known to Host**	**Viruses Known to Host**	**References**
Sus scrofa	Wild boar	LC	NE	56	African_swine_fever_virus	ICTV database
					Banna_virus	Tao *et al*. 2005
					Border_disease_virus	Roehe *et al*. 1992
					Borna_disease_virus	Zhang *et al*. 2014
					Bovine_viral_diarrhea_virus_1	Van Gennip *et al*. 1999
					Classical_swine_fever_virus	Kosmidou *et al* 1998
					Encephalomyocarditis_virus	Tesh and Wallace 1978
					Foot-and-mouth_disease_virus	Ghoneim *et al*. 2010
					Getah_virus	Attoui *et al*. 2007
					Hepatitis_E_virus	Taylor *et al*. 2001
					Human_picobirnavirus	Banyai *et al*. 2008
					Influenza_A_virus	Kaden *et al*. 2008
					Influenza_C_virus	Kimura *et al*. 1997
					Japanese_encephalitis_virus	Nitatpattana *et al*. 2000
					Menangle_virus	Bowden *et al*. 2001
					Ndumu_virus	Masembe *et al*. 2012
					Nipah_virus	Calisher *et al*. 2006
					Norwalk_virus	Farkas *et al*. 2005
					Ovine_herpesvirus_2	Wessels *et al*. 2011
					Patois_virus	Scherer *et al*. 1972
					Porcine_adenovirus_A	Derbyshire *et al*. 1966
					Porcine_adenovirus_B	Kasza *et al*. 1966
					Porcine_adenovirus_C	Tuboly *et al*. 1995
					Porcine_astrovirus	Indik *et al*. 2006
					Porcine_circovirus-1	Kim *et al*. 2001
					Porcine_circovirus-2	Petrini *et al*. 2009
					Porcine_enteric_sapovirus	Martella *et al*. 2008
					Porcine_enterovirus_A	Dunne *et al*. 1971

Genus species	Common Name	IUCN	China Endangered Species List	Number of Viruses Known to Host	Viruses Known to Host	References
					Porcine_enterovirus_B	Moon *et al*. 2012
					Porcine_epidemic_diarrhea_virus	Puranaveja *et al*. 2009
					Porcine_hemagglutinating_encephalomyelitis_virus	Quiroga *et al*. 2008
					Porcine_parvovirus	Cadar *et al*. 2012
					Porcine_reproductive_and_respiratory_syndrome_virus	Dea *et al*. 1992
					Porcine_rubulavirus	Wang *et al*. 2007
					Porcine_teschovirus	Prodelalova 2012
					Porcine_torovirus	Kroneman *et al*. 1998
					Porcine_type-C_oncovirus	Armstrong *et al*. 1971
					Reston_ebolavirus	Barrette *et al*. 2009
					Rotavirus_A	Okadera *et al*. 2013
					Rotavirus_A	Okadera *et al*. 2013
					Rotavirus_B	Medici *et al*. 2010
					Rotavirus_C	Medici *et al*. 2010
					Rotavirus_E	Chasey *et al*. 1986
					Sendai_virus	Faisca *et al*. 2007
					Suid_herpesvirus_1	Hahn *et al*. 2010
					Swine_norovirus	Song *et al*. 2011
					Swinepox_virus	Moorkamp *et al*. 2008
					Thiafora_virus	Chastel 1989
					Torque_Teno_virus	Martelli *et al*. 2006
					Transmissible_gastroenteritis_virus	Morin *et al*. 1973
					Trubanaman_virus	Johansen *et al*. 2005
					Vaccinia_virus	Peres *et al*. 2013
					Vesicular_exanthema_of_swine_virus	Smith and Latham 1978
					Vesicular_stomatitis_Indiana_virus	Fletcher *et al*. 1985
					Wesselsbron_virus	Baba *et al*. 1994

Genus species	**Common Name**	**IUCN**	**China Endangered Species List**	**Number of Viruses Known to Host**	**Viruses Known to Host**	**References**
Ovis aries	Domestic sheep	NE	NE	47	Akabane_virus	Davies *et al*. 1985
					Alcelaphine_herpesvirus_1	Li *et al*. 1995
					Bhanja_virus	Hubalek 2009
					Bluetongue_virus	Allepuz *et al*. 2010
					Border_disease_virus	Valdazo-Gonzalez *et al*. 2007
					Borna_disease_virus	Vahlenkamp *et al*. 2002
					Bovine_herpesvirus_1	Kalman & Egyed 2005
					Bovine_herpesvirus_2	Kalman & Egyed 2005
					Bovine_herpesvirus_4	Kalman & Egyed 2005
					Bovine_respiratory_syncytial_virus	Karesh *et al*. 1998
					Bovine_viral_diarrhea_virus_1	Pratelli *et al*. 2001
					Bovine_viral_diarrhea_virus_2	Pratelli *et al*. 2001
					Bunyamwera_virus	Tauro *et al*. 2009
					Caprine_arthritis_encephalitis_virus	Leroux *et al*. 2010
					Colorado_tick_fever_virus	Emmons 1994
					Crimean-Congo_hemorrhagic_fever_virus	Taylor *et al*. 2001
					Dera_Ghazi_Khan_virus	Darwish *et al*. 1983
					Dugbe_virus	Davies 1978
					Foot-and-mouth_disease_virus	Ghoneim *et al*. 2010
					Gan_Gan_virus	Vale *et al*. 1991
					Hepatitis_E_virus	Meng *et al*.
					Ife_virus	Ezefeika *et al*. 1989
					Jaagsiekte_sheep_retrovirus	York *et al*. 1992
					Louping_ill_virus	Gao *et al* 1993
					Middelburg_virus	Jupp *et al*. 1998
					Orf_virus	Vikoren *et al*. 2008
					Orungo_virus	Brown *et al*. 1991
					Ovine_adenovirus_D	Belak *et al*. 1980
					Ovine_astrovirus	Herring *et al*. 1981
					Ovine_herpesvirus_2	Baxter *et al*. 1996
					Ovine_papillomavirus_1	Trenfield *et al*. 1990
					Palyam_virus	Aradaib *et al*. 2009
					Peste-des-petits-ruminants_virus	Taylor & Abegunde 1979
					Rabies_virus	Lembo *et al*. 2008

Genus species	Common Name	IUCN	China Endangered Species List	Number of Viruses Known to Host	Viruses Known to Host	References
					Rift_Valley_fever_virus	Andriamandimby *et al*. 2010
					Rinderpest_virus	Rossiter *et al*. 1989
					Rotavirus_B	Wani *et al*. 2004
					Salehabad_virus	CDC Arbocat
					Sheeppox_virus	Mangana-Vougiouka *et al*. 2000
					Suid_herpesvirus_1	Salwa 2004
					Thiafora_virus	Chastel 1989
					Thogoto_virus	Jones *et al*. 1987
					Torque_Teno_virus	Leary *et al*. 1999
					Trubanaman_virus	Johansen *et al*. 2005
					Visna/maedi_virus	Cortez-Romero *et al*. 2010
					Wad_Medani_virus	Darwish *et al*. 1983
					Wesselsbron_virus	Baba *et al*. 1994
Capra hircus	Domestic goat	NE	NE	34	Akabane_virus	Davies *et al*. 1985
					Bhanja_virus	Hubalek *et al*. 2009
					Bluetongue_virus	Allepuz *et al*. 2010
					Border_disease_virus	Kim *et al*. 2006
					Borna_disease_virus	Zhang *et al*. 2014
					Bovine_herpesvirus_1	Kalman & Egyed 2005
					Bovine_herpesvirus_4	Kalman & Egyed 2005
					Bovine_parainfluenza_virus_3	Yang *et al*. 2008
					Bovine_viral_diarrhea_virus_1	Pratelli *et al*. 2001
					Bunyamwera_virus	Tauro *et al*. 2009
					Caprine_arthritis_encephalitis_virus	Leroux *et al*. 2010
					Caprine_herpesvirus_1	Roperto *et al*. 2000
					Crimean-Congo_hemorrhagic_fever_virus	Mourya *et al*. 2012
					Dugbe_virus	Davies 1978
					Epizootic_hemorrhagic_disease_virus	Nol *et al*. 2010
					Foot-and-mouth_disease_virus	Ghoneim *et al*. 2010
					Goatpox_virus	Tulman *et al*. 2002
					Hepatitis_E_virus	Meng *et al*.
					Ife_virus	Ezefeika *et al*. 1989
					Jaagsiekte_sheep_retrovirus	York *et al*. 1992
					Orf_virus	Vikoren *et al*. 2008

Genus species	Common Name	IUCN	China Endangered Species List	Number of Viruses Known to Host	Viruses Known to Host	References
					Orungo_virus	Brown *et al*. 1991
					Ovine_herpesvirus_2	Jacobsen *et al*. 2007
					Palyam_virus	Aradaib *et al*. 2009
					Peste-des-petits-ruminants_virus	Taylor & Abegunde 1978
					Rabies_virus	Kuzmin *et al*. 2004
					Rift_Valley_fever_virus	Andriamandimby *et al*. 2010
					Rinderpest_virus	Rossiter *et al*. 1989
					Sepik_virus	Olson *et al*. 1984
					Sheeppox_virus	Bhanuprakash *et al*. 2010
					Suid_herpesvirus_1	Salwa 2004
					Trubanaman_virus	Johansen *et al*. 2005
					Wad_Medani_virus	Darwish *et al*. 1983
					Wesselsbron_virus	Baba *et al*. 1994
Canis lupus familiaris	Domestic Dog	NE	NE	30	African_horse_sickness_virus	Baba *et al*. 1993
					Barmah_Forest_virus	Kay *et al*. 2007
					Borna_disease_virus	Zhang *et al*. 2014
					Bunyamwera_virus	Calisher *et al*. 1986
					Canid_herpesvirus_1	Gadsden *et al*. 2012
					Canine_adenovirus	Ledbetter *et al*. 2009
					Canine_calicivirus	Matsuura*et al*. 2002
					Canine_distemper_virus	Frolich *et al*. 2000
					Canine_minute_virus	Binn *et al*. 1970
					Canine_oral_papillomavirus	Yhee *et al*. 2010
					Crimean-Congo_hemorrhagic_fever_virus	Shepherd *et al*. 1987
					Everglades_virus	Coffey *et al*. 2006
					Hepatitis_E_virus	Vitral *et al*. 2005
					Human_herpesvirus_4	Choi *et al*. 2005
					Human_norovirus-Alphatron	Martella *et al*. 2008
					Human_picobirnavirus	Costa *et al*. 2004
					Mammalian_orthoreovirus	Decaro *et al*. 2005
					Mokola_virus	Sabeta *et al*. 2007
					Mumps_virus	Stone 1969
					Murray_Valley_encephalitis_virus	Calisher *et al*. 2006
					Rabies_virus	Lembo *et al*. 2008

Genus species	Common Name	IUCN	China Endangered Species List	Number of Viruses Known to Host	Viruses Known to Host	References
					Ross_River_virus	Digoutte *et al*. 1976
					Sandfly_fever_Sicilian_virus	Sakhria *et al*. 2014
					St._Louis_encephalitis_virus	Lillibridge *et al*. 2004
					Suid_herpesvirus_1	Eidson *et al*.1953
					Vaccinia_virus	Peres *et al*. 2013
					Venezuelan_equine_encephalitis_virus	Estrada-Franco *et al*. 2004
					Vesicular_stomatitis_Indiana_virus	Webb *et al*. 1987b
					Wesselsbron_virus	Baba *et al*. 1994
Rattus norvegicus	Brown rat	LC	NE	21	Andes virus	Fernandez *et al*. 2008
					Cowpox_virus	Wolfs *et al*. 2002
					Crimean-Congo_hemorrhagic_fever_virus	Darwish *et al*. 1983
					Encephalomyocarditis_virus	Tesh & Wallace 1978
					H-1_parvovirus	Fregolente *et al*. 2009
					Human_picobirnavirus	Easterbrook *et al*. 2009
					Kilham_rat_virus	Kilham & Olivier 1959
					Lassa_virus	Nitatpattana *et al*. 2000
					Murid_herpesvirus_2	Bruggeman *et al*. 1982
					Murine_pneumotropic_virus	Easterbrook *et al*. 2008
					Rat_coronavirus	Parker *et al*. 1970
					Rat_minute_virus_1	Wan *et al*. 2002, 2006
					Rat_parvovirus_1	Darwish *et al*. 1983
					Salehabad_virus	Easterbrook *et al*. 2008
					Sendai_virus	Wang *et al*. 2000
					Seoul_virus	Easterbrook *et al*. 2008
					Theilovirus	Theiler 1934
					Venezuelan_equine_encephalitis_virus	Sudia & Newhouse 1975
					Wad_Medani_virus	Darwish *et al*. 1983
					West_Nile_virus	Gomez *et al*. 2008
					Whitewater_Arroyo_virus	Bennett *et al*. 2000
Felis catus	Domestic Cat	NE	NE	17	Barmah_Forest_virus	Kay *et al*. 2007
					Borna_disease_virus	Kamnieh *et al*. 2008
					Encephalomyocarditis_virus	Smith *et al*. 1992
					Feline_astrovirus	Atkins at al. 2009
					Feline_calicivirus	Ohe *et al*. 2006

