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Investigations on the occurrence of infections with hepatitis E virus and related viruses in zoo animals

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A

ab	antibodies
ALT	Alanine transaminase
anti-HEV-ab	anti-HEV-antibodies
AST	Aspartate aminotransferase

B

BfR	Bundesinstitut für Risikobewertung
BLSD	big liver and spleen disease

C

°C	degree Celsius
CTV	cutthroat trout virus
CVUA	Chemisches und Veterinäruntersuchungsamt

E

e.g.	for example (exempli gratia)
ELISA	enzyme-linked immunosorbent assay
ET-NANBH	enterically transmitted NANBH

F

FLI	Friedrich-Loeffler Institut

G

g	gram
gamma-GT	gamma-glutamyltransferase
GT	genotype
GTs	genotypes

H

HAV	hepatitis A virus
HBV	hepatitis B virus
HE	hepatitis E
HEV	hepatitis E virus

HEV-1 to HEV-7	HEV genotypes 1 to -7
HEV-Ab EIA	HEV-antibody ELISA
HEV-GT	hepatitis E virus genotype
HEV-GTs	hepatitis E virus genotypes
HEVs	hepatitis E viruses

I

ICTV	International Committee on Taxonomy of Viruses
IgG	immunoglobulin G
IgM	immunoglobulin M

K

kb	kilo bases

N

NANBH	non-A-/non-B hepatitis
NBS-RT-PCR	nested broad-spectrum RT-PCR
nt	nucleotide

O

ORF	open reading frame
ORFs	open reading frames

P

PCR	polymerase chain reaction
PEI	Paul-Ehrlich-Institut

R

RdRp	RNA-dependent RNA polymerase
RKI	Robert-Koch-Institut
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
RT-qPCR	reverse transcription real-time polymerase chain reaction

T

TEM	Transmission electron microscopic

U

USA	United States of America

V

VLPs	virus-like particles

W

WHO	World Health Organisation

1 General introduction

Hepatitis E is mainly a self-limiting human disease, basically characterised by acute or chronic hepatitis, which is caused by infection with the hepatitis E virus (HEV) (KAMAR et al. 2015; REIN et al. 2012). Large outbreaks have been described in developing countries, where hepatitis E is known to be endemic (PURCELL and EMERSON 2008). However, the disease is also increasingly recognised in industrialised countries like Germany e.g., with 3.275 notified HE cases in 2018 and it is grouped into emerging infectious diseases (PURCELL and EMERSON 2008; RKI 2019). Generally, the case fatality rate is low, ranging between 1–4%, though mortality rates up to 25% have been described for pregnant women during fulminant outbreaks in endemic regions (KAMAR et al. 2012; KUMAR et al. 2013; KHUROO et al. 1995). Dependent on the distinct genotype, hepatitis E viruses particularly can be transmitted zoonotically or by the consumption of faecally contaminated water, undercooked meat and sausage products (PAVIO et al. 2017). Domestic pig (*Sus scrofa domestica*) and wild boar (*Sus scrofa*) represent the major reservoirs for human-pathogenic, zoonotic genotypes (GTs) 3 and 4 (PAVIO et al. 2010). Besides domestic pig and wild boar, HEV or HEV-related viruses have been detected in several other domestic, wildlife, pet and zoo animal species (DÄHNERT et al. 2018; JOHNE et al. 2014; ZHANG et al. 2008).

1.1 Discovery of HEV

During the 1970s and the 1980s, multiple hepatitis outbreaks enforced the development of novel sensitive and specific diagnostic tools for the identification of human infections with hepatitis A (HAV) and hepatitis B virus (HBV). However, the novel diagnostic tools failed to determine the causative agents in certain cases (KHUROO 1980; WONG et al. 1980). Thus, the unidentified virus causing human hepatitis was named non-A-/non-B hepatitis (NANBH) virus (REYES et al. 1990).

In 1980, KHUROO (1980) and WONG et al. (1980) reasserted the hypothesis of the existence of a NANBH virus transmitted by the faecal-oral route. Neither antigens nor antibodies (ab) of both, HAV and HBV were identified as the aetiological agent of the hepatitis outbreak in Kashmir Valley, India (KHUROO 1980). The authors therefore assumed the existence of an additional virus causing hepatitis in humans (KHUROO 1980). According to epidemiological analysis, a stream used as resource for drinking water was strongly indicated as the source of infection (KHUROO 1980). Retrospective

investigations of human sera from New Delhi, India, date the first HEV outbreak in 1955 (WONG et al. 1980). The Delhi epidemic (1955–1956), the Ahmedabad epidemic (1975–1976) and some sporadic hepatitis cases in Pune (1978–1979) were epidemiologically associated with faecally contaminated drinking water (WONG et al. 1980). The number of hepatitis cases with unknown aetiological agent increased and evidence was growing for the existence of an enterically transmitted virus (ET-NANBH) similar to HAV, but yet unknown, being responsible for a major proportion of hepatitis cases in India (BALAYAN et al. 1983).

In 1983, about 30.000 persons were sickened by a water-borne hepatitis infection nearby Tashkent, caused by ET-NANBH (BALAYAN et al. 1983). After an experimental faecal-oral infection of Mikhail Balayan himself, the Russian Scientologist and his team were able to purify and visualise virus-like particles (VLPS) with diameters of 27 to 30 nm in stool samples, using immune electron microscopy (BALAYAN et al. 1983). Five years later, in 1988, the virus was given its current name “hepatitis E virus” by PURCELL and TICEHURST (1988). Furthermore, the molecular characterisation and cloning of the genome was successfully reached in 1990/1991 for the first time (REYES et al. 1990; TAM et al. 1991).

In the same year, BALAYAN and his colleagues (1990) first claimed the possibility of a zoonotic HEV transmission when the experimental transmission of a human HEV strain resulted in the successful infection of a domestic pig. The first animal strain (swine HEV) was detected in domestic pigs from the United States seven years later (MENG et al. 1997). Since swine HEV and human HEV are closely related to each other, a zoonotic way of transmission was now more evident (ERKER et al. 1999; MENG et al. 1997).

A HEV-like virus associated with Big Liver and Spleen Disease (BLSD) was discovered in chicken (*Gallus gallus domesticus*) from Australia and designated as avian HEV in the year 2001 (HANDLINGER and WILLIAMS 1988; HAGSHENAS et al. 2001; PAYNE et al. 1999). BLSD is associated with decreased egg production and a slightly increased mortality in chicken flocks (GERBER et al. 2014; RITCHIE and RIDDELL 1991). In 2010, rat HEV was first identified in Norway rats (*Rattus norvegicus*) from Hamburg, Germany, using a broad-spectrum RT-PCR assay (EASTERBROOK et al. 2007; JOHNE et al. 2010a; JOHNE et al. 2010b). This virus was subsequently detected worldwide in different rat species (LI et al. 2013b; MULYANTO et al. 2014; PURCELL et al. 2011). Another virus, widely spread in

spawning adult trout from California, USA, was first detected in 1991 (HEDRICK et al. 1991). The virus could be isolated in cell culture and was termed the cutthroat trout virus (CTV) (HEDRICK et al. 1991). In 2011, genome sequence analyses of CTV lead to the affiliation into the family *Hepeviridae*, since then known as fish HEV (BATTS et al. 2011). Avian, rat and fish HEV seem to be mainly host-specific with no or low potential of zoonotic transmission to humans.

1.2 Taxonomy

HEV was first classified within the family *Caliciviridae* due to structural and genomic similarities (BRADLEY and BALAYAN 1988; OKAMOTO 2007). In 2005, the genus *Hepevirus* was created, but not assigned to any virus family (EMERSON et al. 2005). This genus comprised two species: the hepatitis E virus, containing the mammalian HEV isolates, and a tentative species containing avian hepatitis E virus (EMERSON et al. 2005). Soon after, a new taxonomic proposal created the new family *Hepeviridae*, including the genus *Hepevirus* and the type species hepatitis E virus (MAYO and BALL 2006). Since a large variety of HEV-like viruses was identified in animals and humans between 2001 and 2014, Smith et al. suggested to divide the family *Hepeviridae* into two genera: *Orthohepevirus* and *Piscihepevirus* (SMITH et al. 2014) (Tab. 1). Instead, the genus *Hepevirus* was deleted.

Currently, the International Committee on Taxonomy of Viruses (ICTV) assigns all human, mammalian and avian GTs to the genus *Orthohepevirus*, whereas the genus *Piscihepevirus* only contains one single species from cutthroat trout (*Oncorhynchus clarkii*) and related fish species (ICTV 2014) (Tab. 1). The genus *Orthohepevirus* is associated with for species, containing in turn various genotypes: *Orthohepevirus A* (isolates from mammals: human, domestic pig, wild boar, rabbit, deer, mongoose dromedary and Bactrian camel), *Orthohepevirus B* (isolates from chicken), *Orthohepevirus C* (isolates from Norway rat, black rat, greater bandicoot, Asian musk shrew, ferret, mink and red fox) and *Orthohepevirus D* (isolates from different bat species) (SMITH et al. 2014) (Tab. 1). Eight genotypes are assigned to the species *Orthohepevirus A*: HEV-1 and HEV-2 (restricted to humans), zoonotic HEV-3 (human, domestic pig, wild boar, rabbit, deer, mongoose), zoonotic HEV-4 (humans, domestic pig, wild boar), HEV-5 and HEV-6 (restricted to wild boar), zoonotic HEV-7 (dromedary camel and human) and HEV-8 (Bactrian camel) (NIDAIRA et al. 2012) (Tab. 1). *Orthohepevirus B* consists of avian HEV that are divided into four proposed subtypes (I-IV) (SMITH et al. 2014). HEV-C1 (isolates from rats) and HEV-

C2 (isolates from wild carnivores: ferret, mink, red fox) are assessed to the species *Orthohepevirus C* (SMITH et al. 2014) (Tab. 1). *Orthohepevirus D* strains have been detected in various bat species (DREXLER et al. 2012; SMITH et al. 2014) (Tab. 1).

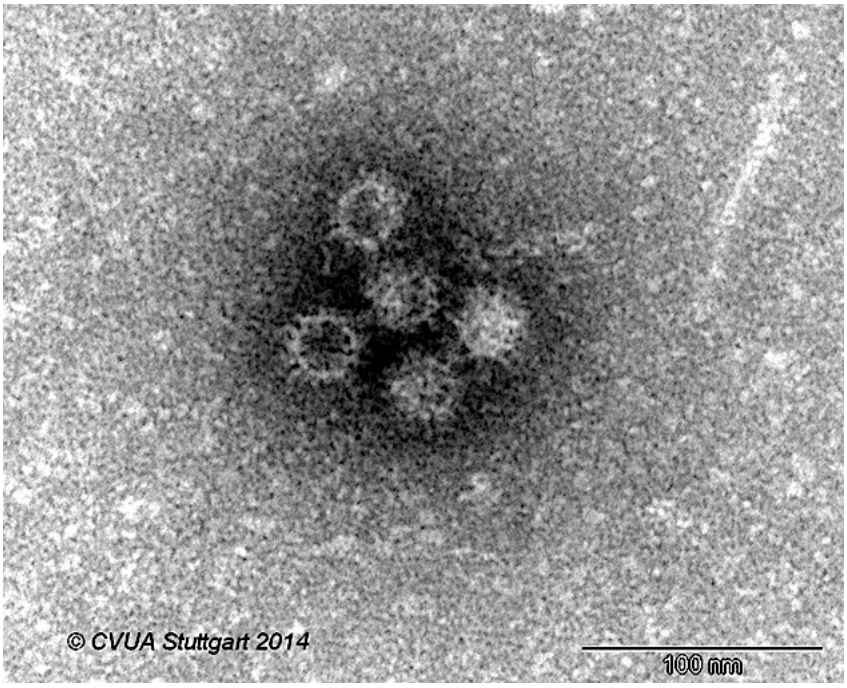
HEPEVIRIDAE	
Genus ***Orthohepevirus***	
Species ***Orthohepevirus A***	
Genotype:	
HEV-1 / HEV-2	human
HEV-3	human, domestic pig, wild boar, rabbit, deer, mongoose
HEV-4	human, domestic pig, wild boar
HEV-5 / HEV-6	wild boar
HEV-7	dromedary, human
HEV-8	Bactrian camel
Species ***Orthohepevirus B***	
Genotype:	
avianHEV	chicken
Species ***Orthohepevirus C***	
Genotype:	
HEV-C1	Norway rat, black rat, bandicoot rat, Asian musk shrew
HEV-C2	ferret, mink, red fox
Species ***Orthohepevirus D***	
Genotype:	
---*	different bat species
Genus ***Piscihepevirus***	
Species ***Piscihepevirus A***	
Genotype:	
---*	trout, related fish

*, no genotype existing.

Table 1: Taxonomic classification of HEV within the family *Hepeviridae*.

1.3 Morphology

The hepatitis E virus is a small non-enveloped RNA-virus with an icosahedral capsid of about 27 to 34 nm in diameter (MENG et al. 2010). Recent analyses suggest the presence of an additional outer membrane in a fraction of HEV particles in patient sera and cell culture supernatant (YIN et al. 2016). The virus' morphology resembles the morphology of caliciviruses (Fig. 1). The virus particle is mainly composed of the capsid protein encoded by the open reading frame (ORF) 2. Enveloped particles additionally contain the small phosphoprotein encoded by ORF3 (JOHNE et al. 2014).

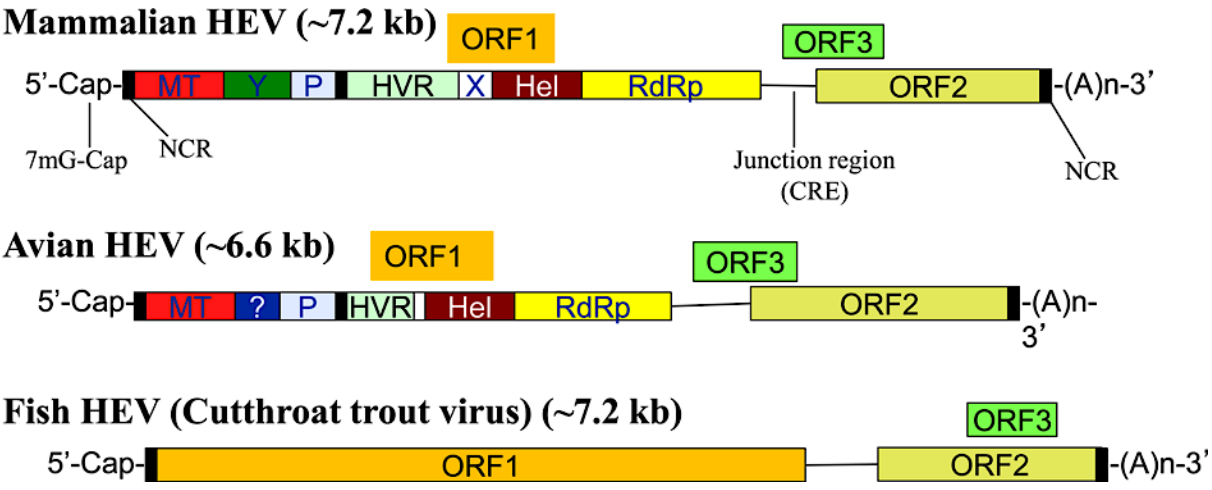


With permission from Dr. Valerij Akimkin, CVUA, Stuttgart, Germany, 2014.

Figure 1: TEM picture of HEV from German wild boar.

1.4 Genomic organisation

The viral genome consists of a linear, single-stranded RNA with positive polarity and a length of 6.6–7.3 kb (kilo bases) (RYLL et al. 2017). The genome contains typical sequence elements of an eukaryotic mRNA: it is capped at the 5´-end with 7-methylguanosine and polyadenylated at the 3´-end (KABRANE-LAZIZI et al. 1999; REYES et al. 1990; TAM et al. 1991). The regions adjacent to the poly A-tail and the cap structure are non-coding regions, which seem to have essential influence on the viral replication and protein translation (CAO et al. 2012; CHANDRA et al. 2008; TAM et al. 1991). The virus genome contains three major open reading frames (ORF1, ORF2 and ORF3) (JOHNE et al. 2014). Strains of genotype HEV-C1 (rat HEV and ferret HEV) contain an additional open reading frame (ORF4), overlapping with the 5'-region of ORF1. The genome of avian HEV is 600 base pairs shorter compared to mammalian HEV or fish HEV and shares only 50% nucleotide (nt) sequence identity with them. A schematic presentation of the genomes of mammalian, avian and fish HEV is presented in Fig. 2.



Adapted from MENG 2016;

CRE, cis-reactive element; Hel, helicase; HVR, hypervariable region; MT, methyltransferase; NCR, non-coding regions; ORFs, open reading frames; P, papain-like cysteine protease; RdRp, RNA-dependent RNA polymerase; X, macro domain.

Figure 2: Genomic organisation of mammalian, avian and fish HEV.

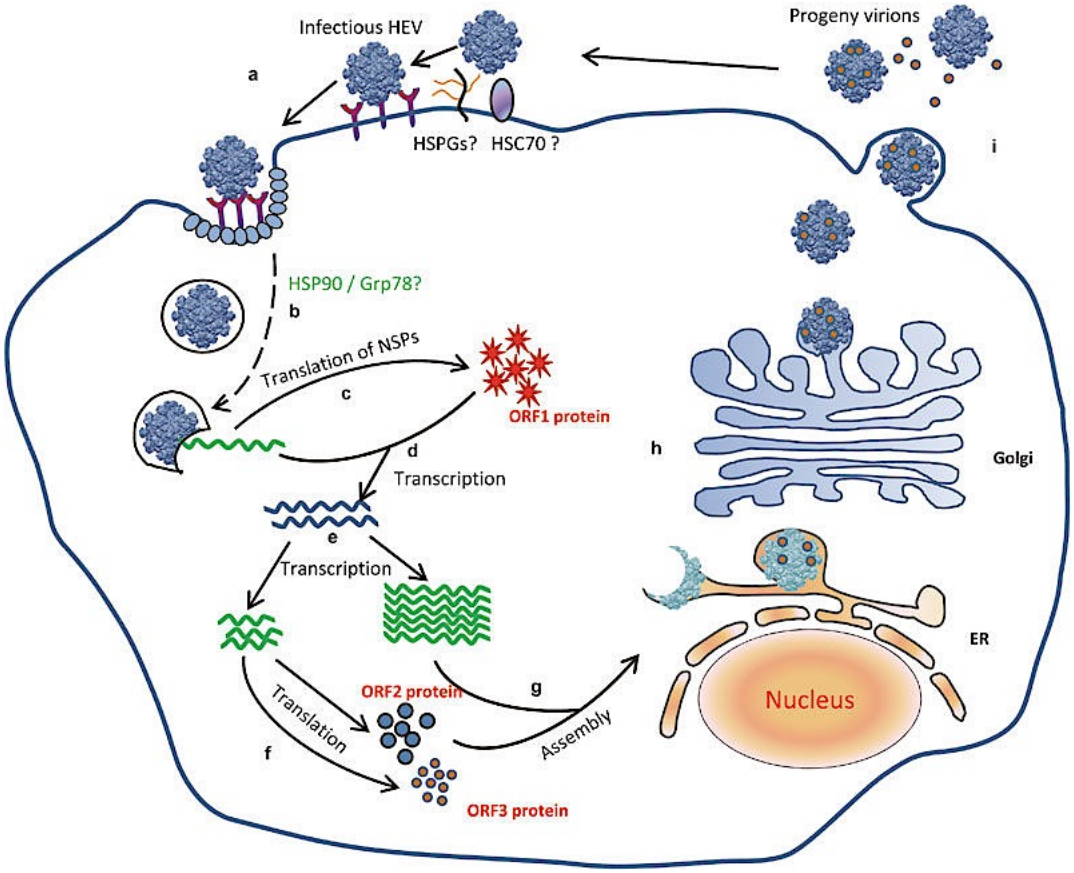
The ORF1 is the largest part of the genome with 4.6–5.2 kb, positioned at the 5´-end of the genomic map and directly translated (MENG et al. 2010). It encodes one polyprotein, which in turn is processed into various non-structural proteins with enzymatic activities (MENG et al. 2010). Between ORF1 and ORF3, there is a junction region for mammalian HEV and avian HEV, containing a stem-loop structure and a cis-reactive element (CRE), which initiates the synthesis of a sub genomic mRNA encoding ORF2 and ORF3.

ORF2 encodes the capsid protein of 600–675 amino acids, with binding activity to cell surface heparin sulphate proteoglycans (YAMASHITA et al. 2009). This capsid protein is positioned at the 3´-end of the genome. The capsid protein is capable of self-assembly into virus-like particles (CHANDRA et al. 2008).

The ORF3 overlaps with ORF2 and encodes a small phosphoprotein of largely varying length in avian, mammalian and fish HEV (HOLLA et al. 2013; JOHNE et al. 2014; ZAFRULLAH et al. 1997). Functions in viral infectivity and immunosuppression of the host have been suggested (CHANDRA et al. 2008). ORF4 is an additional ORF of 522 nt, only described for rat HEV and ferret HEV. This ORF4 overlaps with ORF1 at its 5´-end and its function is still unknown (JOHNE et al. 2010b; RAJ et al. 2012).

1.5 Viral replication

HEV is a hepatotropic virus mainly infecting hepatocytes and Kupffer cells of the liver (LEE et al. 2009). The virus replication cycle is shown schematically in Fig. 3.



Adapted from CAO and MENG 2012;

ER: endoplasmatic reticulum; putative attachment receptors: HSPGs, heparin sulphate proteoglycans; HSC70, heat shock cognate protein 70; HSP90, heat shock cognate protein 90; GrP78, glucose-regulated protein 78; NSP, non-structural polyprotein; Golgi, Golgi apparatus.

Figure 3: Replication cycle of HEV.

Primarily, the HEV particle binds to the cell surface using heparin sulphate proteoglycans and a still unknown receptor molecule before entering the cell. The viral RNA is then released from the capsid into the cytoplasm with the help of heat shock protein 90 and glucose-regulated protein 78 (CAO and MENG 2012). The released positive-sense genomic RNA serves as a template for translation of the ORF1-encoded non-structural polyprotein (NSP), which is subsequently processed by cellular proteases (CHANDRA et al. 2008). One product of the NSP is the viral RNA-dependent RNA polymerase (RdRp). An ER transmembrane domain in the RdRp is involved in the replication complex of HEV and interacts with the 3´-end of the genomic HEV RNA (AGGARWAL et al. 2001). In the first replication cycle, the positive strand is transcribed into a negative strand. This negative strand serves as template for the genomic positive strand and for the sub genomic positive strand in a second replication cycle. The structural proteins (capsid protein and phosphoprotein) encoded by ORF2 and ORF3 are then translated from this bicistronic sub genomic RNA (GRAFF et al. 2006). The

ORF2-encoded capsid protein thereafter packages the genomic viral RNA and new virions are assembled (CAO and MENG 2012). Thereafter, progeny virions are transported to the cell membrane and exit the infected liver cells by the help of the ORF3-encoded phosphoprotein (CAO and MENG 2012). It has been suggested that the released HEV particles originally contain a membrane derived from the cell and associated with the ORF3 protein, which is removed by bile salts and trypsin during the egress of the virus through the bile duct and intestine (OKAMOTO 2013; OKAMOTO, 2011). Therefore, HEV particles shed by stool are non-enveloped (OKAMOTO 2013; OKAMOTO, 2011).

1.6 Hepatitis E in humans

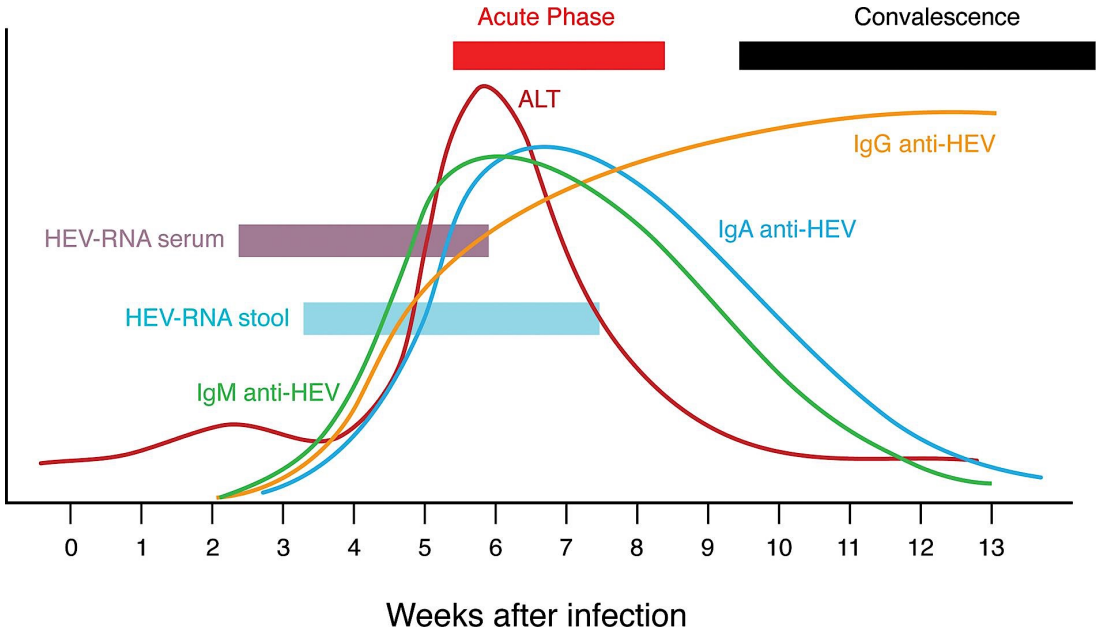
HEV represents one of the five major human hepatotropic viruses (hepatitis A–E), which primarily affect the liver. In particular, infection with HEV is the most common cause for acute hepatitis in humans worldwide (REIN et al. 2012). Annually about 3.5 million patients worldwide come up with an acute hepatitis caused by HEV proceeding to lethal disease in more than 65,000 cases and 3,000 stillbirths (REIN et al. 2012). After incubation for two to eight weeks, mild to moderate influenza-like symptoms arise at first, developing to emesis, fever, pain of the limbs, headache or epigastralgia, before signs of acute hepatitis can occur. On the basis of clinical symptoms only, hepatitis E is hard to distinguish from other viral infections causing hepatitis or non-infectious liver diseases due to abuse of alcohol or medication, toxins, storage diseases, Alpha 1-antitrypsin deficiency or autoimmune hepatitis (RKI 2015). Hepatomegaly and increased levels of the liver enzymes bilirubin, alanine transaminase (ALT) and gamma-glutamyltransferase (gamma-GT) are indicators for an acute liver disease (PAVIO et al. 2010). These changes of the liver function occur within 4–6 weeks post infection and are often accompanied by decolouration of the stool and dark urine (SCHIELKE 2011).

Generally, the case/fatality rate is low, ranging between 0.2% and 4% (KUMAR et al. 2013). In developing countries, large outbreaks of hepatitis E may occur due to improper hygiene, leading to faecal contamination of drinking water with HEV. In these countries, the HEV-IgG seroprevalence within adults ranges between 30–80% (WHO 2015). For endemic regions as China, India, Somalia and Uganda, high mortality rates up to 25% have been observed in pregnant women with fulminant hepatitis after infection with HEV genotype 1 (KAMAR et al. 2012). In contrast, sporadic asymptomatic cases of human hepatitis are common in industrialised European

countries (ADLHOCH et al. 2016). These cases are still comparatively rare, but the disease is also increasingly recognised during the past ten years (ADLHOCH et al. 2016). Here, the HEV-IgG seroprevalence ranges between 16% (Southwestern England) and 53% (Southwestern France) (FABER et al. 2012).

The disease is mostly self-limiting, and the patients fully recover after a few weeks (FABER et al. 2012). However, chronic HEV infections, which may develop to liver cirrhosis, have been repeatedly described in immunosuppressed transplant patients after infection with HEV genotype 3 (KAMAR et al. 2015; KAMAR et al. 2014b; KAMAR et al. 2013).

Since HEV does not cause a cytopathic effect in liver cells or hepatocyte cytolysis, hepatitis E is assumed to be an immune-mediated disease, which is induced by the host immune response against the infected liver cells (PAVIO et al. 2010). The viremic phase starts in the prodromal stage, about 2 weeks after infection, when HEV-RNA can be detected in serum (Fig. 4). About one week later, viral excretion via faeces begins, continuing until 2–3 weeks after the onset of jaundice (PAVIO et al. 2010). Clinical symptoms and liver enzyme values usually decrease within 6 weeks (Fig. 4). IgM ab are first detected in serum after about 2 weeks post infection; however, their concentration declines within 3 months. IgG ab occur in parallel or later and may persist for several years (Fig. 4).



Adapted from PEREZ-GRACIA et al. 2015.

Figure 4: Time course of HEV infection in humans.

1.7 Tools for HEV diagnosis

The haemograms of human patients with clinical symptomatology typically show increased levels of AST and ALT, which are disproportionately high compared to the increase of AP and gamma-GT (RKI 2015). Especially for icteric disease courses, the serum bilirubin level, and the urobilinogen level, are significantly elevated (RKI 2015).

Several immunoassays (e.g. ELISA's or western blots) are available for the detection of HEV antigens or HEV-specific IgG or IgM ab (SCHIELKE 2011). Acute HEV infection in humans is typically detected, using HEV-specific IgM ab ELISAs. For HEV diagnosis in animals, the use of anti-HEV-IgG ELISAs is more common. However, to date, there is no immunoassay, which serves as "gold standard" for the detection of HEV-specific ab.

Nowadays HEV diagnosis is mostly done using molecular methods, such as conventional, nested or real time reverse transcription polymerase chain reaction (RT-PCR) (JOTHIKUMAR et al. 2006; SCHIELKE 2011). Several real time RT-PCR protocols enable the detection of the four major human-pathogenic GTs HEV-1 to HEV-4 (JOTHIKUMAR et al. 2006). A nested broad-spectrum RT-PCR can be used for the broad detection of HEV strains from species *Orthohepevirus A*, *B* and *C* (JOHNE et al. 2010b). Several other specific RT-PCR assays have been developed, e.g. for detection of rat HEV (WOLF et al. 2013).

Generally, it is also possible to detect HEV via isolation in cell culture. However, this diagnostic tool has no relevance for routine laboratory diagnosis, as it is very sophisticated, time-consuming and mostly not successful (RKI 2015). Other techniques like electron microscopy and immunohistochemical staining techniques are only used sporadically for demonstration of HEV in specific tissue samples (RKI 2015).

1.8 Therapy

Ribavirin monotherapy, what is also successfully used for therapy of HIV and HCV, has repeatedly been described as an effective drug that inhibits the replication of HEV *in vivo* and induces a sustained antiviral response in immunocompromised transplant patients with chronic HEV infections (DEBING et al. 2014; KAMAR et al. 2014a; KAMAR et al. 2010). Although it has been shown to be effective in several patients, in Germany the hepatitis E therapy with ribavirin has no approval yet (ANON. 2017; LEE et al. 2016; SRIDHAR et al. 2015).

1.9 Animal infections with HEV and HEV-like viruses

Domestic pig, wild boar, rabbit (*Oryctolagus cuniculus*) and dromedary camel (*Camelus dromedarius*) are known as the main reservoirs of the zoonotic genotypes HEV-3, -4 and -7 (ABRAVANEL et al. 2017; DOCEUL et al. 2016; MENG et al. 2009). Transmission of HEV-3 from deer to humans has also been described repeatedly, although deer most probably undergoes “spillover infections” from wild boar, rather than being a true reservoir (ANHEYER-BEHMENBURG et al. 2017; MATSUURA et al. 2007; TEI et al. 2003). The HEV infection in animals seems to be generally asymptomatic (DE CARVALHO et al. 2013).

In 1997, the first animal strain of HEV, swine HEV, was detected in domestic pigs from the United States (MENG et al. 1997) (Fig. 5). In the following years, several studies from different countries (USA, New Zealand, Mexico, Spain, France) gave evidence for a worldwide distribution of HEV in domestic pigs (CARUSO et al. 2016; DOCEUL et al. 2016; PAVIO et al. 2010). The reported anti-HEV-IgG seroprevalences in swine herds are usually high, ranging between 23% and 100%, with increasing seroprevalence with higher age, suggesting that HEV is enzootic in swine herds all over the world (CARUSO et al. 2016; DOCEUL et al. 2016; PAVIO et al. 2010). Anti-HEV-antibodies (anti-HEV-ab) or HEV-RNA can be detected in sera, faeces, slurry or livers of infected pigs. The close relationship between swine HEV and human HEV strains led to the assumption, that zoonotic transmission may be possible (VAN DER POEL et al. 2001). Therefore, domestic pigs are regarded as the most important reservoir of HEV (SCHIELKE 2011). Successful experimental transmission of HEV-3 strains to domestic pigs and non-human primates provided evidence for zoonotic transmission of human-pathogenic HEV strains (DE CARVALHO et al. 2013; XU et al. 2014).

As anti-HEV-ab were repeatedly detected in domestic pigs, it was speculated, that wild boars may represent a reservoir for HEV, too (DOCEUL et al. 2016; JOHNE et al. 2014; KACI et al. 2008). The detection of HEV-RNA in sera, bile, faeces or liver from wild boars in different countries supported this hypothesis (MENG 2010). The first genome of a wild boar HEV strain from Japan was published in 2004 (SONODA et al. 2004) (Fig. 5). Another four years later, wild boar HEV was even detected in the European wild boar population (KABA et al. 2010; MARTELLI et al. 2008, REUTER et al. 2009). Besides domestic pigs, wild boars are regarded as the second most important animal reservoir of HEV.

Mongoose and rabbit are also known as animal reservoirs of HEV-3. For the first time, anti-HEV-3-ab and a full HEV-3 genome sequence were detected in Javan mongooses (*Herpestes javanicus*) from Okinawa, Japan in 2006, suggesting that these animals represent an additional reservoir for HEV-3 (LI et al. 2006; NAKAMURA et al. 2006) (Fig. 5). In 2009, anti-HEV-ab and HEV-RNA were detected in 57.0% (191/335) and 7.5% (25/335) of the sera from farmed rex rabbits (*Oryctolagus cuniculus domesticus*) from China, respectively (ZHAO et al. 2009). Subsequently, the new HEV genotype was named rabbit HEV (ZHAO et al. 2009) (Fig. 5). In 2014, rabbit HEV was grouped as a distinct subtype of HEV-3 (SMITH et al. 2014). HAMMERSCHMIDT et al. (2017) succeeded in the detection of anti-HEV-3-antibodies in European brown hares (*Lepus europaeus*). HEV-RNA was also detected in livers from wild rabbits (HAMMERSCHMIDT et al. 2017).

A new human-pathogenic HEV-GT (HEV-7) was recently described in dromedary camels from the Middle East, which seems to be widely distributed among these animals (RASCHE et al. 2016; WOO et al. 2014) (Fig. 5). Evidence for the zoonotic potential of HEV-7 was given in 2016, when it was detected in a chronically infected transplant patient from the United Arab Emirates, who regularly consumed camel meat and milk (LEE et la. 2016; SRIDHAR et al. 2017).

Rat HEV was first detected in Norway rats from Germany, in 2010, and subsequently in different rat species, worldwide (JOHNE et al. 2012; RYLL et al. 2017; WIDÉN et al. 2014). Primarily, host specificity of rat HEV was suggested and evidenced, using experimentally infected laboratory rats and other mammals (COSSABOOM et al. 2012; LI et al. 2013c). The detection of rat HEV in bandicoot rats and Asian musk shrews, however, suggested a broader host range or common “spillover infections” (GUAN et al. 2013; MULYANTO et al. 2013; RYLL et al. 2017). Therefore, rats have been suspected as HEV animal reservoir for several years, besides the main reservoirs of zoonotic genotypes HEV-3, -4 and -7, (LI et al. 2013a; LI et al. 2013b; RYLL et al. 2017). Although some serological reports gave incidence for a zoonotic potential of rat HEV and in contrast, few Norway rats were reported to be HEV-3 positive, rats are still discussed controversially as a potential zoonotic reservoir (DREMSEK et al. 2012; LACK et al. 20120; KANAI et al. 2012).