Genus species	Common Name	IUCN	China Endangered Species List	Number of Viruses Known to Host	Viruses Known to Host	References
					Feline_coronavirus	Kipar *et al*. 2010
					Feline_foamy_virus	Winkler *et al*. 1999
					Feline_immunodeficiency_virus	Fujimo *et al*. 2008
					Feline_leukemia_virus	Englert *et al*. 2012
					Feline_papillomavirus	Terai & Burk 2002
					Human_herpesvirus_4	Milman *et al*. 2010
					Human_norovirus-Alphatron	Di Martino *et al*. 2010
					Mokola_virus	Meredith *et al*. 1996
					Rabies_virus	Nadin-Davies *et al*. 1994
					Racoonpox_virus	Yager *et al*. 2006
					Suid_herpesvirus_1	Salwa 2004
					Vaccinia_virus	Peres *et al*. 2013
Cervus elaphus	Red deer	LC	II	13	Bluetongue_virus	Ruiz-Fons *et al*. 2008
					Bovine_herpesvirus_1	Kalman & Egyed 2005
					Bovine_herpesvirus_2	Kalman & Egyed 2005
					Bovine_herpesvirus_4	Kalman & Egyed 2005
					Bovine_viral_diarrhea_virus_1	Glawishnig *et al*. 2010
					Caprine_herpesvirus_1	Frolich *et al*. 2006
					Cervid_herpesvirus_1	Squires 2012
					Deer_adenovirus	Horner & Read 1982
					Epizootic_hemorrhagic_disease_virus	Nol *et al*. 2010
					Ovine_herpesvirus_2	Vikoren *et al*. 2006
					Parapoxvirus_of_red_deer_in_New_Zealand	Horner *et al*. 1987
					Thiafora_virus	Chastel 1989
					Vesicular_stomatitis_Indiana_virus	Webb *et al*. 1987
Vulpes vulpes	Silver fox	LC	NE	5	California encephalitis virus	Parkin *et al*. 1973
					Canine_adenovirus	Thompson *et al*. 2010
					Rabies_virus	Nadin-Davies *et al*. 1994
					St._Louis_encephalitis_virus	Artsob *et al*. 1986
					Trubanaman_virus	Johansen *et al*. 2005
Myocastor coypus	Nutria	LC	NE	3	Encephalomyocarditis_virus	Bollo *et al*. 2003
					Rabies_virus	Childs *et al*. 1997
					Vesicular_stomatitis_Indiana_virus	Aguirre *et al*. 1992

Genus species	**Common Name**	**IUCN**	**China Endangered Species List**	**Number of Viruses Known to Host**	**Viruses Known to Host**	**References**
Nyctereutes procyonoides	Raccoon dog	LC	NE	3	Rabies_virus Rotavirus_A SL-COV	(Kuzmin *et al.* 2006) (Abe *et al.* 2010) (Tu *et al.* 2004)
Prionailurus bengalensis	Leopard cat	LC	NE	2	Feline_calicivirus Feline_panleukopenia_virus	(O'Brien *et al.* 2012) (O'Brien *et al.* 2012)
Paguma larvata	Masked palm civet	LC	NE	2	SL-COV Rotavirus A	(Abe *et al.* 2010) (Tu *et al.* 2004)
Lepus sinensis	Chinese hare	LC	NE	1	Klebsiella pneumoniae	(Du *et al.* 2014)
Melogale moschata	Ferret badger	LC	NE	1	SL-COV	(Tu *et al.* 2004)
Petaurista petaurista	Common Giant flying squirrel	LC	NE	1	Kyasanur virus	(Bhat *et al.* 1979)
Muntiacus reevesi	Reeve's muntjac	LC	NE	1	Gammaherpesvirus	(Dick *et al.* 2012)
Rusa unicolor	Sambar deer	VU	NE	1	Foot-and-mouth_disease_virus	(Barman *et al.* 1999)
Mustela sibirica	Siberian weasel	LC	NE	1	Hantavirus	(Ge *et al.* 2016b)
Marmota baibacina	Grey Marmot	LC	NE	0	-	-
Rhizomys pruinosus	Hoary Bamboo Rat	LC	NE	0	-	-
Rhizomys sinensis	Chinese Bamboo rat	LC	NE	0	-	-
Arctonyx collaris	Hog badger	VU	NE	0	-	-
Erinaceus amurensis	Manchurian Hedgehog	LC	NE	0	-	-
Hystrix brachyura	Malayan Porcupine	LC	NE	0	-	-
Mustela kathiah	Yellow Bellied Weasel	LC	NE	0	-	-

Table 32. List of 143 viruses reported by other research (See Table 31) to have been found in the same species that were observed in this study in Taiping and Foshan live animal wildlife markets in Guangdong Province. The third column from the left indicates the 60 viruses also reported to infect humans (+) as well as the 83 viruses not known to infect humans (-). The rightmost column lists the species observed in the two markets that have been reported carriers or hosts of the listed viruses.

Viruses Reported in Same Species as Observed in Markets	**Viral Family**	**Present in Humans**	**Viral Host Species Observed in Markets**
African horse sickness virus	Reoviridae	-	*Canis lupus familiaris*
African swine fever virus	Asfarviridae	-	*Sus scrofa*
Akabane virus	Bunyaviridae	-	*Capra hircus, Ovis aries*
Alcelaphine herpesvirus 1	Herpesviridae	-	*Ovis aries*
Andes virus	Bunyaviridae	+	*Rattus norvegicus*
Banna virus	Reoviridae	+	*Sus scrofa*
Barmah Forest virus	Togaviridae	+	*Felis catus, Canis lupus familiaris*
Bhanja virus	Bunyaviridae	+	*Capra hircus, Ovis aries*
Bluetongue virus	Reoviridae	-	*Cervus elaphus, Capra hircus, Ovis aries*
Border disease virus	Flaviviridae	-	*Capra hircus, Ovis aries, Sus scrofa*
Borna disease virus	Bornaviridae	+	*Felis catus, Canis lupus familiaris, Capra hircus, Ovis aries, Sus scrofa*
Bovine herpesvirus 1	Herpesviridae	-	*Cervus elaphus, Capra hircus, Ovis aries*
Bovine herpesvirus 2	Herpesviridae	-	*Cervus elaphus, Ovis aries*
Bovine herpesvirus 4	Herpesviridae	-	*Cervus elaphus, Capra hircus, Ovis aries*
Bovine parainfluenza virus 3	Paramyxoviridae	-	*Capra hircus*
Bovine respiratory syncytial virus	Paramyxoviridae	-	*Ovis aries*
Bovine viral diarrhea virus 1	Flaviviridae	-	*Cervus elaphus, Capra hircus, Ovis aries, Sus scrofa*
Bovine viral diarrhea virus 2	Flaviviridae	-	*Ovis aries*
Bunyamwera virus	Bunyaviridae	+	*Canis lupus familiaris, Capra hircus, Ovis aries*
California encephalitis virus	Bunyaviridae	+	*Vulpes vulpes*
Canid herpesvirus 1	Herpesviridae	-	*Canis lupus familiaris*
Canine adenovirus	Adenoviridae	-	*Vulpes vulpes, Canis lupus familiaris*
Canine calicivirus	Caliciviridae	-	*Canis lupus familiaris*
Canine distemper virus	Paramyxoviridae	-	*Canis lupus familiaris*
Canine minute virus	Parvoviridae	-	*Canis lupus familiaris*
Canine oral papillomavirus	Papillomaviridae	-	*Canis lupus familiaris*
Caprine arthritis encephalitis virus	Retroviridae	-	*Capra hircus, Ovis aries*
Caprine herpesvirus 1	Herpesviridae	-	*Cervus elaphus, Capra hircus*
Cervid herpesvirus 1	Herpesviridae	-	*Cervus elaphus*

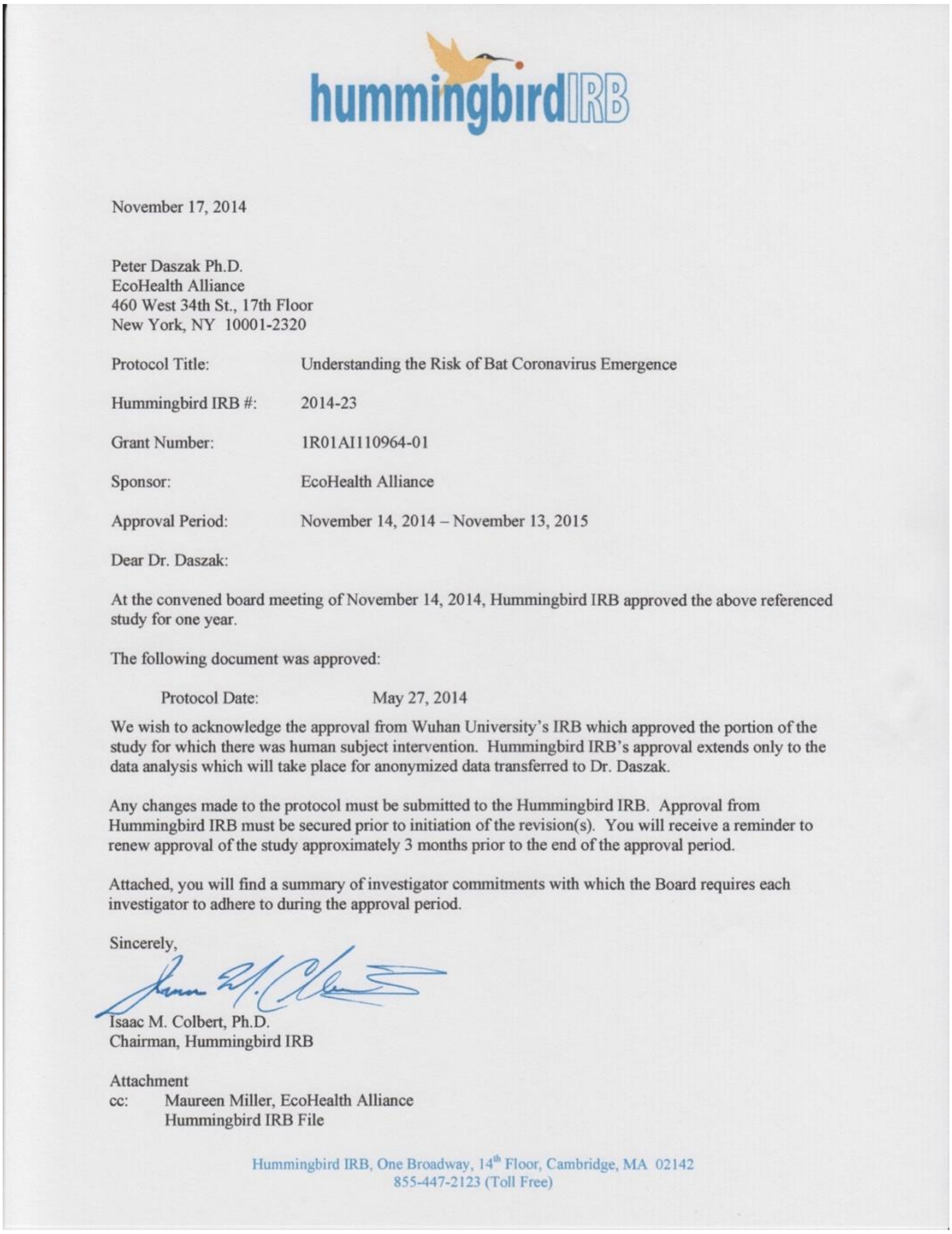
Viruses Reported in Same Species as Observed in Markets	Viral Family	Present in Humans	Viral Host Species Observed in Markets
Classical swine fever virus	Flaviviridae	-	*Sus scrofa*
Colorado tick fever virus	Reoviridae	+	*Ovis aries*
Cowpox virus	Poxviridae	+	*Rattus norvegicus*
Crimean-Congo hemorrhagic fever virus	Bunyaviridae	+	*Rattus norvegicus, Canis lupus familiaris, Capra hircus, Ovis aries*
Deer adenovirus	Adenoviridae	-	*Cervus elaphus*
Dera Ghazi Khan virus	Bunyaviridae	+	*Ovis aries*
Dugbe virus	Bunyaviridae	+	*Capra hircus, Ovis aries*
Encephalomyocarditis virus	Picornaviridae	+	*Myocastor coypus, Felis catus, Rattus norvegicus, Sus scrofa*
Epizootic hemorrhagic disease virus	Reoviridae	-	*Cervus elaphus, Capra hircus*
Everglades virus	Togaviridae	+	*Canis lupus familiaris*
Feline astrovirus	Astroviridae	-	*Felis catus*
Feline calicivirus	Caliciviridae	-	*Prionailurus bengalensis, Felis catus*
Feline coronavirus	Coronaviridae	-	*Felis catus*
Feline foamy virus	Retroviridae	-	*Felis catus*
Feline immunodeficiency virus	Retroviridae	-	*Felis catus*
Feline leukemia virus	Reoviridae	-	*Felis catus*
Feline panleukopenia virus	Parvoviridae	-	*Prionailurus bengalensis*
Feline papillomavirus	Papillomaviridae	-	*Felis catus*
Foot-and-mouth disease virus	Picornaviridae	+	*Rusa unicolor, Sus scrofa, Ovis aries, Capra hircus*
Gammaherpesvirus	Herpesviridae	-	*Muntiacus reevesi*
Gan Gan virus	Bunyaviridae	+	*Ovis aries*
Getah virus	Togaviridae	+	*Sus scrofa*
Goatpox virus	Poxviridae	-	*Capra hircus*
H-1 parvovirus	Parvoviridae	-	*Rattus norvegicus*
Hantavirus	Bunyaviridae	-	*Mustela sibirica*
Hepatitis E virus	Hepeviridae	+	*Canis lupus familiaris, Capra hircus Ovis aries, Sus scrofa*
Human herpesvirus 4	Herpesviridae	+	*Felis catus, Canis lupus familiaris*
Human norovirus-Alphatron	Caliciviridae	+	*Felis catus, Canis lupus familiaris*
Human picobirnavirus	Picornaviridae	+	*Rattus norvegicus, Canis lupus familiaris, Sus scrofa*
Ife virus	Reoviridae	-	*Capra hircus, Ovis aries*
Influenza A virus	Orthomyxoviridae	+	*Sus scrofa*
Influenza C virus	Orthomyxoviridae	+	*Sus scrofa*