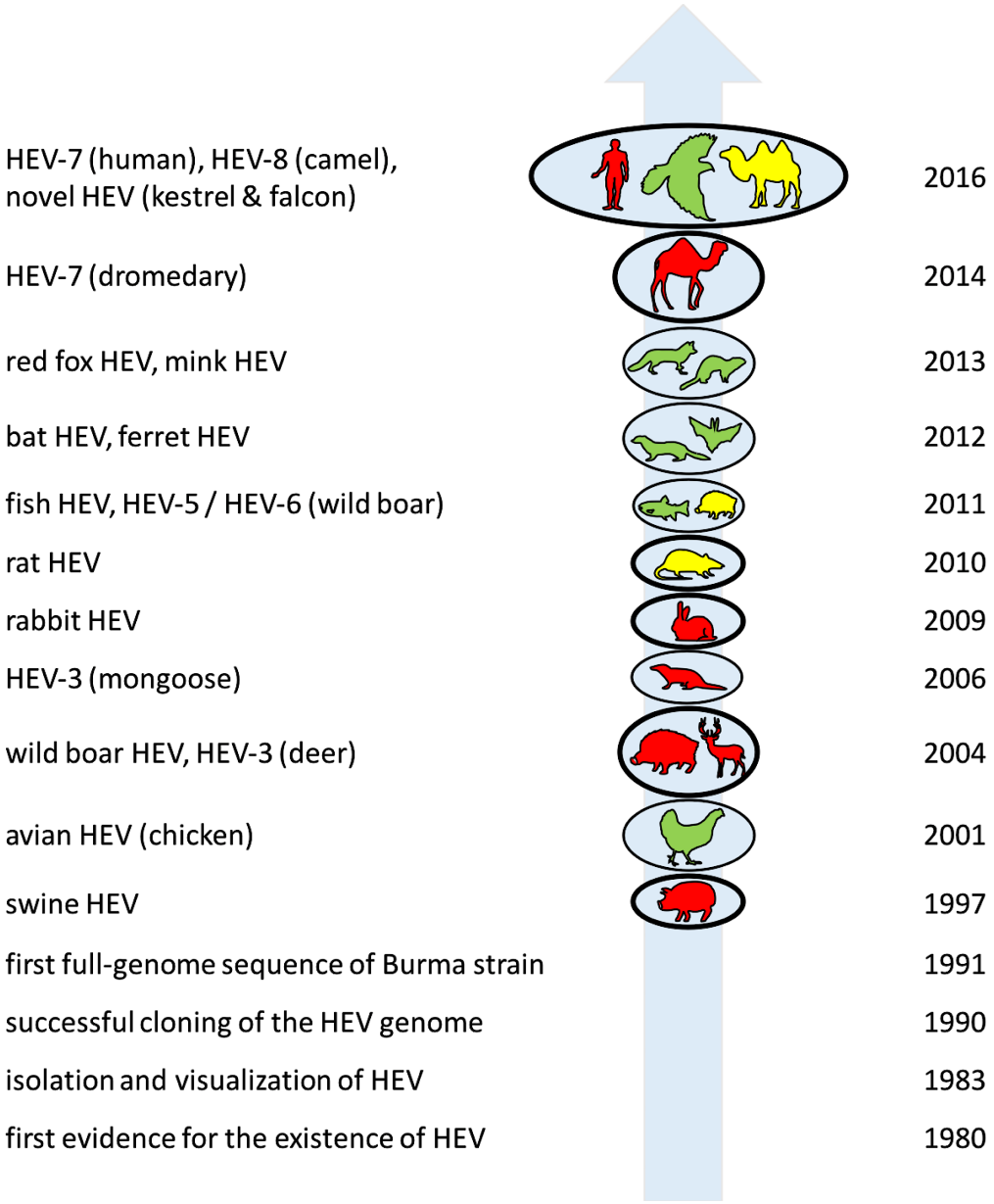


Figure 5: Important discoveries about HEV in humans and animals.

Additional HEV-like viruses have been detected in a wide range of animal species, including chicken, fish, bat, ferret, mink and fox (HSU and TSAI et al. 2014; KROG et al. 2013; RAJ et al. 2012) (Fig. 5). This includes detections in wild, farmed and zoo animals (BODEWES et al. 2013; PERALTA et al. 2009; ZHANG et al. 2008a). So far, these viruses show only a distant genetic relationship to the human-pathogenic genotypes and are therefore suspected to have a low potential of transmission to humans. However, the general involvement of a large variety of animal species in the HEV transmission cycles and their involvement in virus transmission to humans have not been determined so far.

1.10 Experimental infections of animals

Experimental infections of various animals with different HEV species and genotypes have been used to assess the infection routes, excretion, host range, organ tropism and pathogenesis of viruses and the vaccine efficiency (DOCEUL et al. 2016).

Domestic pigs and various non-human primate species, among them macaques, tamarin, langur monkey and chimpanzee (*Pan troglodytes*), served as excellent primary model organisms for successful infections with HEV 1–4, including zoonotic infections (ARANKALLE et al. 1988; MA et al. 2009; TSAREV et al. 1995; TSAREV et al. 1994; VITRAL et al. 1998; YU et al. 2010). Non-human primates infected intravenously with high doses of HEV show typical clinical symptoms resembling those of human hepatitis E, e.g. elevation of liver enzymes, viremia and seroconversion (YUGO et al. 2014; TICEHURST et al. 1992).

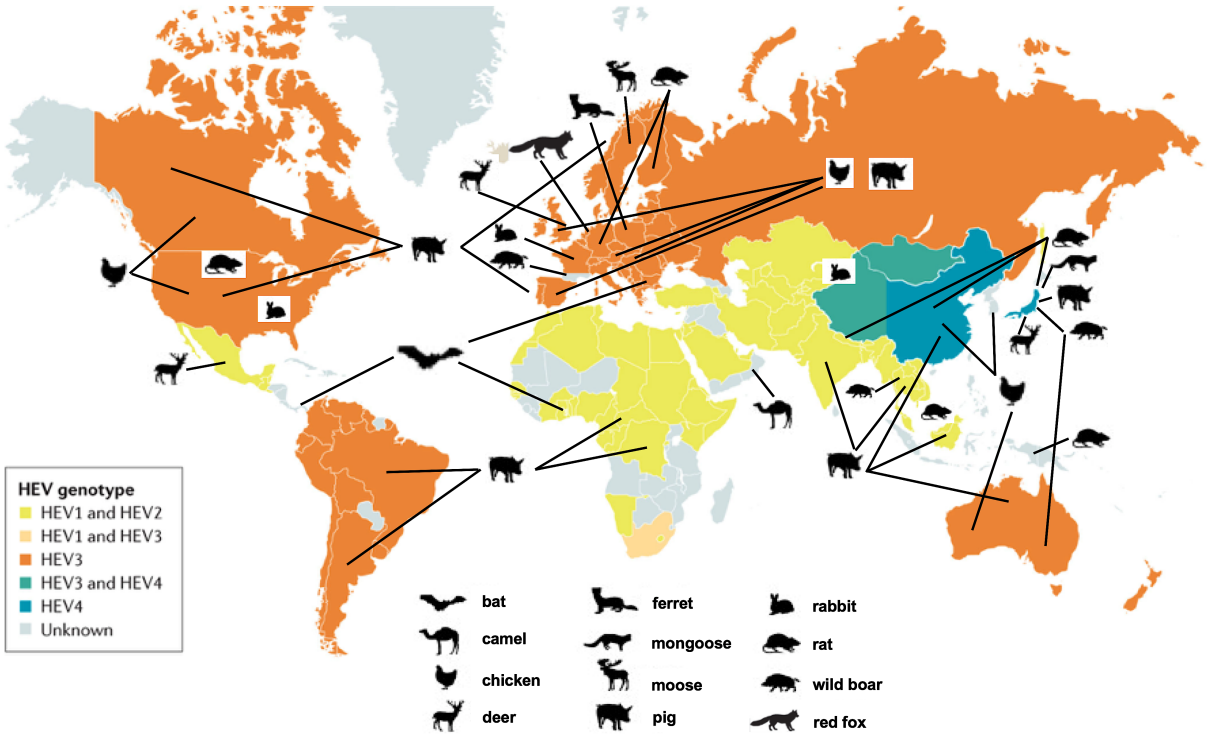
A variety of animal species served as models to assess the zoonotic character of HEV-3 and HEV-4. HEV-3 strains from human and swine were successfully used for experimental cross-species transmission to non-human primates and domestic pigs (FEAGINS et al. 2008; HALBUR et al. 2001; MENG et al. 1998a; MENG et al. 1998b). Intravenously infected domestic pigs showed viremia and seroconversion, however, mostly without clinical signs (LEE et al. 2009; WILLIAMS et al. 2001). Rabbit HEV-3 strains can be transmitted to domestic pigs, non-human primates and rabbits (CHENG et al. 2012; COSSABOOM et al. 2012; LIU et al. 2013). Experimental inoculation of non-human primates, swine and rabbits with human and swine HEV-4 strains has also been demonstrated (CHENG et al. 2012). Mongolian gerbils (*Meriones unguiculatus*) and [immunodeficient] house mice (*Mus musculus*) were used as small animal models demonstrating experimental cross-species transmissions of swine HEV-4 strains (DOCEUL et al. 2016).

1.11 Geographical distribution

HEVs and HEV-related viruses are distributed worldwide. However, the human-pathogenic HEV genotypes (HEV-GTs) are differently distributed in geographically distinct regions of the world. HEV-1 mainly occurs in Eastern Asia (e.g. India, Pakistan, Russia and Japan) as well as in Northern and Eastern Africa (KAMAR et al. 2017) (Fig.6, yellow). HEV-2 has primarily been isolated in Mexico but can also be found in some areas of Africa (KAMAR et al. 2017) (Fig. 6, yellow). HEV-3 has been identified as the major GT in Europe and the USA, but was also detected in Australia, Africa,

Argentina and Japan (DE PAULA et al. 2013; KAMAR et al. 2017) (Fig. 6, orange). In contrast, HEV-4 is mainly confined to South-Eastern Asia with focus on China, Japan and Indonesia (KAMAR et al. 2017) (Fig. 6, blue and green).

HEV and HEV-like viruses do have various animal reservoirs with “complex ecology and genetic diversity” worldwide (SRIDHAR et al. 2015) (Fig. 6). As already mentioned in chapter 1.9, this includes a wide range of different animal species. Domestic pig and wild boar are the main reservoirs of zoonotic HEV-3 and HEV-4 (CARUSO et al. 2016; LAPA et al. 2015; MENG et al. 2009). These animals are widely distributed all over the world and the geographical distributions of HEV-3 and HEV-4 in these animals are similar to that in humans (Fig. 6). HEV-5 and -6 have only been detected in single wild boars from Japan so far and HEV-7 seems to be mainly confined to the Middle East (LI et al. 2017; TAKAHASHI et al. 2014; TAKAHASHI et al. 2011; ZHOU et al. 2015). HEV-8 has so far only been detected in Bactrian camels (*Camelus bactrianus*) from a farm in China (WOO et al. 2016). A significant number of studies describes the detection of avian HEV and rat HEV in different countries of the world suggesting a worldwide distribution of these viruses (RYLL et al. 2017; ZHANG et al. 2017; ZHANG et al. 2014). For the other HEV-like viruses, only scattered information is available on their geographical distribution.

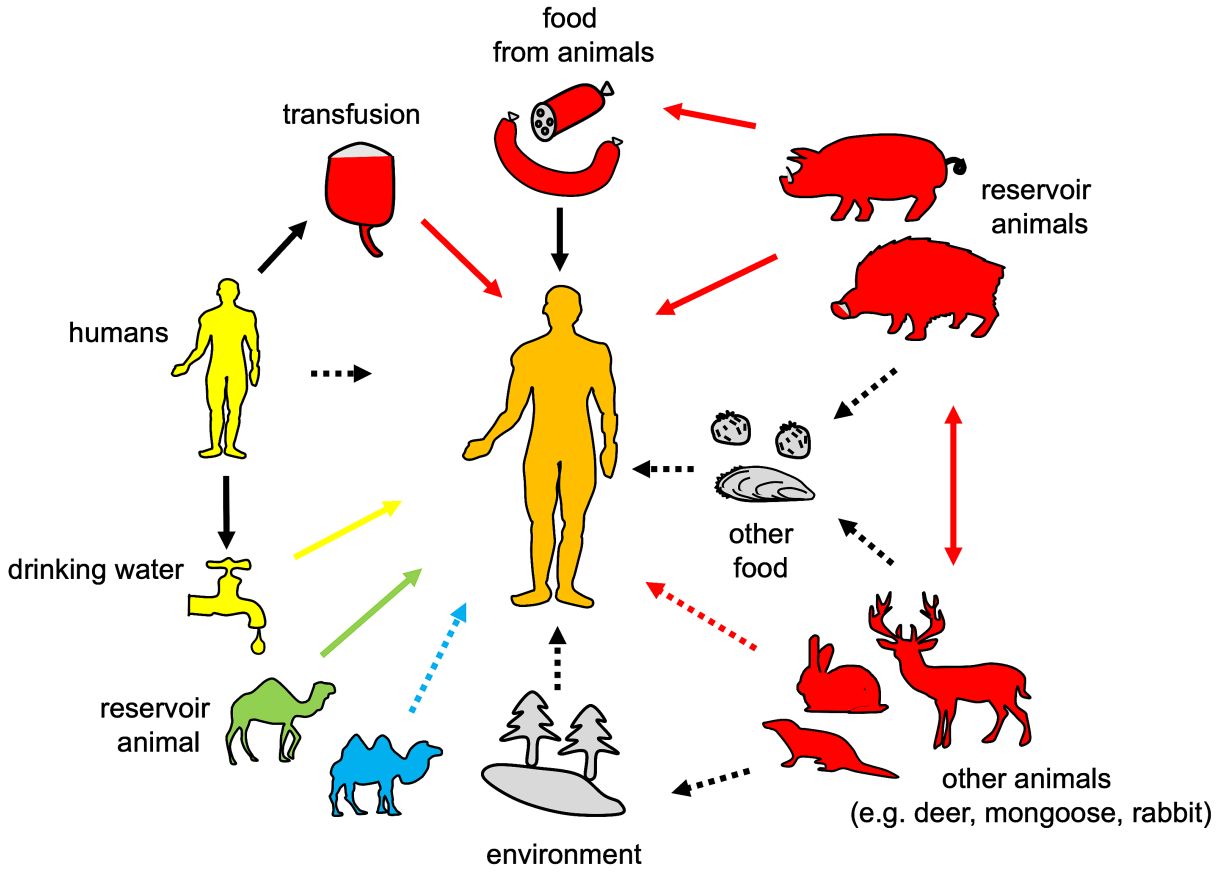


Adapted from KAMAR et al. 2017.

Figure 6: Geographical distribution of HEVs.

1.12 Transmission pathways

The distinct transmission pathways of human-pathogenic HEV are especially dependent on the GT of the virus (JOHNE et al. 2014). HEV-1 and HEV-2 are mainly transmitted via faecally contaminated drinking water (Fig. 7, yellow). For HEV-3, HEV-4 and HEV-7, a foodborne transmission pathway has been shown, which is mainly based on consumption of raw milk and undercooked food prepared from infected animals (CHOI et al. 2013; LEE et al. 2016; RIVERO-JUAREZ et al. 2016) (Fig. 7, red and green/blue). In addition, transmission of HEV-3 and HEV-4 via contaminated blood products or organ transplantation has been shown (KAMAR et al. 2015; KAMAR et al. 2014b; KAMAR et al. 2008). Several other transmission pathways, e.g. through environmental contamination with faeces (berries and shellfish) or direct contact to animals and humans, have been proposed, but often their evidence is proven scarcely (BRASSARD et al. 2012; CROSSAN et al. 2012; GAO et al. 2015; KHUROO et al. 2009; MAUNULA et al. 2013; MESQUITA et al. 2016) (Fig. 7, dotted arrows).



Adapted from SPAHR at al. 2018b.

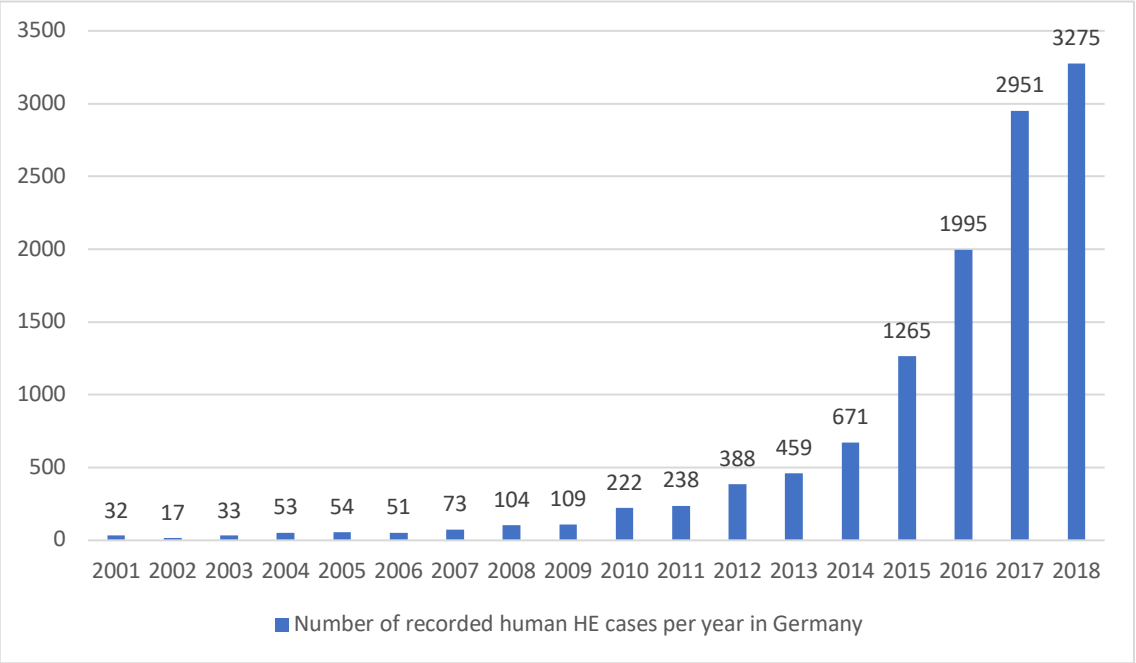
Figure 7: Transmission routes of human-pathogenic HEV-GTs.

1.13 Epidemiology

In developing countries, HEV is responsible for more than 50% of acute viral hepatitis cases including over 50% cases in India, about 25% in Africa and 15–20% in the Eastern Orient (PURCELL and EMERSON 2008). In Africa, Asia and Latin America, human-pathogenic, zoonotic HEV-1 and HEV-2 are known to be endemic (OKAMOTO 2007; PUCRELL and EMERSON 2001). For Africa and Eastern Asia, approximately 20 million HEV infections are reported per year (REIN et al. 2012). One-third of the world's population – comprising more than two billion people – are living in areas highly endemic for HEV-1 and HEV-2 (WHO 2015). In these areas with poor sanitation conditions, e.g. China, India, Sudan, Chad and Uganda, large waterborne outbreaks and epidemics with thousands of cases were reported (AYE et al. 1992; MÉRENS et al. 2009; NAIK et al. 1992; TESHALE et al. 2010). Young to middle-aged male adults (15–40 years) seem to be afflicted predominantly (CHANDRA et al. 2008). Infections with HEV-1 are often associated with high mortality rates (15%–25%) in pregnant women (KHUROO et al. 1995).

In industrialised countries, the human-pathogenic, zoonotic HEV-3 and HEV-4 are predominant. HEV-3 is known to be endemic in European countries and is detected in 5–15% of the acute hepatitis cases (ADLHOCH et al. 2016; DALTON et al. 2008; LAPA et al. 2015). Out of these, most patients got infected, traveling to the above-mentioned developing countries, preserving blood transfusions or regularly consuming pig meat (LAPA et al. 2015). However, the reported anti-HEV-antibody prevalence in European blood donors, up to 52% in France, gave evidence, that subclinical HEV infections are very common in industrialised countries (LAPA et al. 2015). It is estimated, that about 30% of the adult German population undergoes a mostly asymptomatic HEV infection within their lives (ANON. 2017). During the last years, the number of recorded autochthonous clinical hepatitis E cases has steeply been increased in many Western European countries (e.g. France, Germany, England and Wales), whereas in Northern and Southern European countries less cases were notified (ADLHOCH et al. 2016). As the management of the disease is subject to national policies, the notification, prevention and control is not implemented consistently in all countries. Figure 8 illustrates the development of the number of annually reported hepatitis E cases in Germany since 2001, when hepatitis E became a notifiable infectious disease (RKI 2019). The increase of recorded hepatitis cases

may be a result of the availability of novel diagnostic tools, enhanced awareness of human doctors or a raised public attention (ADLHOCH et al. 2016; PAVIO et al. 2010).



Numbers adapted from RKI 2019.

Figure 8: Number of recorded annual HE cases in Germany.

1.14 Prevention

In developing countries, prevention and control of human HEV infections with HEV-1 and HEV-2 is extremely important, as morbidity and mortality of the disease are relatively high. Improvement of hygiene and access to clean drinking water are the most effective ways to control HEV infections in these regions (PAVIO et al. 2010). Additionally, the consumption of insufficiently heated food, which may be contaminated during preparation, should be avoided (BfR FAQs 2016).

In industrialised countries, where higher sanitation standards are common, the focus should be laid on the prevention of zoonotic HEV transmissions.

Food-borne HEV infections with HEV-3 or HEV-4 are most common in industrialised countries. They can be prevented by complying with a good kitchen hygiene and cooking thoroughly meat products from pig, wild boar and deer (BfR FAQs 2016). Heating over 70°C (degree Celsius) for at least 20 minutes inactivates the HEV (BARNAUD et al. 2012; JOHNE et al. 2016).

People occupationally working with animals and animal samples, e.g. veterinarians, keepers, swine handlers, slaughterhouse workers and hunters, are at

higher risk of HEV-3 and HEV-4 infections (BfR FAQs 2016; PAVIO et al. 2017). This group of people should therefore be trained on hygienic measures while handling animals or animal products, e.g. for hunters to use gloves during evisceration of wild boars (SCHIELKE et al. 2015). Specific recommendation for veterinarians and animal keepers may be difficult as long as the distinct host range of HEV and HEV-like viruses and therefore the risk of infection by contact to a specific animal species are not known.

Vaccination of the population, especially of groups of persons with higher risk for HEV infections, such as old, pregnant or immunocompromised persons with liver diseases, would be reasonable. However, a vaccination against HE is currently accredited in China only and has no concession for Europe (RKI 2015).

As up to 50% of immunocompromised transplant patients come up with a chronic HEV infection after receiving repeated blood transfusions, blood donors and organ transplants should be routinely screened for HEV (ANON. 2017). In England, Ireland and the Netherlands HEV screening of donated blood has already been regulated in 2017 (ANON. 2017). To protect immunocompromised patients in Germany likewise, the screening of therapeutic blood products was very recently prescribed by the Paul-Ehrlich-Institut (PEI) (Deutsches Ärzteblatt 2018). Starting September, the 30th 2019, blood products need to be proofed for HEV genome and declared HEV negative before application (Deutsches Ärzteblatt 2018).

2 Aims of the study

The human-pathogenic zoonotic HEV-3, -4 and -7 are well-known to infect reservoir animals, such as domestic pig, wild boar, rabbit, deer and dromedary camel. In addition, other HEV-related viruses have been described in rodents, bats and fish, as well as in many other domestic and wildlife mammal and avian species. In most cases, the presence of HEV in these animals may be explained by “spillover infections”, but the available data are mostly rare. Non-human primates are known to be susceptible to a variety of human pathogens, including influenza, herpes and hepatitis B viruses. Using experimental infection trials, their susceptibility to HEV has been demonstrated. In contrast, little is known about natural HEV infections in non-human primates. Also, the distinct role of other mammal species besides the well-known reservoirs, in transmission of HEV to humans and other animals, is mostly not known so far.

Against this background, the following major aims arose for this study.

1. Review of the current knowledge about HEV infection in various animal species. The collation of published data on this topic should enable an overview on the occurrence of HEV infections in different animal species and taxons.
2. Assessment of the incidence of natural HEV-infections in zoo-housed non-human primates. As these animals are known to be susceptible to human-pathogenic HEVs, the risk of virus transmission to humans may be high.
3. The assessment of the prevalence of HEV in other mammal zoo animal species. Zoos are housing a huge species diversity within a small area, therefore offering excellent opportunities for research on zoonotic agents, which may give new insights into the general host range of HEV.
4. Unravel potential transmission pathways of HEV in a zoo-setting. By investigating the HEV transmission between animals (different species, wild or from zoo) potential new starting points for prevention of HEV infections in animals and humans may be identified.

All animals should be tested for serological and molecular markers of HEV infection, using available detection methods for the analysis of sera and transudates.

Since rats have been suspected as HEV animal reservoir for several years, wild-living and feeder rats from two German zoos should be additionally investigated for the presence of rat HEV-RNA. Genomic and phylogenetic analysis of the detected animal HEV strains should clarify the transmission routes.

The results of the study should help to assess the distribution of HEV and HEV-related viruses among zoo-housed mammals and thus serve for decisions about possible human health risk and pest management in zoological gardens.

3 Publications

3.1 Publication I

Hepatitis E virus and related viruses in wild, domestic and zoo animals: A review

Carina Spahr, Tobias Knauf-Witzens, Thomas W. Vahlenkamp, Rainer G. Ulrich and Reimar Johne

Zoonoses and Public Health 2018 Feb., 65(1):11-29, Epub 2017 Sep. 24, <https://dx.doi.org/10.1111/zph.12405>.

3.1.1 Summary of Publication I

Hepatitis E is a human disease with zoonotic potential, which is increasingly recognised worldwide. Serologic and molecular evidence of HEV infection has been additionally described for many mammalian and avian species, suggesting the possibility of infection with HEV or HEV-like viruses in a wide range of animal species. However, the descriptions are mainly scattered into a large number of single publications, making general conclusions about the host range of HEV and the potential of distinct animal species for zoonotic HEV transmission difficult. Here, a large part of the available scientific literature on this topic has been reviewed and the findings were collated.

According to the available literature, domestic pig, wild boar, rabbit and dromedary camel represent well-known animal reservoirs for human-pathogenic, zoonotic HEV-GTs HEV-3, HEV-4 and HEV-7. In addition, evidence for HEV infection has been described for about 50 other animal species originating from 19 taxonomic orders, including 19 avian species out of 10 taxonomic orders. However, in most of these animal species, HEV or HEV-related viruses have been detected sparsely and with low detection rates, which may be indicative for “spillover infections”. Many of the publications only describe the detection of HEV-reactive antibodies, what does not allow any conclusion on the involved virus strain and its zoonotic potential. In contrast to humans, animals generally seem to be infected asymptomatically with HEV. In addition to field investigations, experimental infections of several animal species have

been performed, which may also be used to clarify the epidemiology, transmission pathways and host range of HEV.

In conclusion, many domestic, wildlife and zoo animal species have to be considered as potential carriers of HEV or HEV-related viruses in addition to the major reservoir animals (domestic pig and wild boar). Therefore, especially persons with occupational contact to these animals, e.g. breeders, hunters, slaughterhouse workers, animal keepers or veterinarians, are at higher risk for HEV infections. Natural HEV infections in animal species apart from the well-known virus reservoirs are clearly under investigated. Therefore, research on HEV infection in those animal species is needed to estimate the risk of zoonotic HEV transmission and to develop effective protection strategies for people in contact with the animals.

3.1.2 Key messages of Publication I

- HEV-3, -4 and -7 can be transmitted to humans from the reservoir animals domestic pig, wild boar, rabbit and dromedary camel
- serological and molecular evidence of infection with HEV or HEV-like viruses is available for a wide range of other domestic, wildlife and zoo animal species
- in contrast to reservoir animals, HEV is only sparsely detected in other animal species, which may indicate “spillover infections”
- natural HEV infections in animals generally seem to be asymptomatic
- experimental animal infections may contribute to elucidate transmission pathways and host range of HEV
- further research on HEV in non-reservoir species is necessary for risk estimation of zoonotic HEV transmission and development of protection strategies for people in contact with the animals

3.1.3 Own contribution to Publication I

For this review, I performed intensive scientific literature research. I prepared all tables, as well as figure 1.

I wrote the following chapters of the paper: the summary, the introduction, the chapters 3.1 and 3.2, 4.1.1 – 4.1.2, 4.2.1, the paragraphs 4, 5, 6, 9, 10, 11 of chapter 4.2.2, as well as the chapters 4.2.3 and 5.

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REVIEW ARTICLE

WILEY

Hepatitis E virus and related viruses in wild, domestic and zoo animals: A review

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Summary

Hepatitis E is a human disease mainly characterized by acute liver illness, which is caused by infection with the hepatitis E virus (HEV). Large hepatitis E outbreaks have been described in developing countries; however, the disease is also increasingly recognized in industrialized countries. Mortality rates up to 25% have been described for pregnant women during outbreaks in developing countries. In addition, chronic disease courses could be observed in immunocompromised transplant patients. Whereas the HEV genotypes 1 and 2 are mainly confined to humans, genotypes 3 and 4 are also found in animals and can be zoonotically transmitted to humans. Domestic pig and wild boar represent the most important reservoirs for these genotypes. A distinct subtype of genotype 3 has been repeatedly detected in rabbits and a few human patients. Recently, HEV genotype 7 has been identified in dromedary camels and in an immunocompromised transplant patient. The reservoir animals get infected with HEV without showing any clinical symptoms. Besides these well-known animal reservoirs, HEV-specific antibodies and/or the genome of HEV or HEV-related viruses have also been detected in many other animal species, including primates, other mammals and birds. In particular, genotypes 3 and 4 infections are documented in many domestic, wildlife and zoo animal species. In most cases, the presence of HEV in these animals can be explained by spillover infections, but a risk of virus transmission through contact with humans cannot be excluded. This review gives a general overview on the transmission pathways of HEV to humans. It particularly focuses on reported serological and molecular evidence of infections in wild, domestic and zoo animals with HEV or HEV-related viruses. The role of these animals for transmission of HEV to humans and other animals is discussed.

KEYWORDS
cross-species transmission, domestic animals, hepatitis E virus, wildlife, zoo animals, zoonosis

1 | INTRODUCTION

The hepatitis E virus (HEV) represents one of the five major human hepatotropic viruses, in addition to hepatitis A, B, C and D virus. It represents the most common cause of acute hepatitis in humans worldwide (Rein, Stevens, Theaker, Wittenborn, & Wiersma, 2012). After incubation for two to 8 weeks, mild-to-moderate influenza-like symptoms arise at first. The symptoms may thereafter develop to emesis, fever, pain of the limbs or headache and epigastralgia before signs of acute hepatitis can occur. Generally, the case fatality rate is low, ranging between 0.2% and 4% (Kumar, Subhadra, Singh, & Panda, 2013). However, case fatality rates of up to 25% were observed in pregnant women due to fulminant hepatitis in outbreak areas of endemic regions in China, India, Somalia and Uganda (Kamar et al., 2012). In contrast, only sporadic cases of hepatitis are common in industrialized countries. As the HEV IgG seroprevalence range here between 16% and 53% (Faber et al., 2012), most of these infections seem to be asymptomatic.

Although still comparatively rare, the total numbers of hepatitis E cases are currently increasing in many industrialized countries. The disease is mostly self-limiting and the patients recover after a few weeks. However, chronic HEV infections, which may develop to liver cirrhosis, have been described in immunosuppressed transplant patients (Kamar, Izopet, & Dalton, 2013; Khuroo, Khuroo, & Khuroo, 2016).

In the last years, the transmission pathways of HEV have been elucidated pointing out the zoonotic nature of some of the HEV genotypes. In particular, pigs and wild boars have been identified as animal reservoirs of the virus. However, evidence of HEV infection has also been identified in several other animal species. This review gives a general overview on the epidemiology of HEV. It particularly focuses on HEV infections in wild and zoo animals and discusses the role of these animals for transmission of HEV to humans and other animals.

Impacts

- Infections with the hepatitis E virus (HEV) can cause severe hepatitis in humans, which may appear in large epidemics in developing countries or in increasingly reported sporadic cases in industrialized countries.
- HEV genotypes 3, 4 and 7 are zoonotically transmitted from subclinically infected reservoir animals, which mainly include domestic pigs, wild boars, rabbits and dromedary camels.
- Many other domestic, wildlife and zoo animal species can be infected by spillover infections, which therefore may also represent a risk of virus transmission through contact with humans.

2 | VIRUS AND TAXONOMY

Hepatitis E virus is a small non-enveloped virus with an icosahedral capsid (Meng, 2010). Recent analyses suggest the presence of an additional outer membrane in a fraction of HEV particles (Yin, Ambardekar, Lu, & Feng, 2016). The virus genome consists of a non-segmented, single-stranded RNA with positive polarity and a length of 6.6–7.3 kb. It contains three major open reading frames encoding a non-structural polyprotein, a capsid protein and a small phosphoprotein (Meng, 2010). Rat HEV and ferret HEV contain an additional open reading frame (ORF4) of still unknown function, which overlaps with ORF1 at its 5′-end (Johne, Plenge-Bönig, et al., 2010; Raj et al., 2012).

There is a large variety of HEV-like viruses identified in animals and humans so far. The current taxonomy (Smith et al., 2014) classifies all of them within the family *Hepeviridae* (Figure 1). This family is divided into the two genera *Orthohepevirus* and *Piscihepevirus*. The genus *Orthohepevirus* contains four species designated as *Orthohepevirus A* to *D*. *Orthohepevirus A* contains seven genotypes (HEV-1 to HEV-7) and a putative new genotype 8 that can infect humans and/or a wide variety of mammals (Lee et al., 2016; Smith et al., 2014; Woo et al., 2016). *Orthohepevirus B* consists of avian viruses and is divided into four proposed subtypes (I–IV), which are mainly detected in domestic chicken. *Orthohepevirus C* includes two genotypes mainly detected in rats (HEV-C1) and carnivores (HEV-C2). *Orthohepevirus D* strains have been detected in different bat species. Additional putative new genotypes within the genus *Orthohepevirus* have been proposed, but not assigned so far. The genus *Piscihepevirus* contains only one single species: *Piscihepevirus A*, which has been identified in cutthroat trout and related species.

3 | TRANSMISSION PATHWAYS TO HUMANS

The transmission pathways of HEV are complex and may involve virus transmissions via faecally contaminated water, blood products, food, environment and direct contact with animals as well as to humans (Figure 2). Some of these pathways are well proven, whereas others are only suspected. Most importantly, the transmission pathways are dependent on the genotype of the virus.

3.1 | Genotypes 1 and 2

HEV-1 and HEV-2 are mainly restricted to humans and have been responsible for large hepatitis E outbreaks in the past. According to the World Health Organization (WHO), one-third of the world's population—comprising of more than two billion people—are living in areas highly endemic for these genotypes, including South-East Asia, the Middle East, India, Central Asia, Middle America or South America (World Health Organization, 2015). Annually, approximately 20 million HEV infections are reported for Africa and Eastern Asia (Rein et al., 2012).

Contamination of drinking water and food with human excretions is suspected to be the major route of transmission for HEV-1 and HEV-2 (Figure 2, yellow). Low hygienic standards and limited access to clean water therefore represent a high risk for the occurrence of hepatitis E outbreaks in developing countries. In households, sharing of utensils for eating and drinking during an HEV outbreak may also contribute to virus transmission. Direct person-to-person transmission of HEV is possible, although this has been described only rarely. Infections with HEV-1 are often associated with high mortality rates (15%–25%) in pregnant women (Khuroo, Kamili, & Jameel, 1995). Vertical transmission from infected mothers to their babies has also been described (Khuroo, Kamili, & Khuroo, 2009).

To analyse the infection routes of HEV-1 and HEV-2, unravel the disease progression and assess vaccine efficiency, non-human primates served as useful animal models in the past (Vitral, Yoshida, & Gaspar, 1998). By this, several primate species have been shown to be susceptible for HEV-1 and HEV-2 including African green monkey (Doceul, Bagdassarian, Demange, & Pavio, 2016) (*Chlorocebus sabaeus*), cynomolgus macaque (Aggarwal, Kamili, Spelbring, & Krawczynski, 2001; Balayan et al., 1983; Bradley et al., 1987; Ticehurst et al., 1992; Tsarev

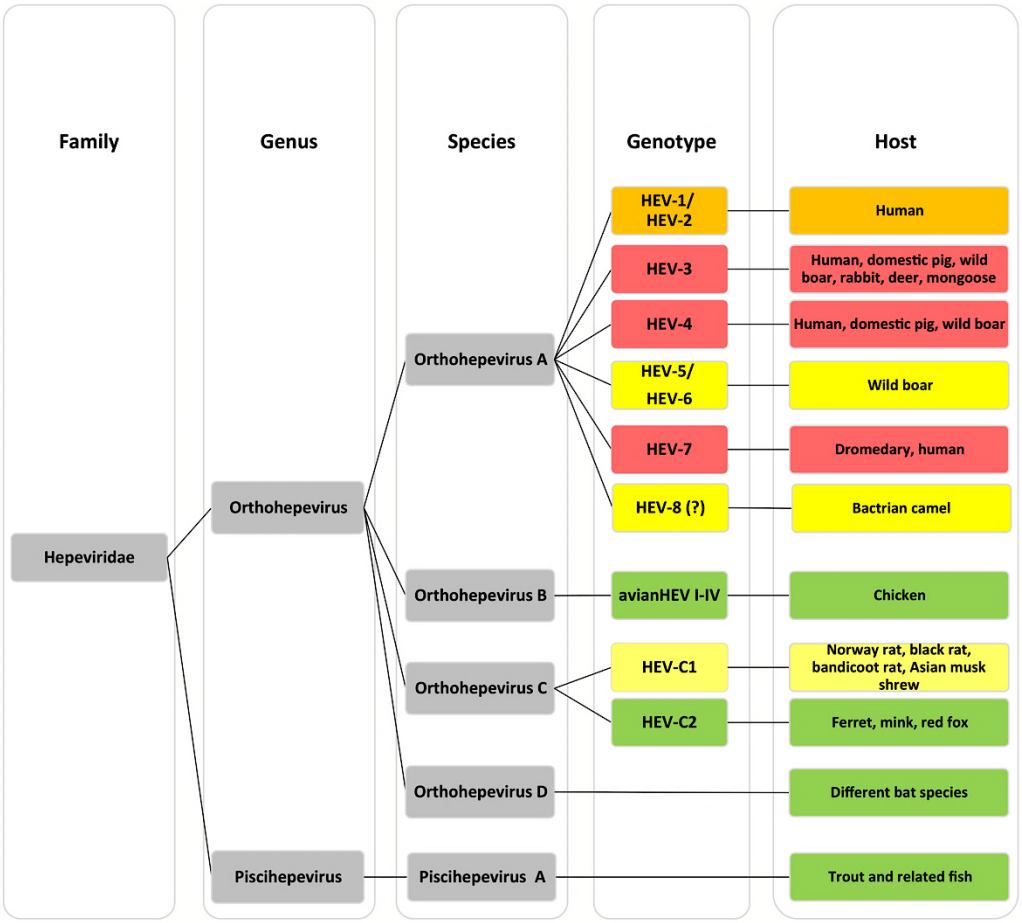

FIGURE 1 Taxonomical classification of hepatitis E virus (HEV) and HEV-related viruses according to Smith et al., 2014. The grouping according to family, genus, species, genotype and the common hosts are indicated. Colours indicate the zoonotic potential of the genotypes: orange (human-to-human transmission only), red (animal-human transmission proven), yellow (animal-human transmission not proven, but maybe possible due to relationship to human strains or serological evidence) and green (no animal-human transmission expected) [Colour figure can be viewed at wileyonlinelibrary.com]

et al., 1994; Arankalle et al., 1993) (*Macaca fascicularis*), eastern-owl monkey (Ticehurst et al., 1992; Yugo, Cossaboom, & Meng, 2014) (*Aotus trivirgatus*), rhesus macaque (Arankalle, Goverdhan, & Banerjee, 1994; Arankalle et al., 1993) (*Macaca mulatta*), squirrel monkey (Tsarev et al., 1994) (*Saimiri sciureus*), moustached tamarin (Bradley et al., 1987) (*Saguinus mystax mystax*) and chimpanzee (Arankalle et al., 1988; Yu et al., 2010) (*Pan troglodytes*) (Table 1). Experimental infection of cynomolgus monkeys leads to faecal excretion of virus particles and clinical symptoms similar to those observed in humans (Aggarwal et al., 2001; Bradley et al., 1987). Interestingly, even Mongolian gerbils (*Meriones unguiculatus*) could be infected successfully with HEV-1 via intravenous inoculation (Hong et al., 2015)