Viruses Reported in Same Species as Observed in Markets	Viral Family	Present in Humans	Viral Host Species Observed in Markets
Jaagsiekte sheep retrovirus	Retroviridae	-	*Capra hircus, Ovis aries*
Japanese encephalitis virus	Flaviviridae	+	*Sus scrofa*
Kilham rat virus	Parvoviridae	-	*Rattus norvegicus*
Klebsiella pneumoniae	Flaviviridae	-	*Lepus sinensis*
Kyasanur virus	Flaviviridae	+	*Petaurista petaurista*
Lassa virus	Arenaviridae	+	*Rattus norvegicus*
Louping ill virus	Flaviviridae	+	*Ovis aries*
Mammalian orthoreovirus	Reoviridae	-	*Canis lupus familiaris*
Menangle virus	Paramyxoviridae	+	*Sus scrofa*
Middelburg virus	Togaviridae	-	*Ovis aries*
Mokola virus	Rhabdoviridae	+	*Felis catus, Canis lupus familiaris*
Mumps virus	Paramyxoviridae	+	*Canis lupus familiaris*
Murid herpesvirus 2	Herpesviridae	-	*Rattus norvegicus*
Murine pneumotropic virus	Pneumoviridae	-	*Rattus norvegicus*
Murray Valley encephalitis virus	Flaviviridae	+	*Canis lupus familiaris*
Ndumu virus	Togaviridae	+	*Sus scrofa*
Nipah virus	Paramyxoviridae	+	*Sus scrofa*
Norwalk virus	Caliciviridae	+	*Sus scrofa*
Orf virus	Poxviridae	+	*Capra hircus, Ovis aries*
Orungo virus	Reoviridae	+	*Capra hircus, Ovis aries*
Ovine adenovirus D	Adenoviridae	-	*Ovis aries*
Ovine astrovirus	Astroviridae	-	*Ovis aries*
Ovine herpesvirus 2	Herpesviridae	-	*Cervus elaphus, Capra hircus, Ovis aries, Sus scrofa*
Ovine papillomavirus 1	Papillomaviridae	-	*Ovis aries*
Palyam virus	Reoviridae	+	*Capra hircus, Ovis aries*
Parapoxvirus of red deer in New Zealand	Poxviridae	-	*Cervus elaphus*
Patois virus	Bunyaviridae	+	*Sus scrofa*
Peste-des-petits-ruminants virus	Paramyxoviridae	-	*Capra hircus, Ovis aries*
Porcine adenovirus A	Adenoviridae	-	*Sus scrofa*
Porcine adenovirus B	Adenoviridae	-	*Sus scrofa*
Porcine adenovirus C	Adenoviridae	-	*Sus scrofa*
Porcine astrovirus	Astroviridae	-	*Sus scrofa*

Viruses Reported in Same Species as Observed in Markets	Viral Family	Present in Humans	Viral Host Species Observed in Markets
Porcine circovirus-1	Circoviridae	-	*Sus scrofa*
Porcine circovirus-2	Circoviridae	-	*Sus scrofa*
Porcine enteric sapovirus	Caliciviridae	-	*Sus scrofa*
Porcine enterovirus A	Picornaviridae	-	*Sus scrofa*
Porcine enterovirus B	Picornaviridae	-	*Sus scrofa*
Porcine epidemic diarrhea virus	Coronaviridae	-	*Sus scrofa*
Porcine hemagglutinating encephalomyelitis virus	Coronaviridae	-	*Sus scrofa*
Porcine parvovirus	Parvoviridae	-	*Sus scrofa*
Porcine reproductive and respiratory syndrome virus	Arteriviridae	-	*Sus scrofa*
Porcine rubulavirus	Paramyxoviridae	-	*Sus scrofa*
Porcine teschovirus	Picornaviridae	-	*Sus scrofa*
Porcine torovirus	Coronaviridae	-	*Sus scrofa*
Porcine type-C oncovirus	Retroviridae	-	*Sus scrofa*
Rabies virus	Rhabdoviridae	+	*N. procyonoides, Myocastor coypus, V. vulpes, Felis catus, C. hircus, O. aries, Canis lupus familiaris*
Racoonpox virus	Poxviridae	-	*Felis catus*
Rat coronavirus	Coronaviridae	-	*Rattus norvegicus*
Rat minute virus 1	Parvoviridae	-	*Rattus norvegicus*
Rat parvovirus 1	Parvoviridae	-	*Rattus norvegicus*
Reston ebolavirus	Filoviridae	+	*Sus scrofa*
Rift Valley fever virus	Bunyaviridae	+	*Capra hircus, Ovis aries*
Rinderpest virus	Paramyxoviridae	-	*Capra hircus, Ovis aries*
Ross River virus	Togaviridae	+	*Canis lupus familiaris*
Rotavirus A	Reoviridae	+	*Paguma larvata, Nyctereutes procyonoides, Sus scrofa*
Rotavirus B	Reoviridae	+	*Sus scrofa, Ovis aries*
Rotavirus C	Reoviridae	+	*Sus scrofa*
Rotavirus E	Reoviridae	-	*Sus scrofa*
Salehabad virus	Bunyaviridae	-	*Rattus norvegicus, Ovis aries*
Sandfly fever Sicilian virus	Bunyaviridae	+	*Canis lupus familiaris*
Sendai virus	Paramyxoviridae	-	*Rattus norvegicus, Sus scrofa*
Seoul virus	Bunyaviridae	+	*Rattus norvegicus*
Sepik virus	Flaviviridae	-	*Capra hircus*
Severe acute respiratory syndrome-related coronavirus	Coronaviridae	+	*Melogale moschata, Nyctereutes procyonoides, Paguma larvata*

Viruses Reported in Same Species as Observed in Markets	**Viral Family**	**Present in Humans**	**Viral Host Species Observed in Markets**
Sheeppox virus	Poxviridae	-	*Capra hircus, Ovis aries*
St. Louis encephalitis virus	Flaviviridae	+	*Vulpes vulpes, Canis lupus familiaris*
Suid herpesvirus 1	Herpesviridae	+	*Felis catus, Canis lupus familiaris, Capra hircus, Ovis aries, Sus scrofa*
Swine norovirus	Caliciviridae	-	*Sus scrofa*
Swinepox virus	Poxviridae	-	*Sus scrofa*
Theilovirus	Picornaviridae	-	*Rattus norvegicus*
Thiafora virus	Bunyaviridae	+	*Sus scrofa, Cervus elaphus, Ovis aries*
Thogoto virus	Orthomyxoviridae	+	*Ovis aries*
Torque Teno virus	Anelloviridae	+	*Ovis aries, Sus scrofa*
Transmissible gastroenteritis virus	Coronaviridae	-	*Sus scrofa*
Trubanaman virus	Bunyaviridae	+	*Vulpes vulpes, Capra hircus, Ovis aries, Sus scrofa*
Vaccinia virus	Poxviridae	+	*Felis catus, Canis lupus familiaris, Sus scrofa*
Venezuelan equine encephalitis virus	Togaviridae	+	*Rattus norvegicus, Canis lupus familiaris*
Vesicular exanthema of swine virus	Caliciviridae	-	*Sus scrofa*
Vesicular stomatitis Indiana virus	Rhabdoviridae	+	*Myocastor coypus, Cervus elaphus, Sus scrofa, Canis lupus familiaris*
Visna/maedi virus	Retroviridae	-	*Ovis aries*
Wad Medani virus	Reoviridae	-	*Rattus norvegicus, Capra hircus, Ovis aries*
Wesselsbron virus	Flaviviridae	+	*Canis lupus familiaris, Capra hircus, Ovis aries, Sus scrofa*
West Nile virus	Flaviviridae	+	*Rattus norvegicus*
Whitewater Arroyo virus	Arenaviridae	+	*Rattus norvegicus*

7.7 Hummingbird IRB Behavioural Protocol Approval



hummingbirdIRB

November 17, 2014

Peter Daszak Ph.D.
EcoHealth Alliance
460 West 34th St., 17th Floor
New York, NY 10001-2320

Protocol Title:	Understanding the Risk of Bat Coronavirus Emergence
Hummingbird IRB #:	2014-23
Grant Number:	1R01AI110964-01
Sponsor:	EcoHealth Alliance
Approval Period:	November 14, 2014 – November 13, 2015

Dear Dr. Daszak:

At the convened board meeting of November 14, 2014, Hummingbird IRB approved the above referenced study for one year.

The following document was approved:

Protocol Date:	May 27, 2014

We wish to acknowledge the approval from Wuhan University's IRB which approved the portion of the study for which there was human subject intervention. Hummingbird IRB's approval extends only to the data analysis which will take place for anonymized data transferred to Dr. Daszak.

Any changes made to the protocol must be submitted to the Hummingbird IRB. Approval from Hummingbird IRB must be secured prior to initiation of the revision(s). You will receive a reminder to renew approval of the study approximately 3 months prior to the end of the approval period.

Attached, you will find a summary of investigator commitments with which the Board requires each investigator to adhere to during the approval period.

Sincerely,

Isaac M. Colbert, Ph.D.
Chairman, Hummingbird IRB

Attachment
cc: Maureen Miller, EcoHealth Alliance
Hummingbird IRB File

Hummingbird IRB, One Broadway, 14th Floor, Cambridge, MA 02142
855-447-2123 (Toll Free)

7.8 Wuhan University IRB Behavioural Protocol Approval

WUHAN UNIVERSITY
299 Bayi Rd., Wuhan 430072, Hubei, P.R. China

Wuhan University Ethics Approval Board

Research Study US NIAID R01AI110964: Understanding the Risk of Bat Coronavirus Emergence

This multidisciplinary study will include human subjects research. The human subjects research is both qualitative and quantitative. The focus is on the type and frequency of animal contact, as well as the range of wildlife observed. The research provides a framework to gain rapid understanding of human-animal interactions. Alignment of the human subjects research will coincide with animal biological surveillance to maximize the understanding of transmission risk with the potentially zoonotic pathogens identified in animal populations.