3.2 | Genotypes HEV-3 and HEV-4

HEV-3 and HEV-4 can be detected in both, humans and animals, and the predominant transmission pathway follows that of a zoonosis (Figure 2, red). Domestic pig (*Sus scrofa domesticus*) and wild boar (*Sus scrofa*) represent the most important animal reservoirs for HEV-3 and HEV-4 (Caruso et al., 2016; Johne et al., 2014). Transmission of HEV-3 from deer to humans has also been described repeatedly, although deer most probably undergoes spillover infections from wild boar, rather than being a true HEV reservoir (Anheyer-Behmenburg et al., 2017). A distinct subtype of HEV-3 has been repeatedly detected in rabbits (*Oryctolagus cuniculus*) and was recently also identified in a few

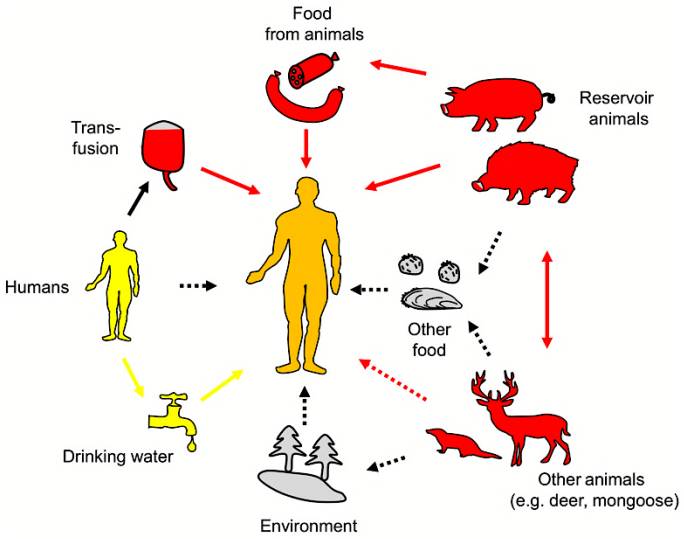

FIGURE 2 Transmission routes of human pathogenic hepatitis E virus genotypes. Transmissions, which are well proven, are indicated by bold arrows. Dotted arrows are used if transmissions are rarely shown or only speculated. Yellow: main transmission routes for genotypes 1 and 2. Red: main transmission routes for genotypes 3 and 4 [Colour figure can be viewed at wileyonlinelibrary.com]

human patients (Abravanel et al., 2017; Cossaboom, Córdoba, Cao, Ni, & Meng, 2012; Cossaboom, Córdoba, Dryman, & Meng, 2011; Izopet et al., 2012).

Evidence for transmission of HEV-3 and HEV-4 by direct contact of humans with animals has been repeatedly described, although the clinical consequence is not clear in most cases. Several studies have shown that persons with occupational contact to domestic pigs such as slaughterers, pig farmers or veterinarians exhibit significant higher anti-HEV antibody prevalences than the general population (De Carvalho et al., 2013; Khuroo et al., 2009). The same has been demonstrated for people with frequent contact with wild boars and their excretions like forestry workers and hunters (Dremsek et al., 2012; Schielke et al., 2015). However, some studies found no significant differences in the seroprevalences between exposed and non-exposed people (De Sabato et al., 2017; Hinjoy et al., 2013). Foodborne infections with HEV-3 and HEV-4 due to consumption of undercooked meat and meat products derived from infected pigs, wild boars and deer have been repeatedly described (Choi et al., 2013; Li et al., 2005; Pavio, Meng, & Doceul, 2015; Rutjes et al., 2010). HEV transmission by milk seems to be another possibility of infection with HEV, although cattle has been only rarely described to be infected with HEV (Rivero-Juarez, Frias, Rodriguez-Cano, Cuenca-López, & Rivero, 2016). However, a recent study from China indicates that viral RNA of HEV-4 can be excreted by cow milk (Huang et al., 2016). Other types of food like berries and shellfish have been suspected to act as vehicles for HEV transmission after environmental contamination with animal faeces, but this has been demonstrated only rarely (Brassard, Gagné, Généreux, & Côté, 2012; Crossan et al., 2012; Gao et al., 2015; Maunula et al., 2013; Mesquita et al., 2016). Transmission of HEV-3 has also been described by parenteral routes due to blood transmission or organ transplantation (Kamar, Abravanel, Lhomme, Rostaing, & Izopet, 2015; Kamar et al., 2008, 2014).

Experimental infection of animals with HEV-3 and HEV-4 has also been studied (Table 1). Successful experimental cross-species transmission of human and swine HEV-3 strains to swine and non-human primates has been reported (De Carvalho et al., 2013; Erker, Desai, Schlauder, Dawson, & Mushahwar, 1999; Meng et al., 1998; Xu et al., 2014). Wild boar HEV-3 strains can be experimentally transmitted to swine and wild boar, and rabbit HEV-3 strains can be transmitted to swine, non-human primates and rabbits (Cheng et al., 2012; Doceul et al., 2016; Liu et al., 2013). Non-human primates, swine and rabbits have also been demonstrated to be susceptible to human and swine HEV-4 strains by experimental inoculation (Cheng et al., 2012; Doceul et al., 2016). Additionally, experimentally cross-species infection of house mice (*Mus musculus*) and Mongolian gerbils with swine HEV-4 strains has been reported (Doceul et al., 2016).

3.3 | Other HEV genotypes and HEV-related viruses

Besides HEV-1 to HEV-4, a new potentially human pathogenic HEV genotype (HEV-7) has been described recently (Rasche et al., 2016; Woo et al., 2014). This genotype seems to be widely distributed in dromedary camels (*Camelus dromedarius*) from the Middle East (Rasche et al., 2016; Woo et al., 2014). HEV-7 was also detected in an immunocompromised transplant patient who regularly consumed camel milk and meat, pointing towards a zoonotic potential of this genotype (Lee et al., 2016). The genotypes HEV-5 and HEV-6 have been detected in wild boar from Japan, but not yet in humans (Takahashi et al., 2011). A putative additional genotype, HEV-8, was detected very recently in farmed Bactrian camels (*Camelus bactrianus*) from Xinjiang,

TABLE 1 Detection of natural and experimental hepatitis E virus (HEV) infections in primates

Animal species	Scientific name	Anti-HEV anti-bodies	Viral particles/genome	HEV genotype	Experimental infection	Natural infection
Non-human primates						
Moustached tamarin	*Saguinus mystax mystax*	+	+	HEV-1	Bradley et al. (1987)[a]	.
		+	+	HEV-2	Bradley et al. (1987)[a]	
Squirrel monkey	*Saimiri sciureus*	n.d.	+	HEV-1	Tsarev et al. (1994)	
		n.d.	+	HEV-2	Tsarev et al. (1994)	
Eastern-owl monkey	*Aotus trivirgatus*	n.d.	+	HEV-1	Yugo et al. (2014)	
		+	+	HEV-2	Ticehurst et al. (1992)[b]	
African green monkey	*Chlorocebus sabaeus*	+	n.d.	HEV-1	Doceul et al. (2016)	
		+	n.d.	HEV-2		
Vervet monkey	*Chlorocebus pygerythrus*	n.d.	+	HEV-1	Tsarev et al. (1994)	
		n.d.	+	HEV-2	Tsarev et al. (1994)	
Patas monkey	*Erythrocebus patas*	n.d.	+	HEV-1	Yugo et al. (2014)	
		n.d.	+	HEV-2	Yugo et al. (2014)	
Cynomolgus macaque	*Macaca fascicularis*	n.d.	+	HEV-1	Balayan et al. (1983)	
		n.d.	+	HEV-1	Bradley et al. (1987)[b]	
		+	+	HEV-1	Tsarev et al. (1994)	
		n.d.	+	HEV-2	Balayan et al. (1983)	
		+	+	HEV-2	Bradley et al. (1987)[a]	
		n.d.	+	HEV-2	Ticehurst et al. (1992)[b]	
		+	+	HEV-2	Aggarwal et al. (2001)	
		+	+	HEV-3	Erker et al. (1999)	
		+	+	HEV-3	De Carvalho et al. (2013)	
		+	+	Rabbit HEV	Liu et al. (2013)[b]	
Bonnet macaque	*Macaca radiata*	+	+	HEV-1		Arankalle et al. (1994)[a]
Rhesus macaque	*Macaca mulatta*	+	+	HEV-1		Arankalle et al. (1994)[a]
		+	+	HEV-2		Arankalle et al. (1994)[a]
		+	+	HEV-3	Yamamoto et al. (2012)[b]	Yamamoto et al. (2012)[b]
		+	+	HEV-3	Meng et al. (1998)[b]	
		+	+	HEV-4	Huang et al. (2011)[a]	Huang et al. (2011)[a]
Japanese macaque	*Macaca fuscata*	+	+	HEV-3		Yamamoto et al. (2012)[b]
Grey langur	*Semnopithecus entellus*	+	+	HEV-1	.	Arankalle et al. (1994)[a]

(Continues)

TABLE 1 (Continued)

Animal species	Scientific name	Anti-HEV anti-bodies	Viral particles/genome	HEV genotype	Experimental infection	Natural infection
Great apes						
Chimpanzee	*Pan troglodytes*	+	+	HEV-1	Yu et al. (2010)[b]	
		n.d.	+	HEV-2	Arankalle et al. (1988)	
		+	+	HEV-3	Meng et al. (1998)[b]	
		n.d.	+	HEV-4	Yugo et al. (2014)	
		n.d.	+	Unknown		Zhou et al. (2014)[c]

+, positive; −, negative; n.d., not determined.
[a]Wildlife animals.
[b]Laboratory animals.
[c]Animals from zoo or zoo-like location.

China, but its zoonotic potential has not been investigated so far (Woo et al., 2016).

Some evidence of a zoonotic transmission of rat HEV or a related member of the species *Orthohepevirus C* to humans arose from two serological studies: antibodies, that showed a higher reactivity with rat HEV than with HEV-1 or HEV-3 antigens, were detected in a low percentage of forestry workers from Germany as well as in febrile patients from Vietnam (Dremsek et al., 2012; Shimizu et al., 2016). However, further evidence like detection of genome sequences is still missing. For the species *Orthohepevirus B*, which originates from birds, there is no evidence for transmission to humans. An experimental infection trial with avian HEV and rhesus macaque did not result in infection (Huang et al., 2004). There is no evidence of human infection for viruses of the species *Orthohepevirus D*, which have been detected in bats (Drexler et al., 2012). Also, the distantly related viruses of the genus *Piscihepevirus* seem to be confined to fish species as hosts (Batts, Yun, Hedrick, & Winton, 2011).

4 | HEV INFECTION IN ANIMALS

Serological and/or molecular analyses indicated infections with HEV or HEV-related viruses in a broad spectrum of different animal species. These investigations included farmed animals, pets, laboratory animals, wild animals or animals from zoo-like locations. For some of the animals like domestic pigs, wild boars, chicken or rats, a frequent and continuous detection of specific HEV types at different geographical areas clearly indicates a function of a true animal reservoir. In other animal species, HEV is detected only sparsely, which may rather suggest spillover infections. However, for many animal species, no systematic studies on HEV infections are available. Additionally, there are large differences in the methods used for the identification of HEV infection, which make direct comparisons of the studies difficult. In some studies, only HEV-specific antibodies were detected, but the causative virus or genotype was not determined. Besides experimental intravenous infections of primates, clinical symptoms due to HEV infection have only been described in chicken infected with avian HEV, whereas all other animal species seem to be infected mainly asymptomatically. In this chapter, we give an overview on the current knowledge on animal species that can be infected with HEV and which may therefore represent sources of infection for humans. As the situation in the typical reservoir animals has already been reviewed extensively, this review mainly focuses on other animal species.

4.1 | HEV infection in domestic and pet animals

4.1.1 | Domestic and pet mammals

A summary of reports on natural HEV infections in mammals including domestic, wildlife and zoo animals is presented in Table 2.

TABLE 2 Detection of anti-hepatitis E virus (HEV) antibodies and HEV-RNA in mammals

Animal species	Scientific name	Anti-HEV antibodies	Viral particles/ genome	HEV genotype	Reference
Artiodactyla					
Dromedary	*Camelus dromedarius*	n.d.	+	HEV-7	Woo et al. (2014)
		+	+	HEV-7	Rasche et al. (2016)
Bactrian camel	*Camelus bactrianus*	n.d.	+	HEV-8	Woo et al. (2016)
Yellow cattle	*Bos taurus primigenius*	+	+	HEV-4	Yan et al. (2016)
Holstein Frisian cattle	*Bos taurus primigenius*	−	+	HEV-4	Huang et al. (2016)
Dairy cattle	*Bos taurus primigenius*	+	−	HEV-1	El-Tras et al. (2013)
Yak	*Bos grunniens*	−	+	HEV-4	Xu et al. (2014)
American bison	*Bison bison*	+	−	HEV-1 to HEV-4	Dong et al. (2011)
Cape buffalo	*Syncerus caffer*	+	n.d.	HEV-1	El-Tras et al. (2013)
Goat	*Capra hircus aegagrus*	+	n.d.	unknown	El-Tras et al. (2013)
		+	n.d.	unknown	Sanford et al. (2013)
		n.d.	+	HEV-3	Di Martina et al. (2016)
		+	n.d.	HEV-3	Peralta, Casas, et al. (2009)
Sheep	*Ovis aries orientalis*	+	n.d.	unknown	El-Tras et al. (2013)
		+	n.d.	HEV-3	Peralta, Casas, et al. (2009)
Swedish moose	*Alces alces*	−	+	moose HEV	Lin et al. (2014)
		+	+	moose HEV	Lin et al. (2015)
Sika deer	*Cervus nippon nippon*	+	n.d.	HEV-3	Sonoda et al. (2004)
		n.d.	+	HEV-4	Zhang, Shen, Mou, Gong, et al. (2008) and Zhang, Shen, Mou, Yang, et al. (2008)[aa]
Tufted deer	*Elaphodus cephalophus*	n.d.	+	HEV-4	Zhang, Shen, Mou, Gong, et al. (2008) and Zhang, Shen, Mou, Yang, et al. (2008)[a]
Reeves' muntjac	*Muntiacus reevesi*	n.d.	+	HEV-4	Zhang, Shen, Mou, Gong, et al. (2008) and Zhang, Shen, Mou, Yang, et al. (2008)[a]
Yezo deer	*Cervus nippon yesoensis*	+	neg.	HEV-3	Tomiyama et al. (2009)
		+	−	HEV-3	Sonoda et al. (2004)
Roe deer	*Capreolus capreolus*	−	+	HEV-3	Reuter et al. (2009)
		n.d.	+	HEV-3	Forgách et al. (2010)
		−	+	HEV-3	Anheyer-Behmenburg et al. (2017)
Red deer	*Cervus elaphus*	n.d.	+	HEV-3	Forgách et al. (2010)
		−	+	HEV-3	Anheyer-Behmenburg et al. (2017)
Domestic pig[c]	*Sus scrofa domestica*	+	+	HEV-3 HEV-4	Breum, Hjulsager, de Deus, Segalés, and Larsen (2010)

(Continues)

TABLE 2 (Continued)

Animal species	Scientific name	Anti-HEV antibodies	Viral particles/ genome	HEV genotype	Reference
Wild boar	*Sus scrofa*	+	+	HEV-3	Sonoda et al. (2004)
		+	+	HEV-3	Adlhoch et al. (2009)
		n.d.	+	HEV-3	Martelli et al. (2008)
		+	+	HEV-3	De Deus et al. (2008)
		n.d.	+	HEV-3	Wiratsudakul and Sariya (2012)
		n.d.	+	HEV-3	Kaci, Nöckler, and Johne (2008)
		+	+	HEV-3	Dong et al. (2011)
		+	+	HEV-3	Anheyer-Behmenburg et al. (2017)
		+	+	HEV-3	
				HEV-4	Takahashi et al. (2014)
		n.d.	+	HEV-5	Takahashi et al. (2011)
				HEV-6	
		+	n.d.	Unknown	Larska et al. (2015)
		+	n.d.	Unknown	Carpentier et al. (2012)
		+	n.d.	Unknown	Chandler, Riddell, Li, Love, and Anderson (1999)
Perissodactyla					
Horse	*Equus caballus ferus*	+	+	HEV-1	Saad et al. (2007)
		+	+	HEV-3	Zhang, Shen, Mou, Gong, et al. (2008) and Zhang, Shen, Mou, Yang, et al. (2008)
Carnivora					
Clouded leopard	*Neofelis nebulosa*	n.d.	+	HEV-4	Zhang, Shen, Mou, Gong, et al. (2008) and Zhang, Shen, Mou, Yang, et al. (2008)[a]
Cat	*Felis catus silvestris*	+	n.d.	n.d.	Liang et al. (2014)
		+	–	n.d.	Mochizuki et al. (2006)
Javan mongoose	*Herpestes javanicus*	+	+	HEV-3	Nakamura et al. (2006)
		+	–	HEV-3	Li and Saito (2006)
		n.d.	+	HEV-3	Nidaira et al. (2012)
Dog	*Canis lupus familiaris*	+	–	HEV-4	Liu et al. (2009)
		+	n.d.	n.d.	Liang et al. (2014)
		+	–	n.d.	McElroy et al. (2015)
		+	n.d.	n.d.	Arankalle et al. (2001)
Red fox	*Vulpes vulpes*	n.d.	+	HEV-C2	Bodewes et al. (2013)
Asiatic black bear	*Ursus thibetanus*	n.d.	+	HEV-4	Zhang, Shen, Mou, Gong, et al. (2008) and Zhang, Shen, Mou, Yang, et al. (2008)[a]
European mink	*Mustela lutreola*	n.d.	+	HEV-C2	Krog et al. (2013)
European ferret	*Mustela putorius*	+	+	HEV-C2	Raj et al. (2012)
Lagomorpha					
Rabbit	*Oryctolagus cuniculus*	n.d.	+	Rabbit HEV	Izopet et al. (2012)
		+	+	Rabbit HEV	Cossaboom et al. (2011)
		n.d.	+	Rabbit HEV	Izopet et al. (2012)[b]
		n.d.	+	Rabbit HEV	Caruso et al. (2015)
		+	+	Rabbit HEV/ HEV-3	Hammerschmidt et al. (2017)[b]