Volunteers will be recruited by word of mouth or by referral from key informants or other participants from the two target groups (ie, wildlife value chain participants and those highly exposed to wildlife, particularly bats) in Guangdong, Guangxi, Yunnan, and Fujian provinces in cooperation with local Bureaus of Public Health and CDCs. We will identify three sites in each province for a total of 12 sites representing the range of settings where the target population may be found (eg, bat caves, wet markets; formal and informal wildlife trade posts; animal transport/travel routes and mechanisms including transport storage and exchange centers, and wildlife value chain supporting industries such as guesthouses, restaurants, medicinal/magical/material animal parts and animal by-product preparers, vendors and purchasers). It is anticipated that eight focus groups (two per province) of approximately 8-10 individuals each (ie, a total of 48-80) and 144 ethnographic interviews (12 per site) will be conducted. Therefore, a total of 192 to 224 individuals will participate in qualitative research. With participant permission, qualitative interviews and focus groups will be recorded.

For the behavioral survey, a sampling frame and recruitment materials for this quantitative research will be developed in Year 2. It is anticipated that approximately 2500 individuals will be interviewed and asked to provide blood (no more than 550ml), sputum, and stool samples. We will screen sera for antibodies to SARS-CoV, other alpha & beta coronaviruses including MERS-CoV, and novel bat-CoVs.

Only adults 18 years or older will be invited to participate. At least one of the focus groups and an estimated 35-40% of the interviews and surveys will be conducted with women. Subjects will be enrolled in this study without regard to ethnicity. The primary enrollment criteria are related to occupational exposure to wildlife and residence near wildlife. All participants will sign an informed consent approved by the Wuhan Ethics Approval Board. In recognition of the time and expertise offered by study participants, each person will be offered a small token of practical, emotional or social significance. The token will not cost a lot of money, nor will it be money.

All data, including notes, recordings, questionnaires, and computer files will be will be coded to strictly preserve confidentiality. Paper files will be scanned electronically and then shredded. Biological samples will be coded to maintain anonymity of sample results. Identifying information such as consent forms and test results will be kept under lock and key in a file cabinet. All electronic data will be encrypted. Data access will be limited to investigators conducting analyses; data will have protections with data access codes required. Data collection is cross sectional and master list data will not be required for the analysis of data. Data will be presented in the aggregate. Original data will be stored for five years after the completion of the study. At that time, electronic files will be permanently deleted.

Chuanhua Yu

Chuanhua Yu, Ph.D
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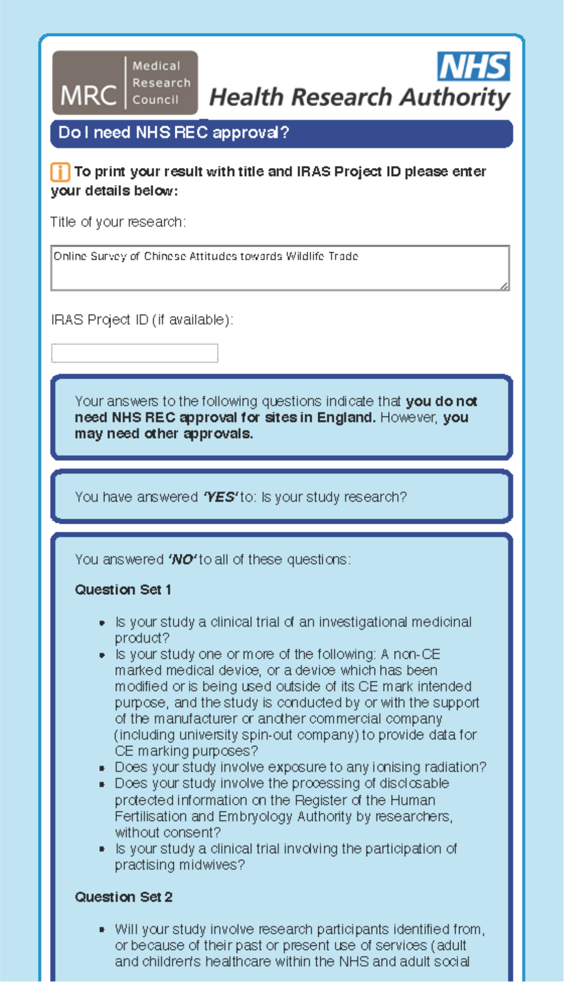
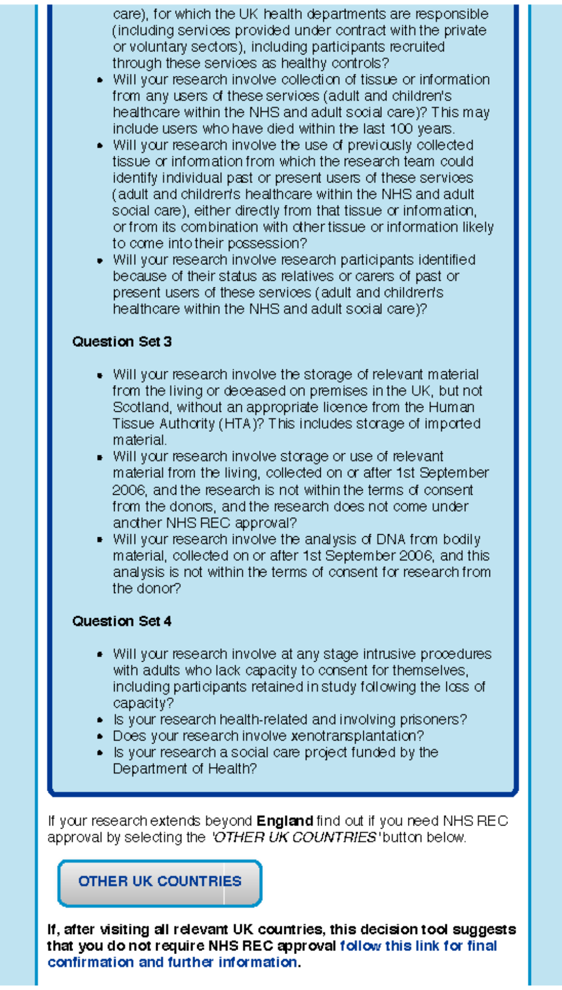
Email: yuchua@163.com

Nov. 11, 2014

7.9 Medical Research Council Online Survey Protocol Approval

Do I need NHS REC approval?

To print your result with title and IRAS Project ID please enter your details below:

Title of your research:

Online Survey of Chinese Attitudes towards Wildlife Trade

IRAS Project ID (if available):

Your answers to the following questions indicate that **you do not need NHS REC approval for sites in England.** However, **you may need other approvals.**

You have answered ***'YES'*** to: Is your study research?

You answered ***'NO'*** to all of these questions:

Question Set 1

- Is your study a clinical trial of an investigational medicinal product?
- Is your study one or more of the following: A non-CE marked medical device, or a device which has been modified or is being used outside of its CE mark intended purpose, and the study is conducted by or with the support of the manufacturer or another commercial company (including university spin-out company) to provide data for CE marking purposes?
- Does your study involve exposure to any ionising radiation?
- Does your study involve the processing of disclosable protected information on the Register of the Human Fertilisation and Embryology Authority by researchers, without consent?
- Is your study a clinical trial involving the participation of practising midwives?

Question Set 2

- Will your study involve research participants identified from, or because of their past or present use of services (adult and children's healthcare within the NHS and adult social care), for which the UK health departments are responsible (including services provided under contract with the private or voluntary sectors), including participants recruited through these services as healthy controls?
- Will your research involve collection of tissue or information from any users of these services (adult and children's healthcare within the NHS and adult social care)? This may include users who have died within the last 100 years.
- Will your research involve the use of previously collected tissue or information from which the research team could identify individual past or present users of these services (adult and children's healthcare within the NHS and adult social care), either directly from that tissue or information, or from its combination with other tissue or information likely to come into their possession?
- Will your research involve research participants identified because of their status as relatives or carers of past or present users of these services (adult and children's healthcare within the NHS and adult social care)?

Question Set 3

- Will your research involve the storage of relevant material from the living or deceased on premises in the UK, but not Scotland, without an appropriate licence from the Human Tissue Authority (HTA)? This includes storage of imported material.
- Will your research involve storage or use of relevant material from the living, collected on or after 1st September 2006, and the research is not within the terms of consent from the donors, and the research does not come under another NHS REC approval?
- Will your research involve the analysis of DNA from bodily material, collected on or after 1st September 2006, and this analysis is not within the terms of consent for research from the donor?

Question Set 4

- Will your research involve at any stage intrusive procedures with adults who lack capacity to consent for themselves, including participants retained in study following the loss of capacity?
- Is your research health-related and involving prisoners?
- Does your research involve xenotransplantation?
- Is your research a social care project funded by the Department of Health?

If your research extends beyond **England** find out if you need NHS REC approval by selecting the *'OTHER UK COUNTRIES'* button below.

If, after visiting all relevant UK countries, this decision tool suggests that you do not require NHS REC approval follow this link for final confirmation and further information.

7.10 Online Survey – Wildlife Trade and You! (Chinese Version)

野生动物贸易中的你我它

人们总是在谈论野生动物贸易，这真的和我有关吗？
那就让我们来一探究竟吧！您只需5分钟就能找到答案。
所有回答都秉着自愿的原则，您所提供的所有信息都会得到保密。

让我们更多地了解您

您的年龄 *

○15岁以下
○15-24岁
○25-39岁
○40-59岁
○60或60岁以上

您所在的省份： *

○安徽 ○北京 ○重庆 ○福建 ○甘肃 ○广东 ○广西 ○贵州
○海南 ○河北 ○黑龙江 ○河南 ○香港 ○湖北 ○湖南 ○江苏
○江西 ○吉林 ○辽宁 ○澳门 ○内蒙古 ○宁夏 ○青海 ○山东
○上海 ○山西 ○陕西 ○四川 ○台湾 ○天津 ○新疆 ○西藏
○云南 ○浙江 ○海外

您每月收入（人民币） *

○2,000以下
○2,001-4,000
○4,001-6,000
○6,001-8,000
○8,000以上

您所取得的，或者现在正在攻读的最高学位 *

○小学
○中学/中专/技校
○大学本科/大专
○硕士、博士及以上

两个小测试，您可以在提交问卷后查看正确答案

您知道下列哪些疾病来源于动物吗？(选择您认为正确的所有选项) * [多选题]

□艾滋病（HIV/AIDS）

□ 非典（SARS）
□ 中东呼吸综合征（MERS）
□ 禽流感（Avian Influenza）
□ 埃博拉（Ebola）
□ 非典（SARS）和禽流感（Avian Influenza）
□ 所有都是

您或许已经意识到有些传染性疾病是源自于动物的，那您知道在我们现在已知的传染性疾病中，有多少是来自于与野生动物的接触呢？ *

○ 20%
○ 40%
○ 60%
○ 80%

一起来了解野生动物贸易

您是否曾经（请勾选所有适合的选项） * [多选题]

□ 去过任何花鸟鱼虫或野生动物交易市场
□ 使用任何野生动物工艺制品作为装饰，如象牙、红珊瑚、或盔犀鸟等
□ 在农贸市场购买活的野生动物
□ 把野生动物当做宠物饲养
□ 使用药用野生动物或制品，如穿山甲壳、虎骨、犀牛角、蛇胆或熊胆制品等
□ 在家或餐馆吃野味
□ 捕猎野生动物
□ 宰杀或处理野生动物
□ 没有任何以上行为

野生动物贸易是指任何人为地出售或者交换野生动物资源，这里面往往伴随着从捕杀到运输，到销售，最终到消费者的过程。人们以多样的方式参与到野生动物贸易的不同环节中，并在此过程中与野生动物产生频繁的接触。

让我们听听您的想法吧

您认为野生动物贸易会带来以下哪些影响吗？（请选择所有您认为正确的选项） * [多选题]

□ 一些野生动物可能会遭受伤害甚至因此灭绝
□ 生态环境会遭到破坏
□ 人们可能会因为与野生动物的接触而染上疾病
□ 不知道

您认为我们应该停止捕杀和买卖野生动物，并将其作为食物、宠物、药材、或工艺品进行交易吗（这就

是我们所说的野生动物贸易）？ *

○是的，应该停止

○不应该或者没必要

如果人们停止野生动物交易的行为，您认为最重要的原因是什么？（请进行排序） * [排序题]

□ 相关的法律越来越严格

□ 非野生动物及其制品更加便宜并且容易获得

□ 保护野生动物和生态环境

□ 预防像非典或者埃博拉这样的来自动物的疾病

□ 购买野生动物及其制品的行为会遭到社会大众和身边人的批判

在减少野生动物贸易的工作中，你相信谁能够真正带来改变？ * [多选题]

□政府相关部门（如林业，卫生，农业等部门）

□社区和当地居民

□非政府公益组织

□商业界

□学术机构

□其它 ____________

7.11 Qualitative Interview Guide (English)

Core Themes

1. Human movement
2. Socioeconomics
3. Biosecurity in human environments
4. Illness, medical care/treatment and death of humans
5. Human-animal contact

HUMAN MOVEMENT

GOAL: To understand living environment and 'home range' (e.g., how far people travel and why).

Home

Where do you live/what kind of dwelling? How many people are in the household? How many rooms? How many are children? Is everyone related? Sleeping arrangements?

How often do you move? Any seasonality of movements?—eg, for work, for food, for safety (e.g., against flood, drought, conflict)?

What are the things you do to protect your home (against predators, animals, outsiders, bad weather)?

Work

What kind of work or activities do you do? What do other household members do? Where do these activities happen?

How do you protect your activities and business interests? (e.g., grazing or crop land, business competition, hunting territory, animal stock)

Travel (*)

How far do household members travel from home and why? (Follow up on animal related issues: shopping, selling/buying/trading, hunting, transport, etc)

How travel (by foot, bike, cart, truck, plane)? Is it ever for overnight? Where stay?

Why traveling? (work/migrant, family, religion, holidays, to sell/trade/buy animals)

Other family members in other areas of the country? Visit often?

Observed environment

Have there been any changes in the environment: new roads, more boats or ports, fields, buildings, population movement (in or out), land clearing or abandonment, new houses, other new buildings

Who is responsible for the changes? Are the changes good or bad?

SOCIOECONOMICS

GOAL: To understand a typical day and how money and social standing impact opportunity and risk.

Daily routine

Tell me about your daily routine (get description of work on a usual day, include purchasing and preparing food, timing of types of meals, responsibilities/duties related to animals, any changes by season)

How do people in the household contribute to earning money and getting food (and water)?

Where do the children play? Who takes care of the children when you are at work?

Animal responsibilities

Describe the animal related jobs and responsibilities for people at every age (i.e., young children, older children, young adults, adults, elderly).

What are the skills/knowledge needed before moving to the next stage of duties/responsibilities?

Are there differences in responsibilities between boys and girls, men and women, by ethnicity or class? (*)

Education

How many children are currently in school? Until what age do your children go to school? (boys and girls?)

What is your level of education? Why did you stop?

Economics

Do you make more money than other people who do the same things as you? Why do you think that is?

Are there times of year when you make less money? What happens then?

Are there times when food is more expensive than others? Tell me about that (eg, different food availability, seasonal, festival related).

Do you think you and your household are better off than most people? Could you do things to make it better?

BIOSECURITY IN HUMAN ENVIRONMENTS

GOAL: To determine if any sanitation or hygiene factors could play a role in disease spillover

Water and food

Is there a central source of water? What is the source? (eg, pond, uncovered well, rainwater, taps, covered well)

Is there a water source you like better?

How far away is the water source? Do animals drink from the same source?

Do you do anything to your drinking water to clean it before you drink it?

How do you store your food? (e.g., open containers, covered, hanging, refrigerate)

Do you eat or drink things where you suspect animal contact? (e.g., teeth/scratch marks, feces or urine seen)

Do you regularly clean your food prep station/kitchen and tools? How?

Sanitation

Are there toilets, latrines or other designated areas for human waste? Are these cleaned and used regularly?

Are butchering and slaughtering areas separate? How often are they cleaned and how? Who does the cleaning?

Are there any official rules or laws about human waste and garbage disposal?

Are there any animal pest control laws? What do you do to control animal pests?

Hygiene

When are the best times to wash your hands? Do you use soap? How much does soap cost and where get it?

Do you wash your hands at home? at work?

How often and where do you and your household members bathe?

ILLNESS, MEDICAL CARE/TREATMENT, DEATH

GOAL: To identify any unusual disease experiences—signs, symptoms and sources

Household illness

Is anyone sick right now?

What do you do when someone in the household gets sick? Who takes care of that person?

The last time someone was seriously sick what happened (explore when, with what, how did they get sick, who told/consulted, anyone else get sick after, final outcome)?

Has anyone ever had an sickness that people don't usually get? What happened? Where did it come from?

Illness from animals

Do you know anyone who has gotten sick from an animal? What animal? What did they get? What happened? Do you know any other diseases/illnesses people can get from animals? How does the animal give the illness to the person? How often does it happen?

Medical care/treatment

How sick would you have to feel to stay home and not do normal routine?

Where do you go when you are sick?

Do you prefer to use traditional medicine, western medicine or a combination?

How sick would you have to feel to go to doctor/clinic/hospital? What does that cost? (in time, lost wages/business, transport costs, etc) How far away?

Death

What is the tradition when someone dies? (Explore if reported to authorities, differ by age or gender, what happens to the body, does the community come together or is it private.)

HUMAN ANIMAL CONTACT

GOAL: To gain knowledge about interactions with animals, animal health and animal perceptions and knowledge.

Encourage but don't lead discussion about which animals. Allow respondent to name the animals. If no birds or bats are mentioned, follow up by asking specific questions about birds and bats.

Indirect contact

What kind of meat do people in your household eat? How do you get it/where does it come from? What is furthest away an animal comes from?

Is meat dead or alive when you get it? If dead(/prepared), how to tell if good/fresh?

If alive, how long are live animals kept before being sold or eaten? How do you get live animals home?

How is meat prepared (raw/undercooked)? Is meat prepared in the same place as other activities? (e.g., preparing vegetables, cleaning babies/changing diapers, where other food or drinking water is stored)

Do animals come in or near the dwelling? How do you know animals are there? Which animals?

Direct contact

Do you or someone in your household handle live animals? In what context? (e.g. ranching/animal husbandry, hunting, wet markets, work, around dwelling/other building, pets)

What are the animals that you keep/raise or sell? How many different kinds of animals? How many of each?

For how long do you have the animals?

Where do live animals come from? Where is the furthest away an animal comes from?

Who buys/trades for your live animals? Where do the animals go?

Have you been bitten, scratched or had bleeding after handling an animal? By a wild animal?

Where are live animals slaughtered? butchered? Do people buy or sell parts?

Do you travel with animals? Explore details of the process, specific routes and encounters (eg, with other animals, with animal transport supporting industries, such as holding areas, restaurants, hotels) along the way.

Explore for differences over time in animal handling, eg, seasonality, legal, religious, animal reproduction

Animal products/rituals

Other uses of animals—e.g., as pets, medicine, magic, fertilizer**,** for trading

Rules for children around wild animals as pets, playing with wild animals or dead animals

Animal health

How do you care for your animals: how are they fed, what do they eat, where do they eat/graze and sleep? Are they segregated or all together? Differences by season? day/night? Does anyone live or stay with the animals?

Is there a central area for animal waste? How often are animal cages, stalls, or penned areas cleaned? Who cleans them?

Do the animals get veterinary care? Vaccinations?

How do you know when an animal is sick? What’s the first thing you do about a sick animal?

Have you seen an animal outbreak or die-off? What happened?

Perceptions and knowledge

What are the most unusual animals anyone can buy?—seasonal? Expensive? Who buys?

Are there any animals you avoid eating? Why? Ever heard of anyone eating/selling dead or infected animals?

Do people ever eat non-domesticated animals/wildlife? Where do they get them?

Who usually buys wildlife products? Have there been changes over time?

What do you do when you find a dead animal?

What laws about animals do you know? (eg, limiting/outlawing hunting, reporting and culling of sick animals)

7.12 Qualitative Interview Guide (Chinese)

人种学访谈向导

核心议题

1. 人的活动
2. 社会经济学
3. 人生活环境中的生物安全
4. 疾病，医疗救助/诊治及人的死亡情况
5. 动物和人的接触

人的活动

目标：理解人的生活环境和“家域范围”（例如：人活动范围有多远以及原因）。

家

你在哪里居住/房屋类型？家里有多少人？有多少间房间？其中有多少人是孩子？所有人都是亲戚吗？如何安排睡觉？搬家的频率？是否季节性的搬迁？ 例如：为了工作，为了获取食物，为了安全(例如：为了防洪，防旱，避免矛盾)？

工作

你从事什么工作？家里其他成员都是做什么工作的？都在哪里工作？你如何保护你的活动和商业利益？(例如：放牧或耕地，商业竞争，狩猎领地，畜牧)

旅行

家庭成员到多远的地方去旅行以及旅行的原因？（跟踪和动物相关的议题：购物，售卖，买动物，贩卖，打猎，运输等等）如何旅行（步行，自行车，马车，卡车，飞机）？曾近在外面住宿吗？为什么住宿？旅行的原因是什么？（工作原因/移居，家庭原因，宗教信仰原因，度假，买卖动物）有住在其他地方的家庭成员吗？他们经常回家来吗？

观察环境

当地有环境改变吗：新修道路，船和港口增多，田地，建筑物，人口流入流出，土地平整或者荒废，新建别墅，其它的新建筑物

谁管理这些环境改变？这些变化是好是坏？

社会经济学

目标：了解典型的日常生活以及金钱和社会地位如何影响机会和风险。

日常生活轨迹

请讲述你的日常生活轨迹（描述日常的工作，包括购买准备食物，进餐时间，和动物相关的责任和职责，是否有季节性的变化）

家庭成员如何为家庭赚钱，获得食物和水？

孩子们在那里玩耍？当你工作的时候谁来照料孩子？

照顾动物的职责

请讲述和动物相关的工作以及不同年龄阶段人群的职责分工（比如：小孩子，大孩子，青年，成人，老人）。

随着成长，需要什么样的知识或者技能才能胜任下一阶段的工作职责？

男孩和女孩在照顾动物的时候有什么不同分工吗？男人和女人呢？

教育

目前有几个孩子在读书？孩子几岁开始上学？（几个男孩，几个女孩？）

现在孩子们都读到几年级了？你当时为什么不读了？

经济学

和其它跟你从事相同工作的人比，你是不是挣得比别人多？你觉得是什么愿意？

有没有一些年你挣的比别人少？什么原因？

有没有一些时间食品比其他东西贵？请讲述（比如：不同食品供给不同，季节变化，春节原因）

你认为你和你的丈夫比大多数人都强？能否把事情做得更好变得更强？

人生活环境中的生物安全问题

目标：确定是否有任何卫生或卫生因素可能在疾病蔓延的作用。

水和食物

这里有重要的水源吗？是什么水源？（例如：池塘，无盖水井，雨水，水龙头，带盖水井）你更喜欢哪个水源的水？水源地离这里有多远？动物也在这些水源地喝水吗？你家里的水在饮用之前经过净化处理吗？你如何储存食物？（例如：敞开的容器，封闭的容器，吊起来的容器，冰冻）