(Continues)

TABLE 2 (Continued)

Animal species	Scientific name	Anti-HEV antibodies	Viral particles/ genome	HEV genotype	Reference
New Zealand White rabbit	*Oryctolagus cuniculus domesticus*	+	n.d.	HEV-3	Birke et al. (2014)
Rex rabbit	*Oryctolagus cuniculus domesticus*	+	+	rabbit HEV	Zhao et al. (2009)
		+	+	rabbit HEV	Geng et al. (2011)
		n.d.	+	rabbit HEV	Xia et al. (2015)
Japanese White rabbit	*Oryctolagus cuniculus domesticus*	n.d.	+	rabbit HEV	Xia et al. (2015)
European brown hare	*Lepus europaeus*	+	−	HEV-3	Hammerschmidt et al. (2017)[b]
Rodentia					
Greater bandicoot rat	*Bandicota indica*	+	+	HEV-C1	Li et al. (2013)
Norway rat	*Rattus norvegicus*	+	n.d.	HEV-C1	Easterbrook et al. (2007)
		n.d.	+	HEV-C1	Johne, Heckel, et al. (2010) and Johne, Plenge-Bönig, et al. (2010)
		−	+	HEV-C1	Johne, Heckel, et al. (2010) and Johne, Plenge-Bönig, et al. (2010)
		+	+	HEV-C1	Johne et al. (2012)
		+	n.d.	unknown	Kabrane-Lazizi et al. (1999)
		+	+	HEV-C1	Li et al. (2013)
		+	+	HEV-C1	Purcell et al. (2011)
		n.d.	+	HEV-C1	Widén et al. (2014)
		+	+	HEV-3	Kanai et al. (2012)
		n.d.	+	HEV-3	Lack et al. (2012)
		n.d.	+	HEV-C1/rabbit HEV	Ryll et al. (2017)
Yellow-breasted rat	*Rattus flavipectus*	+	+	HEV-C1	Li et al. (2013)
Taiwan rat	*Rattus rattoides losea*	+	+	HEV-C1	Li et al. (2013)
Black rat	*Rattus rattus hainanus*	+	−	HEV-C1	Li et al. (2013)
Black rat	*Rattus rattus*	+	+	HEV-C1	Mulyanto et al. (2014)
		n.d.	+	HEC-C1	Ryll et al. (2017)
Eulipotyphla					
Asian musk shrew	*Suncus murinus*	+	+	HEV-C1	Guan et al. (2013)
Chioptera					
Aba roundleaf bat	*Hipposideros abae*	n.d.	+	bat HEV	Drexler et al. (2012)
Great stripe-faced bat	*Vampyrodes caraccioli*	n.d.	+	bat HEV	Drexler et al. (2012)
Serotine bat	*Eptesicus serotinus*	n.d.	+	bat HEV	Drexler et al. (2012)
Bechstein's bat	*Myotis bechsteinii*	n.d.	+	bat HEV	Drexler et al. (2012)
Daubenton's bat	*Myotis daubentonii*	n.d.	+	bat HEV	Drexler et al. (2012)
Cetacea					
Bottlenose dolphin	*Tursiops truncatus*	+	+	HEV-3	Montalvo Villalba et al. (2017)

+, positive; −, negative; n.d., not determined.
[a]Animals from zoo or zoo-like location.
[b]Wildlife animals.
[c]For further studies on domestic pigs, see other reviews, for example Doceul et al. (2016); Lapa et al. (2015); Pavio et al. (2010).

Domestic pig represents a major animal reservoir for zoonotic HEV-3 and HEV-4 worldwide, which has already been reviewed explicitly elsewhere (Doceul et al., 2016; Lapa, Capobianchi, & Garbuglia, 2015; Pavio, Meng, & Renou, 2010). The reported anti-HEV IgG seroprevalences in swine herds are usually high, ranging between 23% and 100%, with increasing seroprevalence with higher age (Caruso et al., 2016; Doceul et al., 2016; Pavio et al., 2010).

Whereas pig is a well-known reservoir animal for HEV, comparatively few studies report evidence of HEV infection in domesticated bovids. HEV-specific antibodies have been found in breeds of domestic cattle (*Bos taurus primigenius*) like yellow cattle (Yan et al., 2016), Holstein Frisian cattle (Huang et al., 2016) and other dairy cattle (El-Tras, Tayel, & El-Kady, 2013) as well as in domesticated wild bovids, such as yak (*Bos grunniens*) (Xu et al., 2014), buffalo (*Syncerus caffer*) (El-Tras et al., 2013) or bison (*Bison bison*) (Dong et al., 2011). Reported anti-HEV IgG prevalences were 4.4%–6.9% in India, 1.4% in Brazil, 10.4% to 37% in China and up to 15% in the USA. In contrast, HEV-RNA was detected in cattle only in a few studies. HEV-4 strains were identified in Holstein cows kept on a mixed farm together with pigs in Southwest China and in yellow cattle from Eastern China (Huang et al., 2016; Yan et al., 2016). As mixed raising of domestic livestock is popular in the investigated regions of China and the detected strains are closely related to pig strains, spillover infections are a likely explanation for the observed findings.

Hepatitis E virus-specific antibodies have also been detected in goat (*Capra hircus aegagrus*) and sheep (*Ovis aries*). An Egyptian study reported seropositivity rates of 9.4% and 4.4% for goat and sheep, respectively (El-Tras et al., 2013). For goats from the USA, HEV-specific antibodies were detected in 16% (Sanford et al., 2013). In Italy, HEV-3 strains closely related to regional wild boar strains were detected in 11 of 119 goat samples (9.24%) (Di Martina et al., 2016). HEV-3-specific IgG antibodies were detected in 0.6% and 1.92% of Spanish goats and sheep, respectively (Peralta, Casas, et al., 2009). 16.3% of domesticated farmed goats from Eastern China were positive for anti-HEV IgG antibodies (Zhang, Shen, Mou, Yang, et al., 2008). In a mixed farm in Eastern China, 8 of 70 sheep were tested positive for HEV-RNA (11.43%) (Yan et al., 2016). The detected HEV-4 strains were also closely related to pig and cattle strains indicating spillover infections.

HEV-7 sequences were first reported from three dromedaries sampled in the United Arab Emirates (UAE) in 2013 (Woo et al., 2014). Later, a retrospective study investigated 2,438 dromedaries from UAE, Somalia, Sudan, Egypt, Kenya and Pakistan (Rasche et al., 2016). Overall, 45.7% of the sera were anti-HEV antibody positive, which is similar to the HEV-3 seroprevalence of pigs in developing countries (Khuroo et al., 2016; Rasche et al., 2016). RNA of HEV-7 was also detected in a sample collected in 1983 suggesting a long evolutionary history of this virus type in dromedaries (Rasche et al., 2016). As a similar virus strain was also found in a liver transplantation patient, zoonotic potential of HEV-7 must be suspected (Lee et al., 2016). Very recently, a novel HEV genotype, tentatively designated as HEV-8, has been identified in Bactrian camels from a farm in China (Woo et al., 2016).

Ferret and mink are small carnivores of the family Mustelidae, which are kept as pets or farmed as fur-bearing animals. RNA of ferret HEV, belonging to species *Orthohepevirus C*, was first identified in ferrets kept as household pets at four locations in the Netherlands (Raj et al., 2012). Additionally, the authors detected HEV-specific antibodies in serum from farmed ferrets in the Netherlands. In farmed minks from Denmark, RNA sequences of strains most closely related to but not identical with ferret HEV were detected (Krog, Breum, Jensen, & Larsen, 2013).

Pet dogs (*Canis lupus familiaris*) and pet cats (*Felis catus silvestris*) are obviously susceptible to infections with HEV or HEV-like viruses, as repeatedly evidenced by serological analysis (Table 2). However, as only antibodies have been detected so far, the distinct viruses causing the infection are unknown.

Rabbits are commonly farmed in many countries for meat consumption, fur production and as pets. In Virginia, USA, farmed rabbits from eight breeds were investigated for anti-HEV antibodies and HEV-RNA (Cossaboom et al., 2011). The IgG seroprevalence was 36.5%, and rabbit HEV-RNA was detected in serum and faeces in 22% of 85 rabbits. IgG seroprevalences in farmed Rex rabbits from China were reported between 55% and 57% (Geng et al., 2011; Zhao et al., 2009). Rabbit HEV-RNA was detected in 7.5% (serum) and 7.0% (faeces) of these animals. Furthermore, IgG seroprevalences in New Zealand White rabbits from two US vendors were reported 40% and 50%, respectively (Birke et al., 2014). Additionally, rabbit HEV-RNA was detected in 5% of Japanese White rabbits and Rex rabbits from China (Xia et al., 2015). Rabbits from France, slaughtered for consumption, had a rabbit HEV-RNA prevalence of 7% in liver specimens (Izopet et al., 2012). Rabbit HEV strains are related to other HEV-3 strains but represent a distinct subtype. However, this subtype has been recently also detected in a few human patients from France (Abravanel et al., 2017; Izopet et al., 2012). In addition, a rabbit HEV strain very closely related to one of these French human patient isolates was also identified in a pet house rabbit (Caruso et al., 2015).

Domesticated horses (*Equus caballus ferus*) were investigated sparsely for HEV. In one study, 200 sera from work horses in Egypt were surveyed for the presence of HEV (Saad et al., 2007). 13% of sera were positive for anti-HEV IgG antibodies and 4% also for HEV-RNA. The detected sequences were closely related to human HEV-1 isolates from Egypt from 1993/1994. Another study investigated 101 horses from Eastern China for the presence of anti-HEV IgG antibodies (Zhang, Shen, Mou, Gong, et al., 2008). 17.8% of these horses were seropositive, and even 2% were positive for HEV-3-RNA. These descriptions are indicative for accidental spillover infections.

4.1.2 | Domestic and pet birds

A summary of reported natural HEV infections in birds including domestic, wildlife and zoo animals is presented in Table 3.

Avian HEV, which belongs to the species *Orthohepevirus B*, was initially described in barnyard fowl (chicken) from the United States, in 2001 (Haqshenas, Shivaprasad, Woolcock, Read, & Meng, 2001). Interestingly, barnyard fowl infected with avian HEV can reach clinical

TABLE 3 Serological and molecular detection of natural hepatitis E virus (HEV) infections in birds

Animal species	Scientific name	Anti-HEV antibodies	Viral particles/ genome	HEV genotype	Reference
Wild-living birds					
Common kestrel	*Falco tinnunculus*	n.d.	+	Novel	Reuter et al. (2016,b)[a]
Red-footed falcon	*Falco vespertinus*	n.d.	+	Novel	Reuter et al. (2016,b)[a]
Common buzzard	*Buteo buteo*	n.d.	+	Avian HEV	Zhang et al. (2017)[a]
Little owl	*Athene noctua*	n.d.	+	Avian HEV	Zhang et al. (2017)[a]
Little egret	*Egretta garzetta*	n.d.	+	Novel	Reuter et al. (2016a)[a]
Song thrush	*Turdus philomelos*	n.d.	+	Avian HEV	Zhang et al. (2017)[a]
Feral pigeon	*Columba livia*	n.d.	+	Avian HEV	Zhang et al. (2017)[a]
Zoo animals					
Silver pheasant	*Lophura nycthemera*	n.d.	+	HEV-4	Zhang, Shen, Mou, Gong, et al. (2008) and Zhang, Shen, Mou, Yang, et al. (2008)[b]
Crowned crane	*Balearica regulorum*	n.d.	+	HEV-4	Zhang, Shen, Mou, Gong, et al. (2008) and Zhang, Shen, Mou, Yang, et al. (2008)[b]
Himalayan griffons	*Gyps himalayensis*	n.d.	+	HEV-3	Li et al. (2015)[b]
Poultry					
Chicken	*Gallus gallus domesticus*	n.d.	+	Avian HEV	Haqshenas et al. (2001)
		n.d.	+	Avian HEV	Agunos et al. (2006)
		+	+	Avian HEV	Huang et al. (2002)
		+	+	Avian HEV	Peralta, Biarnés, et al. (2009)
		+	+	Avian HEV	Hsu and Tsai (2014)
Turkey	*Meleagris gallopavo*	+	+	Avian HEV	Sun et al. (2004)[c]
Duck	*Anas platyrhynchos domesticus*	+	n.d.	Unknown	Zhang, Shen, Mou, Gong, et al. (2008) and Zhang, Shen, Mou, Yang, et al. (2008)
Pigeon	*Columba livia domestica*	+	n.d.	Unknown	Zhang, Shen, Mou, Gong, et al. (2008) and Zhang, Shen, Mou, Yang, et al. (2008)
Pet birds					
Eurasian siskin	*Carduelis spinus*	+	n.d.	Avian HEV	Cong et al. (2014)
Oriental skylark	*Alauda gulgula*	+	n.d.	Avian HEV	Cong et al. (2014)
Black-tailed grosbeak	*Coccothraustes migratorius*	+	n.d.	Avian HEV	Cong et al. (2014)
Budgerigar	*Melopsittacus undulates*	+	n.d.	Avian HEV	Zhang et al. (2014)
Alexandrine Parakeet	*Psittacula eupatria*	+	n.d.	Avian HEV	Zhang et al. (2014)

+, positive; −, negative; n.d., not determined.
[a]Wildlife animals.
[b]Animals from zoo or zoo-like location.
[c]Laboratory animals.

manifestations (Haqshenas et al., 2001; Morrow et al., 2008; Payne, Ellis, Plant, Gregory, & Wilcox, 1999). Clinical signs of an acute infection with avian HEV comprise hepatomegaly, splenomegaly, kidney modifications, growth depressions, decline of laying performance, accumulation of abdominal blood fluid, hepatic vasculitis and amyloidosis as well as increased deaths among poultry (Agunos et al., 2006;

Morrow et al., 2008). Avian HEV is widespread in chicken flocks worldwide (Gerber, Trampel, & Opriessnig, 2014). The reported seropositive rates in chicken flocks are high: 95.1% in Taiwan, 71% in the USA, 90% in Spain and 57% in Korea (Gerber et al., 2014; Hsu & Tsai, 2014; Huang et al., 2002; Peralta, Biarnés, et al., 2009). The HEV-RNA prevalence is similarly high, with 72-100% reported in one study from the USA (Gerber et al., 2014).

Experimental cross-species transmission of avian HEV from chicken to turkeys (*Meleagris gallopavo*) has been reported (Sun et al., 2004). Avian HEV does not seem to have a potential for transmission to humans. Using an experimental infection approach with rhesus macaque as a model, avian HEV was unable to infect primates (Huang et al., 2004). HEV-specific antibodies were detected in domestic farmed duck (*Anas platyrhynchos domesticus*) and pigeon (*Columba livia domestica*) from Eastern China, with anti-HEV IgG prevalences of 12.8% and 4.4%, respectively (Zhang, Shen, Mou, Gong, et al., 2008).

Pet birds can also be infected with HEV. In 2014, 685 serum samples of three pet bird species from China were investigated (Cong et al., 2014). This included the Eurasian siskin (*Carduelis spinus*), the Oriental skylark (*Alauda gulgula*) and the black-tailed grosbeak (*Coccothraustes migratorius*). Collectively, 8.31% birds were positive for avian HEV-specific antibodies. In the same year, another study investigated four parrot species from China and two of them were positive for avian HEV-specific antibodies: budgerigar (*Melopsittacus undulatus*) and Alexandrine Parakeet (*Psittacula eupatria*) (Zhang et al., 2014). The ascertained seroprevalence was 6.43.

4.2 | HEV infection in wildlife and zoo animals

Besides the reservoir animals like wild boar and commonly hunted game species like deer, only little is known about HEV infection in wildlife. Also, HEV infection in zoo animals has been investigated only sparsely.

In the following paragraph, primates will be particularly emphasized, due to their close relationship to humans and the consequential importance for zoonotic virus transmission.

4.2.1 | Primates

Primates in the wild

Natural HEV infection in wild-living primates has been reported sparsely so far. A report from the rural Yunnan province, China, describes the testing of 480 stool samples and 92 serum samples of wild rhesus macaques (Huang et al., 2011). The prevalence of HEV-specific IgG was quite high (35.87%), and three of 31 rhesus macaques were even positive for HEV-specific IgM indicating acute infections. However, the authors concluded that "*Macaca mulatta* may not be a natural reservoir of HEV-4" and that "HEV-4 infection might have been acquired from contact with HEV infected wild boars, wild rats or humans."

Natural infection was also reported in three Indian monkey species: wild rhesus macaques, bonnet macaques (*Macaca radiata*) and grey langur (*Semnopithecus entellus*) were reported with HEV IgG seroprevalences of 36.7%, 19.1% and 2%, respectively (Arankalle et al., 1994). A summary on reported experimental and natural HEV infections in wildlife and zoo primates is presented in Table 1.

Primates in zoos

In a study from China, 37 faecal samples of chimpanzees from two zoos were tested by RT-PCR for the presence of HEV-RNA (Zhou, Li, & Yang, 2014). Overall, 29.2% of the samples were HEV-RNA positive. The HEV-RNA sequences showed 99% identity to each other. Surprisingly, the detected RNA shared only 45%–58% sequence similarity with known HEV strains. The authors therefore suggested that they had found a novel type of HEV. However, only small RNA fragments were amplified and independent confirmation of the sequences has not been done so far.

Natural infection and transmission of HEV-3 has been described in a monkey facility in Japan, housing non-human primates of the species Japanese macaque (*Macaca fuscata*) and rhesus macaque (Yamamoto et al., 2012). None of the animals showed any clinical signs of illness. Initially, neither IgG nor IgM anti-HEV antibodies could be found in any of the primates. Within 2 years, both IgG (78.5%) and IgM (6.6%) reached their maximum and slowly decrease within another 3 years to 35.3% (IgG) and 0% (IgM). HEV-3 RNA was detected in serum and faeces of these animals.