你吃或者喝 你怀疑被动物接触过的东西吗？（例如;牙印，抓恒，粪便，可见尿液）

你定期清理厨房，操作台和厨具吗？如何清理？

卫生设施

这里有厕所吗？废物站或其他指定的地方？这些定期清洗和使用吗 ？

屠宰区和分割区分开吗？他们经常清洗吗？如何清洗？谁清洗？

有没有正式的关于人类的废物和垃圾处理规则或法律？

有什么动物病虫害防治的法律？你如何控制有害动物吗？

卫生状况

什么时候是您洗手的最佳时间？您洗手时用香皂吗？肥皂多少钱，哪儿能够买到？

你在家吸收吗？在工作的地方洗手吗？

你的家庭成员在哪里洗澡，频率多高？

生病，医疗关怀/诊治，死亡

目标: 发现不同寻常的疾病发生情况—迹象，症状和来源。

生活疾病

目前家里有人生病吗？

家里有人生病了怎么处理？家里谁来照料病人？

上次家里有人生重病是什么时候（什么时候，因为什么，怎么样生病的，生病后谁去询问最终的结果？）

有人得过一般人平常不会得的病吗？发生了什么？这个病是从哪里来的？

动物源疾病

你知道谁被动物传染疾病吗？什么种的动物？他生的什么病？发生了什么？

你知道动物可能传染给人的其它疾病吗？动物如何把疾病传播给人类？这种事请多久发生一次？

医疗关怀/诊治

病的多重你会觉得不得不呆在家里，而不去做日常工作？

生病的时候你会去哪里？

生病的时候你会优先选择中医，西医还是中西结合？

病的多重你才会觉得需要去看医生/门诊/医院？看病要花费多少钱？（时间上，损失补贴/影响生意，交通成本等），医院有多远？

死亡

按照传统，人死亡后怎么处理？（探讨如果向当局报告，年龄或性别不同的死者，尸体如何处理，整个社区的人都来参加葬礼还是仅仅是亲戚朋友）

动物和人接触

目标：了解人和动物相互行为，动物健康以及关于动物的观念和知识。

鼓励但是不领导关于动物的讨论。允许发言者命名动物，如果鸟和蝙蝠没有提到，问问鸟和蝙蝠和人的接触情况。

间接接触

你家里人吃什么肉？怎么得到这些肉？这些肉是从哪里来的？这些肉最远来自于哪里？

你得到的肉是活的动物的还是宰杀了的动物的？如果是死的动物的肉，怎么辨别是否新鲜？

如果是活的动物的肉，这些活的动物在被售卖或者吃掉前养多久？你如何在家里得到活动动物的肉？

肉怎么处理（生的/半生不熟）？有固定的地方处理肉吗（例如：处理蔬菜，清洗宝宝，请洗尿布的地方也都有固定的水池吗）？

动物会进入居所或者居所附近吗？你怎么知道动物在哪里？那些种的动物？

直接接触

你或者你家里人处置过活的动物吗？在什么环境下？（例如：牧场/畜牧场，打猎，市场，工作中，居所附近/其它建筑，宠物）

你养或者卖动物的原因是什么？有多少种不同的动物种类？每种有多少数量？

这些动物你已经养了多久了？

这些活的动物是从哪里引进的？最远的动物来自于哪里？

谁买/贩卖 你的活体动物？动物都销往哪里？

你处理过动物之后有过被咬伤，抓伤，或者出血的情况吗？就是被一个野生动物搞伤？

活体动物在哪里被宰杀？在哪里分割？人们买或者卖动物的分割的肉块吗？

你会带着动物旅行吗？揭示旅行的细节过程，具体路线和遭遇（例如：在动物转运部，在餐馆，酒店遭遇其它动物）

揭示不同时间维度上处理动物的不同，例如：季节性，法律，信仰，动物产品变化

动物产品/宗教仪式

动物的其它利用形式，比如：宠物，入药，魔术，蓄肥料，卖卖

小孩子将野生动物作为宠物，和野生动物或者死的动物玩耍。

动物健康

你怎么照看你的动物：怎么喂它们？它们吃什么？在哪里进食/放牧和休息？动物们是分开来的还是混合在一起的？不同季节，昼夜之间有什么不同？有人和动物一起住吗？有专门的动物废弃物区域吗？动物笼舍多久清理一次？谁清洗它们？

动物们接受兽医治疗吗啊？接种疫苗吗？

你如何能够发现动物生病？你对生病的动物采取的第一步措施是什么？

你遇到过动物突然爆发疾病吗？发生了什么状况？

观念和知识

人们能买到的最不寻常的动物是什么？---季节的？昂贵的？谁会购买？

你有什么动物是不吃的吗？为什么？听到过有人吃/卖死的或者受感染的动物吗？

人们吃非家养动物吗？是野生动物吗？去哪里得到野生动物？

谁通常会买野生动物产品？随着时间推移这些情况有变化吗？

你发现死亡的动物时怎么处理？

你知道关于动物的法律有哪些？（例如：限制非法打猎，报告并且处死生病的动物）

7.13 Qualitative Interview Checklist (English)

Participant ID: ____________________________

Interviewer: ______________________________

INTERVIEW CHECKLIST

CORE THEMES

☐ **Human movement**

- ☐ Home
- ☐ Work
- ☐ Travel
- ☐ Observed environment

☐ **Socioeconomics**

- ☐ Daily routine
- ☐ Animal responsibilities
- ☐ Education
- ☐ Economics

☐ **Biosecurity in human environments**

- ☐ Water and food
- ☐ Sanitation
- ☐ Hygiene

☐ **Illness, medical care/treatment and death**

- ☐ Household illness
- ☐ Illness from animals
- ☐ Medical care/treatment
- ☐ Death

☐ **Human-animal contact**

- ☐ Indirect contact
- ☐ Direct contact
- ☐ Animal products/rituals
- ☐ Animal health
- ☐ Perceptions/knowledge

7.14 Qualitative Interview Checklist(Chinese)

受访人编号----------------------

访问人---------------------------

访谈清单

核心议题

- □ 人的活动
  - □ 家
  - □ 工作
  - □ 旅行
  - □ 观察到的环境
- □ **社会经济学**
  - □ 日常活动路线
  - □ 照顾动物职责
  - □ 教育
  - □ 经济学
- □ **人生活环境中的生物安全**
  - □ 水和食物
  - □ 卫生设施
  - □ 卫生状况
- □ 疾病，医疗关怀/诊治，死亡
  - □ 家庭疾病
  - □ 动物源疾病
  - □ 医疗关怀/诊治
  - □ 死亡
- □ 人和动物接触
  - □ 间接接触
  - □ 直接接触
  - □ 动物产品/宗教仪式
  - □ 动物健康
  - □ 观念/知识

7.15 Coding Keyword Guide for Ethnographic Interviews

Human Movement

Home

Dwelling, living quarters, sleeping quarters

Children, family

Daily movement/travel

Flood

Drought

conflict

Protection from predators/ animals

Safety

Work

Work activities

Agriculture areas

Grazing areas

Hunting territories

boundaries

Livestock areas

Markets

Crops

business

Travel

Traveling to Shop/buy/sell/trade

Hunting trips

Transporting animals

Transportation: Walking, biking, cart, truck, plane, boat, trains

Overnight trips

Reasons for travel

Travel destinations

Border crossings

Travel obstacles/issues

Transportation of resources/moving

Observed Environment

Town roads/ports/ trains

New buildings/roads/construction

Route changes

Abandoned land

Socioeconomics

Daily routine

Meal preparation

Shopping

Childcare

Market trips

Groceries

Purchases

Errands

Animal responsibilities

Animal duties/responsibilities

Feeding/grazing

Tasks/roles by age or gender

Sick animals

Slaughtering/Butchering

Education

School/education/graduation

Reading/understanding numbers

Dropping out

Economics

Livelihood

Earning/earning changes throughout year

Large purchases

income

Purchases for event/holiday

Social standing (compared to neighbors/others)

Number of jobs/activities

Biosecurity in Human Environments

Water and food

Water source (where does it come from?)

Water taste/quality/purification

Rain/rainwater/water taps/well

Storing food/storing water

Pests/rats/pesticides/cockroaches/insects

Kitchen

Cleaning

Water usage

Sanitation

Waste management/garbage

Toilets/latrines/bathroom

Cleaning bathroom/kitchen

Feces

Urine

Pesticides

Hygiene

Washing hands

Showering/bathing

Soap

Leave shoes/footwear outside

Illness, medical care/treatment and death

Household illness

Sick relatives

Caretaking of sick

Types of sickness

unusual illness

symptoms of illness

Ebola

SARS

MERS

(other endemic zoonotic diseases)

dispensaries/medication

Illness from animals

Illness from animals

Medical Care and Treatment

Doctor/clinic visit

Medicine/Treatment

Cost of medicine/doctor/treatment

Professionals (doctor, nurse, religious leader, healthcare worker etc…)

Traditional medicine

Ethno botany

Death

Reporting death

Burial/ burial rites

Funeral tradition/rites

Dead body/corpse

Body preparation

Human Animal Contact

Indirect Contact/Food:

Meat/animal consumption

Acquisition of meat

Preparing meat

Meat/animal storage

Butchering

Animal taboos

Infected animals

Wildlife consumption

Purchasing meat or wildlife

Cleaning up after animals

Meat/dead animal markets

Animals around dwelling/pests

Signs of animals (hear, smell)

Faeces

Animal tracks

Garbage disturbance

Observed animals

Direct Contact

Ownership of animals

Live animals

Pets

Playing with animals (wild or domestic, alive or dead)

Animal caretaking

Feeding animals

Grazing animals

Working with animals

Live animal markets/wet markets

Ranching

Animal husbandry

Buying/selling/trading live animals

Bite

scratch

animal handling

Killing live animals/slaughtering

Handling of wildlife

Animal products/rites

Animal by-products (milk, leather, magic, medical)

Magic involving animals

Fertilizer

Animal health

Animals eating/sleeping/grazing

Sick animals

Animal caretaking activities/roles

Animal waste

Cleaning animal areas

Veterinary care

Vaccinations

Outbreak

Die off

Perceptions and knowledge

Exotic or expensive animals

Wildlife consumption

Regulations/laws regarding animals (eg. Hunting, eating, poaching regulations)

Danger from animals

Conservation

Taboos

Special occasions/holidays/ feasts/ holy days

7.16 Ethnographic Interview Informed Consent Form (English)

Introduction: My name is__________and I work for a project called *"Human and Animal Contact Study."* The project is funded by the US Government and conducted in collaboration with Wuhan University School of Public Health, the Chinese CDC and other local partners. We are studying how the health of wildlife, livestock and people affect each other and to better understand the patterns of local trade and consumption of meat and food animals.

Interview: As part of this research we are speaking with all kinds of people to better understand the types of interactions people have with wildlife and their domestic animals, as well how people live their lives, do their jobs, and take care of their families and their animals. While you may not benefit directly from this research, the information you share with us may help to improve the health of other people who live near or work with animals. The main risk to you would come from a loss of confidentiality. To decrease any risk of someone else seeing your personal information, we give the information you share with us and the recording of the interview, a code number and use that instead of your name on all information that you provide. Your information is also kept secure in locked files and is considered confidential. We will use this information to better understand disease risks from wild and domestic animals to humans and share this with local and national leaders, non-governmental organisations and the scientific community. When we write about the study, we will not use your name or anyone else's name, or anything about you that someone could recognise. At the end of the interview, we may ask you to refer other people to the study.

I am here today to ask if you are willing to participate in this study by talking with me. Your participation is voluntary. You do not have to answer a question if you do not want to. The interview will take approximately 60 to 90 minutes of your time, and we will record the conversation. If you are not comfortable with your interview being recorded, you are free to decline to participate in this study.

Additional information: An Institutional Review Board and an Ethics Board that is responsible for making sure that research subjects are protected from harm, has approved this project. If you have any questions now or in the future about your participation in this study or your rights as a research subject, you may contact Guanjian Zhu at zhu@ecohealthalliance.org or by phone at this number 13818140967.

7.17 Ethnographic Interview Consent Statement (English)

Subject ID Number

I have had a chance to ask questions about the study. If I do not want the interview recorded, I may say no to participating in the study. Also, someone has explained to me that:

☐ My name will not appear on interview or other data collection forms: only a code number will be used;

☐ The information I provide is confidential and will be kept in locked files that only the professional research staff can see;

☐ All written and published information will not use my name, anyone else's name, or anything about me that someone could recognise.

☐ There is no punishment or disgrace with saying no to participating in this study. If you decide not to participate, your decision will not be used against you in any way.

______________________________ ______________________
Participant Signature Date

____
Participant Name (please print)

______________________________ ______________________
Interviewer Date

If the individual declines to participate, thank them for their time.

Reason for not participating ______________________________

Certified by Interviewer
Initials Date

7.18 Ethnographic Interview Informed Consent (Chinese)

人和动物接触研究项目

采访知情同意书

介绍：我的名字叫做_________我们现在研究的项目叫做“人和动物接触研究”，项目由美国政府资助，和武汉大学公共卫生学院，中国疾病预防与控制中心及其他当地合作伙伴一起合作开展。我们的项目研究野生动物，家畜和人的健康是如何相互影响的，更好地理解地区贸易和肉类和动物的食物消费的模式。

访谈：作为研究的一部分，我们采访各类人来理解这些饲养动物或者野生动物的人和动物之间的关系，以及他们如何生活，工作，照顾家庭和饲养的动物。你可能无法直接从这个研究中获益，但是你和我们分享的信息可能会帮助提高哪些和动物一起生活或者工作的人们的健康状况。你的最大风险是失去保密性。为了降低其他人看到你个人信息的风险，我们会对你分配编码，并用编码代替你所有信息。你的信息也将得到保密，我们仅仅会使用这些信息更好的理解野生动物家和家养动物到人的疾病传播风险，这些研究结果将会和当地以及国家主管部门领导，非政府组织以及科学团体分享。当我们撰写研究报告时将不会使用你或其他人的姓名，或者任何别人可以识别的信息。在访谈结束时，我们可能会请你为我们的研究推荐其他的参与者。

今天，在这里请问你是不是自愿和我交谈来参加这项研究。你不必回答你不想回答的问题。访谈大约会持续 60-90 分钟。我们会对访谈录音。如果你不愿访谈被录音，你可以自由的退出本研究。

其他信息：负责确保研究对象不受到损害的审查委员会已经准许了这个项目。你在任何时候有任何问题吗？如果你以后对于你在这项研究中的参与或者你作为研究对象的权利有任何问题，可以通过电子邮件 zhu@ecohealthalliance.org 或者手机联系 13818140967

7.19 Ethnographic Interview Consent Statement (Chinese)

研究对象身份编号_______________

知情声明

我已经了解所有对我呈现的信息，我明白我的参与出自自愿，并且谈话将会被录音。研究相关的所有问题，我都得到了满意的回答。

我明白：
我的名字不会出现在访谈记录或者其它数据收集形式当中：仅仅用一个编码来代替；
我提供的信息将得到保密并且封存在只有专业研究人员能够看到的文件中；
所有的资料和出版信息都不会使用我的名字，其他人的名字，或者别人可以识别的一切信息。

________________________________ __________________________

受访人签名 日期

受访人名字 (请打印)

________________________________ __________________________

访问人 日期

如果有人不愿意参加，谢谢他们耗费他们的时间，并问问他们不愿意参加的原因

不接受访问的原因:____.

访问人证明 _________________________ 草签 __________ 日期____________________

7.20 Quantitative questionnaire (English)

Code Number：__________________ Today's Date: ___ ___ / ___ ___ / ___ ___

Month / Day / Year

Consent Form Administered & Signed **YES** **NO**

SECTION A: Background Information

A1	Gender	Female **1** Male **2** Other **3**	A1
A2	Age		A2
A3	Mother's Last Name Respondents birth month Respondents birth day	Name#### *Example: Fisher 0222*	A3
A4	Location ***Interviewer***: Please collect GPS coordinates	Village ________________ Town/City ________________ Province ________________	A4
A5	How long have you lived here?	< 1 month **1** 1 month – 1 year **2** 1 year – 5 years **3** > 5 years **4**	A5
A6	What is your family annual per capita income (RMB)	<1000 **1** 1001-3000 **2** 3001-5000 **3** 5001-10000 **4** 10001-300000 **5** 300001-500000 **6** >500000 **7**	A6

SECTION B: Essential Information

B1	Your occupation level	Owner/Manager **1** Worker **2** Student **3** Live and work at home **4** Other **5**	B1

			YES/NO		
B2	Since this time last year, what type of activities have you done to earn your livelihood? Check all that apply	1. Extraction of minerals, gas, oil, timber	Y	N	B2
		2. Crop production	Y	N	
		3. Wildlife restaurant business	Y	N	
		4. Wild/exotic animal trade/market business	Y	N	
		5. Rancher/farmer animal production business	Y	N	
		6. Meat processing, slaughterhouse, abattoir	Y	N	
		7. Zoo/sanctuary animal health care	Y	N	
		8. Protected area worker	Y	N	
		9. Hunter/trapper/fisher	Y	N	
		10. Forager/gatherer/non-timber forest product collector	Y	N	
		11. Migrant laborer	Y	N	
		12. Nurse, doctor, healer, community health worker	Y	N	
		13. Construction	Y	N	
		14. Other			
B3	If more than one activity was selected, what is the activity on which you spent the most time since this time last year? *Write down the **activity number** from the above list.*				B3
B4	What is the highest level of education that you completed?	None **1** Primary School **2** Secondary school/Polytechnic school **3** College/university/professional **4**			B4
B5	What is the highest level of education that your mother completed?	None **1** Primary School **2** Secondary school/Polytechnic school **3** College/university/professional **4**			B5
B6	Do you live with your family?	**YES** **NO**			B6
B7	How many other people live in the dwelling where you live (not including you)?				B7
B8	How many in the dwelling are children less than 5 years old?				B8
B9	How many in the dwelling are male?				B9
B10	How many rooms are there in the dwelling where you live (do not include bathroom or kitchen)?				B10

SECTION C: Medical History

In this section, I'm going to ask you about any illness or sickness that is not known or recognised in the community, including by medical or treatment providers.

C1	Where do you usually go to get treatment for illness or infection? *Check all that apply.*	Clinic **1** Hospital **2** Mobile clinic **3** Community health worker **4** Traditional healer **5** Pharmacy/dispensary **6** Do not receive medical treatment **7** Other **8**	C1
C2	Have you EVER had an unusual illness with any of the following symptoms? Check all that apply *Note: READ ONLY SYMPTOMS—don't read the illness*	**1.** Fever with headache and severe fatigue or weakness (encephalitis) **2.** Fever with bleeding or bruising not related to injury (hemorrhagic fever) **3.** Fever with cough and shortness of breath or difficulty breathing (Severe Acute Respiratory Infection) **4.** Fever with muscle aches, cough, or sore throat (Influenza Like Illness) **5.** Fever with diarrhea or vomiting **6.** Fever with rash **7.** Persistent rash or sores on skin **8**. Had symptoms, but none of these -describe ____________________________	C2
C3	Since this time last year, have you had any of these symptoms? ***If NO,** skip to **C6**, the question about people you lived with*	YES **1** No **2**	C3
C4	**If YES** for **C3,** which ones *Select all that apply.*	**1.** Fever with headache and severe fatigue or weakness (encephalitis) **2.** Fever with bleeding or bruising not related to injury (hemorrhagic fever) **3.** Fever with cough and shortness of breath or difficulty breathing (Severe Acute Respiratory Infection) **4.** Fever with cough and shortness of breath or difficulty breathing (Severe Acute Respiratory Infection) **5.** Fever with diarrhea or vomiting **6.** Fever with rash **7.** Persistent rash or sores on skin **8.** Had symptoms, but none of these -describe ____________________________	C4
C5	What caused this sickness? Select all that apply.	**1.** Contact with sick people **2.** Contact with wild animals	C5

		3. Contact with other animals **4.** Bad food or water **5.** Bad spirits/witchcraft **6.** Wound or injury **7.** I don't know **8.** Other: ___________________________	
C6	Since this time last year, have any of the people you lived with had any of these symptoms? *If NO, skip to Section D*	YES **1** No **2**	C6
C7	**If YES** for **C6,** which ones *Select all that apply.*	**1.** Fever with headache and severe fatigue or weakness (encephalitis) **2.** Fever with bleeding or bruising not related to injury (hemorrhagic fever) **3.** Fever with cough and shortness of breath or difficulty breathing (Severe Acute Respiratory Infection) **4.** Fever with muscle aches, cough, or sore throat (Influenza Like Illness) **5.** Fever with diarrhea or vomiting **6.** Fever with rash **7.** Persistent rash or sores on skin **8.** Had symptoms, but none of these -describe ____________________________	C7
C8	Since this time last year, did anyone you lived with die from this illness?	YES **1** NO **2**	

SECTION D: Travel

D1	***Since this time last year*** have you traveled outside of your village, town, or city?	YES **1** NO **2**	D1
D2	How many times have you traveled?	Number of Times _______________	D2
D3	What is the farthest location you have traveled?	Village _______________ Town/City _______________ Province _______________	D3
D4	How many kilometers away is that?	#km _______________	D4

SECTION E: Contact with Animals

In this section, I'm going to ask you about the animals in your life since this time last year.

Since this time last year...

E1	Has anyone that you live with had an animal as a pet?	**YES** **NO**	E1

E2	Have you handled live animals?	**YES** **NO**	E2
E3	Have you raised live animals?	**YES** **NO**	E3
E4	Have you shared a water source with animals for washing?	**YES** **NO**	E4
E5	Have you seen animal feces in or near food before you have eaten it?	**YES** **NO**	E5
E6	Have you eaten food after an animal has touched or damaged it? *For example, chew marks or scratches*	**YES** **NO**	E6
E7	Do any animals come inside the dwelling where you live?	**YES** **NO**	E7
E8	Have you cooked or handled meat, organs or blood from a recently killed animal?	**YES** **NO**	E8
E9	Have you eaten raw or undercooked meat or organs or blood?	**YES** **NO**	E9
E10	Have you eaten an animal that you knew was not well /sick?	**YES** **NO**	E10
E11	Have you found a dead animal and collected it to eat, share or sell?	**YES** **NO**	E11
E12	Have you been scratched or bitten by an animal?	**YES** **NO**	E12
E13	Have you slaughtered an animal?	**YES** **NO**	E13
E14	Have you hunted or trapped an animal?	**YES** **NO**	E14

If the respondent has answered "yes" to any of the previous questions, select the animal taxa associated with the activity. *Select all that apply.*

		Pets	Handled	Raised	In house	Cooked/ handled	Eaten raw/ under-cooked	Eaten sick	Found dead collected	Scratched/bitten	Slaughtered	Hunted/trapped	
E15	Rodents/ Shrews												E15
	Bats												
	Non-human primates												
	Birds												
	Carnivores												
	Ungulates												
	Poultry												
	Goats/ Sheep												
	Swine												
	Cattle/ Buffalo												
	Dogs												
	Cats												

E16	The last time you were scratched or bitten by an animal or you cut yourself while butchering or slaughtering, what did you do?		**YES/NO**	E16
		1. Nothing	Y N	
		2. Rinse wound with water	Y N	
		3. Wash wound with soap and water	Y N	
		4. Covered wound with bandage	Y N	
		5. Visit doctor	Y N	
E17	Are you worried about diseases or disease outbreaks in animals at wet markets?		YES **1** NO **2**	E17
E18	Since this time last year, have you purchased live animals from a wet market?		YES **1** NO **2**	E18

E19	**If YES** for **E18**, have you changed your behaviour when you go to a wet market?		**YES/NO**		E19
		1. Wear a mask	Y	N	
		2. Wear gloves			
		3. Wash hands after	Y	N	
		4. Sometimes shop for meat at supermarket	Y	N	
		5. Buy live animals less often	Y	N	
		6. Buy only farmed wildlife	Y	N	
		7. No longer buy wildlife at wet markets	Y	N	
E20	Do you think that animals can spread disease?			**YES** **NO**	E20

Thank you for participating in this study.

END

Please return this completed questionnaire to the Project Coordinator

7.21 Quantitative questionnaire (Chinese)

社区居民健康管理状况调查t

终版 2015年8月31日

编号：_________________ 调查日期: ___ ___ / ___ ___ / ___ ___

月 / 日 / 年

签署/托管知情同意书 是 否

A 部分: 背景信息

A1	性别	女性 1 男性 2 其它 3	A1
A2	年龄		A2
A3	母亲的姓 出生的月 出生日期	姓#### 举例: 李 0222	A3
A4	地点 *调查者*: 请记录GPS信息	村 _______________ 镇/市 ______________ 省 _______________	A4
A5	您在这里住了多久?	< 1 月 **1** 1 月 – 1 年 **2** 1年– 5年 **3** > 5年 **4**	A5
A6	家庭年均人收入 (人民币)	<1000 **1** 1001-3000 **2** 3001-5000 **3** 5001-10000 **4** 10001-300000 **5** 300001-500000 **6** >500000 **7**	A6

B部分: 基本信息

B1	您的职业身份	老板/管理者 **1** 工人 2 农民 3 学生 4 企业或事业单位职工 5 医生/兽医/医疗工作者 6 个体经营（商贩/自由职业者）7 其他 8			B1
			是/否		
B2	去年这个时候开始，你都做过哪些工作来维持生计，请选择所有符合的选项	14. 采矿，提炼石油，开采燃气，砍伐	是	否	B2
		15. 农业生产	是	否	
		16. 经营野味餐馆	是	否	
		17. 野生或者外国输入动物贸易或者在市场上买卖	是	否	
		18. 农牧场动物产品销售	是	否	
		19. 肉类加工, 屠宰, 屠宰场	是	否	
		20. 在动物园或者动物救护中心救助动物	是	否	
		21. 保护区工人	是	否	
		22. 猎人/诱捕动物者/渔民	是	否	
		23. 捕食/采集/非木林业产品采集	是	否	
		24. 民工	是	否	
		25. 护士,医生, 治疗师, 社区健康工作人员	是	否	
		26. 建筑	是	否	
		14. 其它			
B3	如果从去年这个时候开始，您从事过多项工作，哪项工作是从事时间最长的？*请在后面空格处标明从事时间最长的工作的序号*				B3
B4	请问您的受教育水平?	没有 **1** 小学 **2** 中学/中专 **3** 大专/本科/职业教育 **4**			B4

B5	请问您母亲受教育水平?	没有 **1** 小学 **2** 中学/中专 **3** 大专/本科/职业教育 **4**	B5
B6	您和家人住在一起吗?	**是** **否**	B6
B7	和您一起居住的家庭成员有几个人 (不包括您本人)?		B7
B8	和您一起居住的成员中年龄小于5岁的成员有几个人?		B8
B9	和您一起居住的家庭成员中男性成员有几人?		B9
B10	在您家里有几间房间 (不包括厨房和卫生间)?		B10