4.2.2 | Other mammals

Mammals in the wild

Natural HEV infection of wildlife animals has been reported in a variety of mammalian species. Among the affected species, there are ungulates like wild boar, bovids, deer and moose (*Alces alces*), as well as some carnivores like mink, red fox, mongoose and ferret, but also rodents, lagomorphs and bats.

Wild boar are a well-known reservoir for zoonotic HEV strains. This has been already reviewed elsewhere and selected studies are given as examples in Table 2 (Doceul et al., 2016; Johne et al., 2014; Pavio et al., 2015). The reported HEV-specific antibody prevalences range from 3% to 42.7% in the USA and Spain, respectively (De Deus et al., 2008; Dong et al., 2011). Using RT-PCR, HEV-3 was mostly identified, with detection rates up to 68% in wild boar in Germany. HEV-4 has been reported in wild boar from Japan (Takahashi et al., 2014). Also, the single detection of HEV-5 and HEV-6 has been reported in wild boar from Japan (Takahashi et al., 2011). However, these genotypes have not been detected in humans so far.

Hepatitis E virus infection has been repeatedly identified in several deer species (Anheyer-Behmenburg et al., 2017; Forgách et al., 2010; Reuter, Fodor, Forgách, Kátai, & Szucs, 2009; Sonoda et al., 2004; Zhang, Shen, Mou, Yang, et al., 2008). The prevalence of HEV-specific antibodies in Europe and Japan range from 2% to 35% (Pavio et al., 2010). In Japan, wild Sika deer (*Cervus nippon nippon*) showed an HEV IgG seroprevalence of 2%, whereas that of Yezo deer (*Cervus nippon yesoensis*) was 34.8% (Sonoda et al., 2004; Tomiyama, Inoue, Osawa, & Okazaki, 2009). Generally, HEV-3 strains that were identified in deer species were closely related to wild boar and human strains (Pavio et al., 2010; Takahashi, Kitajima, Abe, & Mishiro, 2004;

Tei, Naoto, Kazuaki, & Shunji, 2003). In a study in Germany, similar strains were detected in wild boar, roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) from the same hunting area (Anheyer-Behmenburg et al., 2017). As consistently lower prevalences and lower virus loads were detected in the deer species as compared to the wild boar, spillover infections have been proposed as explanation for the deer HEV infections.

A novel HEV-like virus was detected in liver and kidney samples from Swedish moose (*Alces alces*) (Lin, Norder, Uhlhorn, Belák, & Widén, 2014). Further investigations of sera and faecal samples in an additional study showed HEV-specific antibody prevalences of 19% (Lin et al., 2015). RNA of the virus was detected in 15% of the samples. Genome sequencing showed that moose HEV shares a common ancestor with HEV-1 to HEV-6. It has not been finally classified within the species *Orthohepevirus A* and the zoonotic potential of the virus remains unclear so far.

HEV-3 has also been detected in mongoose, which are small carnivores belonging to the family Herpestidae (Li & Saito, 2006; Nakamura et al., 2006; Nidaira et al., 2012). HEV-specific antibodies were detected in 21% of wild small Asian mongoose (*Herpestes javanicus*) from Japan (Nakamura et al., 2006). In addition, a full HEV-3 genome sequence was recovered from one sample. Another study determined an antibody prevalence of 8.3% for wild mongooses from Japan, whereas HEV-RNA detection failed (Li & Saito, 2006). Six years later, another 209 wild mongooses from the same region were investigated resulting in 2.9% HEV-3-RNA-positive samples (Nidaira et al., 2012). As the mongooses inhabit the same region as wild boar and domestic pigs, the authors concluded that "they may be exposed to HEV in the faeces of pigs and wild boars."

Wild rabbits in warren housing from several departments of France were found to be positive for rabbit HEV-RNA (Izopet et al., 2012). Overall, 47 of 205 liver samples (23%) were positive for rabbit HEV-RNA. All animals were reported to be clinically healthy. In a recent study, 2.2% of 669 European brown hares and 37.3% of 164 wild rabbits hunted in Germany were tested positive for anti-HEV antibodies (Hammerschmidt et al., 2017). HEV-RNA was detected in 17.1% of the rabbits. Although most of the determined sequences clustered with rabbit HEV strains, one sequence was most closely related to pig HEV genotype 3 strains, probably representing a spillover infection.

The red fox represents the most widespread member of the order Carnivora (Bodewes, Van Der Giessen, Haagmans, Osterhaus, & Smits, 2013). Faeces of wild-living foxes from a perirural and periurban area in the Netherlands were investigated by a metagenomics approach for the detection of faecally shed viruses. By this, HEV-like sequences, sharing the highest similarity to rat HEV, were detected. The detected virus has not been finally classified, but belongs most probably to the species *Orthohepevirus C*. A transmission of the virus from prey to fox seems to be reasonable. HEV-like sequences belonging to *Orthohepevirus C* have also been detected in faecal and liver samples from wild mink in Denmark (Krog et al., 2013).

In wild Norway rats (*Rattus norvegicus*), infections with an HEV-like virus were first reported from Germany in 2009 (Johne, Heckel, et al., 2010; Johne, Plenge-Bönig, et al., 2010). The rat HEV has been classified into species *Orthohepevirus C*. Meanwhile, rat HEV has been detected in wild Norway rats (Easterbrook et al., 2007; Johne, Heckel, et al., 2010; Johne, Plenge-Bönig, et al., 2010 Johne et al., 2012; Kabrane-Lazizi et al., 1999; Kanai et al., 2012; Lack, Volk, & Van Den Busche, 2012; Li et al., 2013; Purcell et al., 2011; Widén, Ayral, Artois, Olofson, & Lin, 2014), Black rats (Mulyanto et al., 2014) (*Rattus rattus*) and Bandicoot rats (Li et al., 2013) (*Bandicota indica*) in Germany, Denmark, Vietnam, Indonesia, the United States and France, suggesting a worldwide distribution. The reported prevalences of HEV-specific antibodies in rats from the USA range from 73% to 90% (Easterbrook et al., 2007; Kabrane-Lazizi et al., 1999; Purcell et al., 2011). HEV-specific antibodies and rat HEV-RNA sequences have also been identified in Asian musk shrews (*Suncus murinus*) from China (Guan et al., 2013), most probably representing spillover infections from rats. However, the zoonotic potential of rat HEV is discussed controversially. Experimental infection of rhesus macaques with rat HEV was not successful (Purcell et al., 2011). In contrast, antibodies, which showed a higher reactivity to rat HEV than to HEV-3, were found in healthy forestry workers from Germany (Dremsek et al., 2012). Also, antibodies with high reactivity to rat HEV were recently detected in patients from Vietnam with acute febrile diseases including mild liver dysfunction, hepatomegaly and mild elevation of liver enzymes AST and ALT (Shimizu et al., 2016). The zoonotic potential of rat HEV therefore deserves more investigation in the future. Whereas rat HEV has been repeatedly identified in rats worldwide, detection of HEV-3 in those animals has only been described twice in Japan and the USA (Kanai et al., 2012; Lack et al., 2012). Recently, a rabbit-like subtype of HEV-3 has been detected in a Norway rat from Belgium (Ryll et al., 2017).

Bats worldwide carry HEV strains, too (Drexler et al., 2012). Bat HEV strains form distinct viruses classified within the species *Orthohepevirus D* (Smith et al., 2014). In total, 3,869 bat specimens from 85 species from five continents were investigated in one study (Drexler et al., 2012). HEV-RNA was detected in blood or faeces of seven specimens from Africa, Central America and Europe, whereas no serological investigations were performed. The positive animals derived from five bat species out of three families. As the authors did not detect bat HEV-RNA in human blood donors, there is currently no evidence of transmission to humans so far.

Mammals in zoos

HEV infections in zoo animals have been reported rarely. One study investigated 38 faecal samples from 22 animal species from a zoo-like location in China for the presence of HEV-RNA (Zhang, Shen, Mou, Yang, et al., 2008). A total of 28.9% were HEV-RNA positive. All sequences clustered with HEV-4 strains, with 96%–100% identity to each other. Among the HEV-positive mammalian species, there were three species of deer, Sika deer, tufted deer (*Elaphodus cephalophus*) and Reeves' muntjac (*Muntiacus reevesi*), as well as two carnivores, one Asiatic black bear (*Ursus thibetanus*) and one clouded leopard (*Neofelis nebulosa*), respectively. Additionally, one veterinarian and six feeders from the centre were tested for HEV-specific antibodies resulting in the exceptionally high seroprevalence of 71.4%. The authors conclude

"that cross-species infection of HEV had probably occurred in this zoo-like location."

Very recently, evidence for HEV infection in dolphins, maintained in a National Aquarium in Cuba, was described (Montalvo Villalba et al., 2017). The authors found HEV-specific antibodies in 10 of 31 (32%) and HEV-3-RNA in 5 of 31 (16%) of the animals. The dolphins showed lethargy and elevated liver enzymes. The authors speculated about infection of the animals through contamination of food or water.

4.2.3 | Birds

Birds in the wild

A larger study investigating infection of wild birds with avian HEV, which belongs to the species *Orthohepevirus B*, was published very recently (Zhang, Bilic, Troxler, & Hess, 2017). In this study, swabs from 626 wild birds originating from 62 species from Austria were investigated by RT-PCR. A total of eight samples from four different bird species including song thrush (*Turdus philomelos*), little owl (*Athene noctua*), feral pigeon (*Columba livia*) and common buzzard (*Buteo buteo*) turned out to be positive for HEV-RNA. Sequencing of the RT-PCR products indicated the presence of two different genotypes of avian HEV.

Another recent study demonstrated HEV-like sequences in wild birds of prey (Reuter et al., 2016b). The faeces of 2 of 11 common kestrels (*Falco tinnunculus*) and 1 of 7 red-footed falcons (*Falco vespertinus*) from Hungary were tested positive for HEV-RNA using RT-PCR. The RNA sequences were closely related to each other, but showed only low similarities to known HEV sequences with highest similarities to rat HEV, ferret HEV and HEV-4. The significance of the novel virus for animals and humans is unknown.

Using a viral metagenomics approach with next generation sequencing techniques, HEV-like sequences were also recently identified in the wild waterfowl species little egret (*Egretta garzetta*) in a sample from Hungary (Reuter et al., 2016a). The virus was most closely related to avian HEV, however, with only 61%–71% sequence identity (Reuter et al., 2016a). A final classification and assessment of the zoonotic potential of this virus needs to be elaborated in the future.

Birds in zoos

Two studies report the detection of HEV in birds from zoos. In 2008, faecal samples of eight bird species from a zoo-like location in China were investigated by RT-PCR (Zhang, Shen, Mou, Yang, et al., 2008). HEV-RNA was detected in one crowned crane (*Balearica regulorum*) and one silver pheasant (*Lophura nycthemera*). Sequencing identified the virus as HEV-4.

Another case report described a coinfection with *Aspergillus* sp. and HEV in Himalayan griffons (*Gyps himalayensis*) (Li et al., 2015). Overall, four animals from Beijing Zoo, China, were investigated by RT-PCR and two of them were HEV-3-RNA positive. In conclusion, HEV-3 and HEV-4 can obviously be present in samples from zoo birds. Further studies should clarify if they originate from productive infection of the birds and if a zoonotic transmission to humans can occur.

5 | CONCLUSION

Serologic and molecular evidence of HEV infection has been described for many mammalian and avian species, suggesting that infection with HEV or HEV-like viruses is likely in a wide range of animal species. The potentially human pathogenic genotypes HEV-3, HEV-4 and HEV-7 were found in many domestic, wildlife and zoo animal species. Therefore, contact between humans and animals may represent a common transmission pathway for HEV or HEV-related viruses. However, there are large differences in the HEV prevalences and the detected virus types between the distinct animal species.

Pigs and wild boar represent the major reservoir animals of HEV-3 and HEV-4. Reported data show high detection rates of HEV-3 and HEV-4 in swine and wild boar indicating the highest risk for zoonotic infection for humans, which may especially apply to persons with occupational contact to these animals, for example pig breeders or hunters. For both, HEV-7 from dromedary camels and the HEV-3 subtype from rabbits, at least one case of zoonotic transmission to a human patient is known. Also, deer was frequently identified as a source of human HEV-3 infection.

Except these, no direct evidence for HEV transmission to humans was given. Considering the close relationship to humans, primates are reported to have surprisingly low HEV prevalences. However, natural HEV infection in primates is clearly under investigated. HEV-3 and HEV-4 have been detected in many mammal species, but most of them can be considered as spillover infections as supported by low prevalences and reported contacts to pigs or wild boars. Also, *Orthohepevirus C* and *Orthohepevirus D* strains were detected in rats, carnivores and bats, which seem to have a low zoonotic potential. For birds, HEV-3 and HEV-4 were detected sporadically; instead, avian HEV and novel HEV types seem to play a major role.

As a consequence of the reported HEV infections in animals, people occupationally working with animals and animal samples should be aware of HEV. The situation in zoos appears to be largely under investigated and information on the HEV prevalence in zoo animals and their keepers is scarce. Also, the importance of domestic animals like cattle, sheep and goat, or wild animals like mongoose or rat for HEV transmission to humans needs to be studied in more detail. Generally, more research on the HEV transmission pathways via different animal species apart from domestic pigs and wild boars is needed to estimate the risk of zoonotic transmission of HEV from these animals and to develop effective protection strategies for people in contact with the animals.

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CONFLICT OF INTEREST

None.

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3.2 Publication II

Detection of HEV-specific antibodies in four non-human primate species, including great apes, from different zoos in Germany

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3.2.1 Summary of Publication II

The number of diagnosed cases of human infections with zoonotic HEV-GTs is currently increasing in many European countries. In order to unravel the HEV transmission pathways, contact to non-human primates should be considered, as these animals are known to be susceptible for a variety of other human pathogens like influenza, herpes and hepatitis B virus. It is also well-known that various non-human primate species can be experimentally infected with HEV, showing viremia and virus shedding, sometimes accompanied by clinical symptoms of hepatitis. However, little is known about natural HEV infections in non-human primates and the corresponding risk of zoonotic infections.

In this study, 259 individual sera of clinically healthy non-human primates of 14 species, from nine German zoos, were serologically and molecularly investigated for the presence of HEV. Using a double-antigen-sandwich ELISA (AXIOM® HEV-AB EIA, Bürstadt, Germany) and an IgG-ELISA (Mikrogen® recomWell HEV-IgG, Neuried, Germany), ten animals (3.9%) reacted positive in at least one assay. The HEV-specific antibodies were found in Western lowland gorillas (*Gorilla gorilla gorilla*), bonobos (*Pan paniscus*), lar gibbons (*Hylobates lar*) and drills (*Mandrillus leucophaeus*). No history of clinical symptoms of hepatitis was recorded in these animals. Testing for anti-HEV-IgM antibodies by ELISA (Mikrogen® recomWell HEV-IgM, Neuried, Germany) and for viral RNA by RT-qPCR resulted in negative results.

It can be concluded that non-human primates in zoos can get infected with HEV or HEV-related viruses, without showing obvious clinical signs of hepatitis. Compared

to three other studies, the non-human primates analysed in this study showed surprisingly low detection rates of markers of HEV infection. The source of infection with HEV or HEV-related viruses for the primates is unknown but may be through contact with excretions or contaminated food/water. Despite the low detection rates observed, the possibility of virus transmissions from non-human primates to humans in contact with them should be considered.

3.2.2 Key messages of Publication II

- evidence of natural infection with HEV or HEV-related viruses of three great ape species and one ape species in European zoos:
  - bonobo (Pan paniscus)
  - gorilla (Gorilla gorilla gorilla)
  - lar gibbon (Hylobates lar)
  - drill (Mandrillus leucophaeus)
- 3.9% (10/259) of the investigated animals were anti-HEV-IgG positive
- no history of clinical signs of hepatitis in the seropositive animals
- negative PCR and anti-HEV-IgM results indicate absence of acute HEV infections
- comparatively low HEV seroprevalences in non-human primates
- nevertheless, the possibility of virus transmissions from non-human primates to humans in contact with them should be considered

3.2.3 Own contribution to Publication II

I was responsible for the serum collection, including own sampling, request for samples from the participating zoos and shipment of samples. Additionally, I gathered information on the physical health status of each non-human primate, using ZIMS Species 360 and databases of the zoos. I performed the Axiom® HEV-Ab EIA, RNA isolation and RT-qPCR. I analysed the data and wrote the following parts of the paper: summary, introduction, material part, results, discussion and conclusion.

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SHORT REPORT
Detection of HEV-specific antibodies in four non-human primate species, including great apes, from different zoos in Germany

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SUMMARY

The hepatitis E virus (HEV) has been described in humans and various animal species in different regions of the world. However, the knowledge on natural HEV infection in non-human primates and the corresponding risk of zoonotic transmission is scarce. To determine whether primates in captivity are affected by HEV infection, we investigated 259 individual sera of clinically healthy non-human primates of 14 species from nine German zoos. Using a commercial double-antigen-sandwich ELISA and a commercial IgG ELISA, 10 animals (3·9%) reacted positive in at least one assay. Three ape species and one Old World monkey species were among the seropositive animals: bonobo (*Pan paniscus*), gorilla (*Gorilla gorilla gorilla*), lar gibbon (*Hylobates lar*) and drill (*Mandrillus leucophaeus*). Testing for anti-HEV-IgM antibodies by commercial ELISA and for viral RNA by reverse-transcription real-time polymerase chain reaction resulted in negative results for all animals indicating the absence of acute HEV infections. In the past, no clinical signs of hepatitis were recorded for the seropositive animals. The results suggest that non-human primates in zoos can get naturally and subclinically infected with HEV or related hepeviruses. Future studies should evaluate potential sources and transmission routes of these infections and their impact on human health.

Key words: ELISA, hepatitis E virus, human, non-human primates, seroconversion, zoonosis.

Hepatitis E is a worldwide occurring, notifiable emerging infectious disease caused by hepatitis E virus (HEV), which comprises different genotypes with different transmission modes and geographic distributions [1]. The number of recorded human cases has steadily been increasing in several European countries during the past 10 years [2]. HEV-RNA and anti-HEV-antibodies have also been detected in a considerable variety of wild-living, farmed and pet animal species worldwide. Additional hepeviruses from animals have been discovered in previous years, mostly with unknown zoonotic potential [1].

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Recently, a novel taxonomical classification of hepeviruses was introduced [3]. HEV belongs to the family *Hepeviridae*, which taxonomically is divided into two genera: *Orthohepevirus* and *Piscihepevirus*. The genus *Orthohepevirus* contains all mammalian and avian HEV genotypes. The genus *Piscihepevirus* only contains the HEV-like agent from cutthroat trout (*Oncorhynchus clarki*) and related fish species. The species *Orthohepevirus A* comprises genotypes HEV-1 to HEV-4 and HEV-7 which are human pathogenic. Out of these, HEV-3, HEV-4 and HEV-7 are zoonotic pathogens, with wild boar, domestic pig, rabbit, deer and dromedary representing their reservoirs. In contrast, little is known about HEV infections in non-human primates. Experimental infections with human-pathogenic, zoonotic HEV-3 were successful in Japanese macaques (*Macaca fuscata*) and rhesus macaques (*Macaca mulatta*) [4]. Primates kept in zoos are in close contact with their keepers and are known to be susceptible for diverse human pathogens, like influenza, herpes or hepatitis B viruses [5–7]. Therefore, the objective of our study was to investigate if HEV infections occur within primate populations in zoos.