C部分: 医疗历史

在这部分内容中，我们将会问您一些不被社区居民普遍认知的疾病和不适症状，包括治疗药物和医治者信息

C1	在生病或者感染后，您一般去哪里接受治疗？*请核查所有列出选项*	诊所 **1** 医院 **2** 流动诊所 **3** 社区健康工作人员 **4** 传统治疗师 **5** 药房/药店 **6** 不接受医疗 **7** 其它 **8**	C1
C2	您是否有过不寻常的生病经历，有以下列出的症状? *请核对所有列出的选项* *注意: 调查者在调查时只能读出症状不能读出括号内的疾病名称*	**8.** 发烧并伴有头疼和严重疲劳或者是虚弱 (脑炎) **9.** 发烧伴出血或与受伤不相关的瘀伤 (出血热) **10.** 发烧伴有咳嗽和气短或者呼吸困难(严重急性呼吸道感染) **11.** 发烧伴有肌肉痛，咳嗽或者咽喉痛(流感样病例) **12.** 发烧伴有腹泻或者呕吐 **13.** 发烧伴有皮疹 **14.** 皮肤持续出现皮疹或者溃疡 **8.** 症状和上述所列项不同 -请描述 ____________________________	C2

C3	从去年这个时候开始，您是否有过某些症状? ***如果没有请跳到问题 C6***	是 **1** 否 **2**	C3
C4	如果对**问题 C3** 的回答是**是,** 请选择符合条件的所有选项.	**8.** 发烧并伴有头疼和严重疲劳或者是虚弱 (脑炎) **9.** 发烧伴出血或与受伤不相关的瘀伤 (出血热) **10.** 发烧伴有咳嗽和气短或者呼吸困难(严重急性呼吸道感染) **11.** 发烧伴有肌肉痛，咳嗽或者咽喉痛(流感样病例) **12.** 发烧伴有腹泻或者呕吐 **13.** 发烧伴有皮疹 **14.** 皮肤持续出现皮疹或者溃疡 **8.** 症状和上述所列项不同 -请描述____________________________	C4
C5	您的不适是由什么原因引起的? *选择所有符合条件的选项.*	**9.** 接触病人 **10.** 接触野生动物 **11.** 接触其它动物 **12.** 不干净的食物或者水 **13.** 情绪不好 **14.** 创伤或者损伤 **15.** 不清楚 **16.** 其它: ___________________________	C5
C6	去年这时候开始，有没有和你同住的人出现这些症状? *如果没有，请跳转到 D 部分问题*	是 **1** 否 **2**	C6
C7	*如果对问题* **C6** *的回答是***是,** 出现了哪些症状？. *选择所有符合条件的选项*	**8.** 发烧并伴有头疼和严重疲劳或者是虚弱 (脑炎) **9.** 发烧伴出血或与受伤不相关的瘀伤 (出血热) **10.** 发烧伴有咳嗽和气短或者呼吸困难(严重急性呼吸道感染) **11.** 发烧伴有肌肉痛，咳嗽或者咽喉痛(流感样病例)	C7

12. 发烧伴有腹泻或者呕吐

13. 发烧伴有皮疹

14. 皮肤持续出现皮疹或者溃疡

8. 症状和上述所列项不同
-请描述____________________________

C8	去年这时候开始，和您同住的人有没有出现过上述症状后死亡的?	是 **1** 否 **2**

D部分: 旅行

D1	**去年这个时候起，**您有没有出村，镇和市去旅行?	是 **1** 否 **2**	D1
D2	您旅行了多少次?	次数 _______________	D2
D3	你旅行到达过的最远地方村庄名?	村庄名 _______________ 镇/市 _______________ 省 _______________	D3
D4	那个地方距离您家有多少公里?	#km _______________	D4

E部分: 接触动物

在该部分我们将讨论的是去年这个时候起，您生活中接触到的动物

自去年这个时候起...

E1	有没有和你同住的人养动物作为宠物吗?	是 否	E1
E2	您有没有处理过活的动物?	是 否	E2
E3	您有没有养过活的动物?	是 否	E3
E4	您没有和动物共用水源用来洗漱?	是 否	E4
E5	在吃东西之前，你有没有在食物中或者食物附近发现动物粪便?	是 否	E5
E6	你吃过动物碰过的食物吗? *例如，咀嚼过或者抓过的实物*	是 否	E6
E7	有没有什么动物进入过你居住的地方?	是 否	E7
E8	你有没有烹饪或者处理过刚杀的动物的肉，内脏或者血?	是 否	E8

E9	您有没有吃过生的或者未煮熟的肉，内脏或者血?	是 否	E9
E10	您有没有吃过不健康或者生病的动物?	是 否	E10
E11	您是否把死的动物捡来吃或者将其送给别人或者出售给别人？	是 否	E11
E12	您有没有被动物抓伤或者咬伤过?	是 否	E12
E13	你有没有屠宰过动物?	是 否	E13
E14	你有没有猎杀或者诱捕过动物? *(如果该问题的回答是是，对受访对象调查 human 调查问卷中的**猎人**部分问题 18 页)*	是 否	E14

如果受访者对上述任何问题的回答是"**是**", 请他选择动物种类和对应的行为活动. *选择所有符合条件的选项*

		宠物	经手处理过	饲养	出现在家里	烹饪/烹饪前处理	生吃/半生不熟	吃后生病	发现死亡的动物 并收集	抓伤/咬伤	屠宰	打猎/诱捕	
E15	啮齿类/鼩												E15
	蝙蝠												
	非人灵长类												
	鸟类												
	肉食动物												
	有蹄类												
	家禽												
	山羊/绵羊												
	猪												
	黄牛/水牛												
	狗												

	猫			
E16	上次当你被动物抓伤或者咬伤，或者你在屠宰场割伤自己的时候，您是怎么处理的?		**是/否**	E16
		6. 什么也没做	是 否	
		7. 用水冲洗伤口	是 否	
		8. 用肥皂水清洗伤口	是 否	
		9. 用绷带包扎伤口	是 否	
		10. 医生	是 否	
E17	您会担心在市场上售卖的动物中爆发流行性疾病吗?		是 **1** 否 **2**	E17
E18	去年这个时候开始您从菜市场购买过活的动物吗?		是 **1** 否 **2**	E18
E19	如果您对**E18**的回答是**是**，那么当您去菜市场时会有一些行为上的变化吗？		**是/否**	E19
		8. 戴口罩	是 否	
		9. 戴手套	是 否	
		10. 洗手	是 否	
		11. 有时候从超市购买肉类	是 否	
		12. 购买活体动物的频率降低	是 否	
		13. 只购买养殖动物	是 否	
		14. 不再在菜市场购买野生动物	是 否	
E20	您认为动物能够传播疾病吗?		**是** **否**	E20

感谢您参与此项研究

完

请将完成的调查问卷发回给项目协调员

7.22 Quantitative Consent Form (English)

HUMAN AND ANIMAL CONTACT STUDY

Survey Informed Consent

Introduction: My name is ________ and I work for a project called "*Human and Animal Contact Study.*" The project is funded by the US Government and conducted in collaboration with Wuhan University School of Public Health, Chinese CDC., and other local partners. We are studying how the health of wildlife, livestock and people affect each other and to better understand the patterns of local trade and consumption of meat and food animals.

Interview: As part of this research we are conducting a survey to better understand the types of interactions people have with wildlife and their domestic animals, as well how people live their lives, do their jobs, and take care of their families and their animals. While you may not benefit directly from this research, the information you share with us may help to improve the health of other people who live near or work with animals. The main risk to you would come from a loss of confidentiality. To decrease any risk of someone else seeing your personal information, we give you a code number and use that instead of your name on all information that you provide. Your information is also kept secure in locked files and is considered confidential. We will use this information to better understand disease risks from wild and domestic animals to humans and share this with local and national leaders, non-governmental organisations and the scientific community. When we write about the study, we will not use your name or anyone else's name, or anything about you that someone could recognise.

You will also be asked to provide a small amount of blood, sputum, and stool so that we may test for infections that you may have had. Any leftover samples will be stored for future studies conducted by the research team. These future studies may help us better understand the results of this study and to plan research to help us learn more. Your stored blood will be identified only by the code number that we assign.

Additional information: I am here today to ask if you are willing to participate in this study by talking with me. Your participation is voluntary. You do not have to answer a question if you do not want to. The interview and specimen collection will take approximately 40 minutes of your time.

An Institutional Review Board that is responsible for making sure that research subjects are protected from harm, has approved this project. Do you have any questions at any time? If you have any questions in the future about your participation in this study or your rights as a research subject, or if you change your mind and no longer agree to have your specimen stored, you may contact Guangjin Zhu zhu@ecohealthalliance.org or by phone at 13818140967

Study Code Number ___ ___ ___ ___

Statement of Consent

I have read and understood all of the items on the information sheet. I understand that my participation is voluntary All questions about the study have been answered to my satisfaction.

I understand that:

- ✓ My name will not appear on interview or other data collection forms: only a code number will be used;
- ✓ All information will be kept in locked files that only the professional research staff can see;
- ✓ All written and published information will not use my name, anyone else's name, or anything about me that someone could recognise;

______ (initials) I agree to have my specimens stored for use in future studies that are related to this research study.

________________________________ ________________________

Participant Signature Date

Participant Name (please print)

________________________________ ________________________

Interviewer Date

▪▪

If the individual declines to participate, thank them for their time. Ask why they choose not to participate.

Reason for not participating:____.

Certified by _________________________ Initials ________________ Date____________________

7.23 Quantitative Consent Form (Chinese)

人和动物接触研究

调查知情同意书

介绍：我的名字叫____________，我现在研究的项目是“人和动物的接触研究”，这个项目是由美国政府资助，由武汉大学公共卫生学院，中国疾病控制与预防中心，以及其它的本地合作伙伴一起承担的。我们目前主要研究野生动物，牲畜和人的健康如何相互影响，最终期待更好的理解当地的肉类，以及供给市场动物的贸易和消费模式。

综述：作为研究的一部分，我们希望通过调查，能够更好的理解人和他们饲养的野生动物和家养动物之间的相互作用模式，同时，我们也期望更好的理解人们如何生活，如何工作，如何照顾家庭，如何照顾他们的动物。接受访问的人可能并不能直接从这个研究中获益，但是您和我们分享的信息可能能够帮助那些近距离接触动物或者工作中接触动物的人提高健康状况。对受访者来说，最大的风险源自于私人信息的泄露，所以，为了降低可能导致别人获得受访者私人信息的风险，我们对您提供的所有信息，都将用代码替代您的名字。您的私人信息也将完全受到保密。我们使用以上信息是为了更好的理解疾病由野生和家养动物到人的传播危险，我们将和当地以及国家相关部门，以及非政府组织和科学研究团体分享这些信息。当我们撰写研究报告的时候，我们不会使用任何人的姓名，或者任何可能泄露您身份的信息。

在该项调查当中，需要采集少量的血液，唾液和粪便用来监测可能的感染状况。任何检验后剩余的样品都会被保存，用于以后研究团队进行的相关研究。今后的这些研究可能能够帮助我们更好、更深的理解该项研究结果。从您身上采集并保存的血样会和我们给你的编号保持一致。

补充信息：在这里我正式和你确认：您是否愿意通过和我交谈参加该项研究。请您自愿决定是否参与研究。你不需要回答你不想回答的问题。访谈和收集标本大约会持续 40 分钟时间。

我们有一个机构审查委员会负责保证研究参与者免于受到伤害，该项研究已经获得该委员会的批准。您在任何时候对项目的研究内容、对您在研究过程中享有的权利有任何疑问；或改变主意不愿意保存标本，您可以通过电话 13818140967 或者电子邮件 zhu@ecohealthalliance.org 联系 Guangjian Zhu 博士。

研究编号_____________

知情声明

我已经通读并且了解信息表所有条目内容，我明白我的参与是自愿的，所有的疑问都得到了满意的回答。

我明白：

- ✓ 我的姓名将不会出现在访谈或者其它形式的数据收集过程中，编号将用来替代我的私人信息；
- ✓ 所有的信息都将加密保存，并且只有专业的研究人员能够看到这些信息；
- ✓ 所有撰写或者出版的信息都不会使用任何人的姓名信息，也不会使用其供其他人识别研究对象的信息；

___________(姓名的首字母) 我同意保存我的标本，用于今后和本项目相关的研究。

________________________________ ________________________________

参加者签字 日期

参加者姓名（请打印）

________________________________ ________________________________

采访人 日期

……

如果有人退出访谈，感谢他花费时间了解项目。询问不愿参加访谈得原因。

不愿参加的原因：____

签字____________________姓名首字母__________________日期__________________________

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