Within the years 2015 and 2016, a total of 259 individual sera of 14 non-human primate species were collected from nine German zoos. The sera were obtained during immobilisations for different purposes or taken from serum collections of the participating zoos. Full blood was centrifuged and the supernatant stored at −20 °C until further use. The physical health status of all animals was routinely checked and documented by their keepers on a daily basis.

Three commercial anti-HEV antibody ELISAs were performed and rated according to the protocols of the manufacturers: Axiom® HEV-Ab-EIA (Axiom Diagnostik, Bürstadt, Germany), Mikrogen® recomWell HEV-IgG and Mikrogen® recomWell HEV-IgM (both Mikrogen Diagnostik, Neuried, Germany). The double antigen sandwich-based Axiom assay detects all classes of antibodies and is based on a recombinant open reading frame 2 (ORF2)-derived antigen of HEV-1. The other two assays are specific for IgG and IgM and are based on recombinant ORF2-derived antigens of HEV-1 and HEV-3. The 95% confidence intervals (CIs) were calculated in R (v3.4.0) using the binom.confint function of the binom package (exact method). For Axiom® HEV-Ab-ELISA, serum samples were diluted 1 : 1, for initially positive samples serial dilutions were tested (1 : 10, 1 : 50, 1 : 250, 1 : 1250). For Mikrogen® recomWell HEV-IgG and -IgM assays, serum samples were diluted 1 : 101. RNA was extracted from serum using a commercial kit following the manufacturer's instructions (NucleoMag®VET MACHEREY-NAGEL, Düren, Germany). One hundred microlitres of each clarified sample was used for RNA extraction. During RNA isolation, RNA bacteriophage MS2 was added as an indicator for successful extraction [8]. Finally, the RNA was stored at −80 °C until further use.

A reverse-transcription real-time polymerase chain reaction (RT-qPCR) was performed with 256 samples, using the QuantiTect Probe® RT-PCR kit (QIAGEN GmbH, Hilden, Germany) in a 20 µl reaction volume with primer concentration of 1 µM. For negative and positive controls, 5 µl of RNase-free water and 5 µl of HEV-RNA from a domestic pig, corresponding to 3·7 genome copies, were used. The RT-qPCR followed the thermal profile of a standard protocol with 45 cycles [9]. The evaluation of the RT-qPCR results followed the published standard protocol. The RT-qPCR was performed in a multiplex format to detect simultaneously HEV and phage MS2 RNA. All samples were positive for phage MS2 RNA confirming successful RNA extraction.

A total of 259 individual serum samples were tested for the presence of anti-HEV antibodies, using the Axiom® HEV-Ab-EIA (Table 1). Seven of 259 (2·7%, 95% CI 1·1–5·5%) sera were anti-HEV positive, including four gorillas (*Gorilla gorilla gorilla*), one bonobo (*Pan paniscus*), one lar gibbon (*Hylobates lar*) and one drill (*Mandrillus leucophaeus*). The positive samples originated from five of the nine zoos (A, B, F, G and H) (Supplementary Tables S1 and S2). In total, 257 of the 259 samples were additionally tested using the Mikrogen® recomWell ELISAs, as for two of the 259 samples, there was no more material available. Six of 257 sera (2·3%, 95% CI 0·9–5%) were positive ($n = 4$) or equivocal ($n = 2$) for anti-HEV-IgG; all six samples originated from gorillas. For both tests combined, the overall seroprevalence in gorillas was 15·2% (95% CI 6·3–28·9%; Table 1), whereas the seroprevalence for this species based on the single tests was 8·7% (95% CI 2·4–20·8%) and 13% (95% CI 4·9–26·3%) for Axiom® HEV-Ab-EIA and Mikrogen® recomWell HEV-IgG-ELISA, respectively (Table 1). Three of the gorilla samples were seroreactive in both tests (Supplementary Table S1). One of these three samples was also reactive in the Axiom® HEV-Ab-EIA at higher dilutions (Supplementary Table S3). In contrast, one bonobo, one lar gibbon and one drill were exclusively

Table 1. *Prevalence of anti-hepatitis E virus antibodies in non-human primates from nine zoos in Germany determined by two ELISAs*

			Axiom® HEV-Ab-EIA			Mikrogen® *recom*Well HEV-IgG			No. individuals pos. or equi. in at least one assay/total no. individuals investigated		
Family	Species		pos./total	%	95% CI	pos./total	%	95% CI	pos./total	%	95% CI
Hominidae	Gorilla	*Gorilla gorilla gorilla*	**4/46**	**8·7**	**2·4–20·8**	**6/46**	**13**	**4·9–26·3**	**7/46**	**15·2**	**6·3–28·9**
	Bonobo	*Pan paniscus*	**1/25**	**4·0**	**0·1–20·4**	0/25	–	0–13·7	**1/25**	**4·0**	**0·1–20·4**
	Chimpanzee	*Pan troglodytes*	0/70	–	0–5·1	0/70	–	0–5·1	0/70	–	0–5·1
	Sumatran orangutan	*Pongo abelii*	0/16	–	0–20·6	0/15	–	0–21·8	0/16	–	0–20·6
	Bornean orangutan	*Pongo pygmaeus*	0/4	–	0–60·2	0/4	–	0–60·2	0/4	–	0–60·2
Hylobatidae	Lar gibbon	*Hylobates lar*	**1/11**	**9·1**	**0·2–41·3**	0/11	–	0–28·5	**1/11**	**9·1**	**0·2–41·3**
Cercopithecidae	Drill	*Mandrillus leucophaeus*	**1/7**	**14·3**	**0·4–57·9**	0/7	–	0–41	**1/7**	**14·3**	**0·4–57·9**
	Gelada baboon	*Theropithecus gelada*	0/61	–	0–5·9	0/61	–	0–5·9	0/61	–	0–5·9
	Javan silvered leaf monkey	*Trachypithecus auratus*	0/6	–	0–45·9	0/6	–	0–45·9	0/6	–	0–45·9
	Japanese macaque	*Macaca fuscata*	0/2	–	0–84·2	0/2	–	0–84·2	0/1	–	0–97·5
	White-crowned mangabey	*Cercocebus atys lunulatus*	0/1	–	0–97·5	0/0	–	–	0/1	–	0–97·5
Atelidae	Black howler monkey	*Alouatta caraya*	0/3	–	0–70·8	0/3	–	0–70·8	0/3	–	0–70·8
	White-fronted spider monkey	*Ateles hybridus*	0/4	–	0–60·2	0/4	–	0–60·2	0/4	–	0–60·2
Cebidae	Black-capped squirrel monkey	*Saimiri boliviensis*	0/1	–	0–97·5	0/1	–	0–97·5	0/1	–	0–97·5
Pitheciidae	White-faced saki	*Pithecia pithecia*	0/2	–	0–84·2	0/2	–	0–84·2	0/2	–	0–84·2
Total			**7*/259**	**2·7**	**1·1–5·5**	**6**/257**	**2·3**	**0·9–5**	**10/259**	**3·9**	**1·9–7**

pos., positive; total, total number of samples analysed; equi., equivocal; 95% CI, 95% confidence interval; 7*, result contains 6/172 (3·5%) apes and 1/87 (1·2%) of the residue monkeys; 6**, result contains four pos. and two equivocal samples from gorillas.

reactive in the Axiom® HEV-Ab-EIA, but not in the Mikrogen® recomWell HEV-IgG-ELISA. None of the orangutans (*Pongo abelii* and *Pongo pygmaeus*) and chimpanzees (*Pan troglodytes*) showed anti-HEV-antibodies in any of the applied tests (Table 1). In addition, none of the investigated samples were positive for anti-HEV-IgM or HEV-RNA-positive in the RT-qPCR (data not shown).

Here we describe a HEV seroprevalence study in non-human primates from nine zoos in Germany. HEV-reactive antibodies were detected in 10 of 259 (3·9%; 95% CI 1·9–7%) individuals, which represents a low seroprevalence compared with the results of serosurveys in humans, wild boar or pigs in Germany [10, 11]. None of the seropositive animals displayed signs of an acute infection at the time of sampling, as evidenced by the absence of HEV-RNA and HEV-specific IgM antibodies. The absence of these parameters of an acute infection might be caused by a lower sensitivity of the IgM assay, licensed for human diagnostics, or by the high specificity of the RT-qPCR assay, that probably is unable to detect RNA of HEV cross-reactive, but different, so far unknown hepeviruses. In line with the absence of descriptions of natural HEV infection in non-human primates displaying clinical illness, the seroreactive animals in this study had never been reported to show hepatitis E-related clinical symptoms in the past. Three species of apes, including two great ape species and one lar gibbon, plus one Old World monkey species were found to be anti-HEV antibody positive. Interestingly, none of the 70 chimpanzees and 61 gelada baboons (*Theropithecus gelada*) were found to be anti-HEV positive, including 17 chimpanzees and all gelada baboons from zoo 'H', where HEV-specific antibodies were detected in four gorillas, one bonobo and one drill (Supplementary Table S2). The observed local variation in HEV seroprevalence may be most likely caused by a sampling bias, as most samples (166/259, 64·1%) were collected from zoo 'H' (see Supplementary Table S2).

The number of non-human primates tested for natural HEV infection is scarce so far, but the reported prevalences were mostly higher. An investigation of 92 sera from wild rhesus macaques in the rural city of Kunming, Yunnan Province, China, resulted in a HEV-IgG-seroprevalence of 35·87% [12]. As many pigs and wild boars are housed in Yunnan Province, the authors of this study suggested that contact of monkeys and swine may have led to cross-species transmission of HEV-4. Furthermore, a HEV-3 outbreak in a Japanese outdoor breeding monkey facility was reported [4]. The afflicted animals were Japanese macaques and rhesus macaques. Within a period of 5 years, the HEV-IgG-seroprevalence ranged between 20% (2005), 78·5% (2006) and 35·3% (2009). During this time, no clinical hepatitis E-like symptoms were observed, neither in the macaques, nor in the staff. The source of HEV infection in this study could not be identified. Natural HEV infection in great apes was reported only once; in a Chinese zoo, HEV-RNA was detected in 29·2% (7/24) of faecal samples from chimpanzees [13]. Phylogenetic analyses suggested the virus strain to represent a novel HEV type, but its taxonomical classification is still pending.

The source and route of transmission of HEV or a related hepevirus in the animals investigated here and the potential reason for the observed difference in seroprevalence between gorillas (7/46; 15·2%, 95% CI 6·3–28·9) and chimpanzees (0/70; 0%, 95% CI 0–5·1) remain obscure, but might be also due to sampling bias. Three old gorillas were wild born (individuals no. 1, 4, 7) and it cannot be excluded that they got infected during their earlier life in the wild. In these cases, direct contact to infected group members from the same species, or contact with faecal excretions or contaminated water or food may portray a risk for infection. In contrast, seven seroreactive animals were born in captivity, indicating that they must have been infected in a zoo. Direct contact to the keepers cannot be ruled out as a source and route of transmission. Even though hygiene standards in the holding institutions were high and pest control programmes enforced, contact to wild rabbits or wild rats in outdoor enclosures, either direct or via excretions, poses a potential route of transmission. As gorillas kept in zoos are strictly fed vegetarian, foodborne infection via contaminated meat seems to be unlikely, although wild gorillas, similar to chimpanzees, have been observed to consume wild caught mice and rats. In addition, housing swine and primates in the same zoo may be suspected to enable indirect transmission of HEV via faecally contaminated shoes, utensils or vehicles, carried by the personnel. Interestingly, we investigated two anti-HEV-seroreactive female gorillas from zoo 'H' (Supplementary Table S1), being mother (no. 4) and her 19-year-old child (no. 6). Vertical transmission and breast-feeding may be further possible explanations for HEV infections in great apes, too. The presence of maternal antibodies in the younger animal can be excluded as the animal was already 19 years old during the time of sample collection.

As reflected in Supplementary Table S1, results of the Axiom® HEV-Ab-EIA and Mikrogen® recomWell HEV-IgG-ELISA are only partially matching with the latter assay showing a lower seroprevalence. This observation is in line with previous investigations in humans [14, 15] and pigs [16, 17] and might be caused by the different test principles used [10, 11, 18], but also partially influenced by the use of an IgG assay adapted for human sera. In addition, the long-term storage and frequent freeze–thaw cycles of the samples from serum banks might have resulted in a decrease of antibody amounts in the serum samples.

In conclusion, the present study indicates that non-human primates in zoos are susceptible to natural infection with HEV or related hepeviruses. The observed seroprevalences were found to be very low. This low prevalence in primates is in contrast to the assumption of an anthropozoonotic transmission from non-human primates to humans, but may underline the transmission of HEV by consumption of contaminated groceries or direct contact of humans to domestic pigs. In particular, gorillas were afflicted more often than other apes or non-human primates. Further investigations are needed to prove potential differences in the susceptibility of certain primate species, to identify the hepevirus origin of their infections and the potential transmission routes and to evaluate the veterinary and public health risk consequences. For this purpose, future HEV monitoring in non-human primates and potential reservoir species, such as pigs, deer, rabbits and rats, in zoos is highly recommended.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0950268817002606.

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DECLARATION OF INTEREST

None.

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3.3 Publication III

Serological evidence of hepatitis E virus infection in zoo animals and identification of a rodent-borne strain in a Syrian brown bear

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3.3.1 Summary of Publication III

Hepatitis E is a human disease, which can be zoonotically transmitted from animals. HEV infections have been repeatedly described with high detection rates in domestic pig and wild boar, representing the major reservoir animals for HEV. Furthermore, HEV and HEV-related viruses have been detected in a variety of other mammal and avian species. However, the occurrence of natural HEV infections in zoo animals has only scarcely been investigated so far.

In this study, 244 individual sera of 66 zoo-housed mammal species from three zoos in Germany were serologically investigated. A double antigen sandwich ELISA, based on the HEV-1 capsid protein, was used for species-independent detection of HEV-specific antibodies. Molecular analysis for detection of HEV- or rat HEV-RNA was performed using three different PCR protocols. As a result, 16 animal species were tested positive for HEV-specific antibodies, with the highest detection rates in suids (33.3%) and carnivores (27.0%). RNA of human-pathogenic HEV-1 to HEV-4 was not detected in any of the samples. However, rat HEV-RNA was detected in the serum of a clinically healthy female Syrian brown bear (*Ursus arctos syriacus*). Analysis of subsequent serum samples confirmed a HEV seroconversion in this animal. Closely related rat HEV-sequences were found in pest rats (*Rattus norvegicus*) from the same location, whereas molecular and serologic investigations of feeder rats (*Rattus norvegicus forma domestica*) resulted in negative results.

In conclusion, evidence for infection with HEV or HEV-related viruses was shown for many mammalian zoo animal species. Therefore, the possibility of virus

transmissions to other animals and humans has to be considered. Besides the expected high seroprevalence in suids, the high detection rate in carnivores warrants further investigations on their possible function as reservoir animals. The detection of rat HEV in the Syrian brown bear suggests a higher zoonotic potential of this virus as expected in earlier studies. A “spillover infection” from pest rats living in the zoo is most likely. Further investigations on consequences of HEV and rat HEV infections for zoo animals and humans should be initiated.

3.3.2 Key messages of Publication III

- evidence of natural infection with HEV or HEV-related viruses in 16 zoo animal species from European zoos
- highest seroprevalences were found in suids (33.3%) and carnivores (27.0%)
- detection of rat HEV-RNA in the serum of a Syrian brown bear, that also showed seroconversion
- closely related rat HEV-strains were found in pest rats from the same location indicating a “spillover infection”
- many zoo animals have to be considered susceptible to HEV or HEV-related viruses
- the role of carnivores as potential additional reservoir animals for HEV should be investigated in future

3.3.3 Own contribution to Publication III

I was responsible for the serum and rat collection, including own sampling, request for samples at the participating zoos and shipment of samples. I implemented the dissection of rats and participated in the dissection of died zoo animals and sampling of livers at the CVUA Fellbach. Additionally, I gathered information on the physical health status of the female Syrian brown bear using databases of the zoo. I performed the Axiom® HEV-Ab EIA, RNA isolation from sera and liver samples, as well as PCR analyses. I analysed the data and wrote the following chapters of the paper: the abstract and the chapters 1, 2.1 – 2.3, 3.1, 3.3, 4 and 5.

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Short communication

Serological evidence of hepatitis E virus infection in zoo animals and identification of a rodent-borne strain in a Syrian brown bear

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ABSTRACT

Hepatitis E virus (HEV) is the causative agent of hepatitis E, an emerging infectious disease of humans. HEV infections have also been described in various animal species. Whereas domestic pigs and wild boars are well-known animal reservoirs for HEV, the knowledge on natural HEV infection in zoo animals is scarce so far. Here, we analysed 244 sera from 66 mammal species derived from three zoos in Germany using a commercial double antigen sandwich ELISA. HEV-specific antibodies were detected in 16 animal species, with the highest detection rates in suids (33.3%) and carnivores (27.0%). However, RNA of the human pathogenic HEV genotypes 1–4 was not detected in the serum samples from suids or carnivores. Using a broad spectrum RT-PCR, a ratHEV-related sequence was identified in a sample of a female Syrian brown bear (*Ursus arctos syriacus*). Subsequent serum samples within a period of five years confirmed a HEV seroconversion in this animal. No symptoms of hepatitis were recorded. In a follow-up investigation at the same location, closely related ratHEV sequences were identified in free-living Norway rats (*Rattus norvegicus*), whereas feeder rats (*Rattus norvegicus forma domestica*) were negative for HEV-specific antibodies and RNA. Therefore, a spillover infection of ratHEV from free-living Norway rats is most likely. The results indicate that a wide range of zoo animals can be naturally infected with HEV or HEV-related viruses. Their distinct role as possible reservoir animals for HEV and sources of HEV infection for humans and other animals remains to be investigated.

1. Introduction

Hepatitis E virus (HEV) infections represent the most common cause of acute hepatitis in humans worldwide (Rein et al., 2012). In several European countries, the number of recorded human hepatitis E cases steadily increased during the past ten years (Adlhoch et al., 2016). The disease is mostly characterized by mild to moderate acute hepatitis; subclinical infections appear to be frequent. However, pregnant women in endemic regions with HEV-1 and persons with underlying liver disease portray a risk group for severe acute hepatitis including lethal outcomes. In addition, chronic infections, which can develop to liver cirrhosis, have been identified in immunosuppressed transplant patients (Kamar et al., 2012).

HEV belongs to the family *Hepeviridae* and possesses an RNA genome containing three open reading frames (ORFs). ORF1 encodes a non-structural polyprotein, ORF2 the capsid protein and ORF3 a small phosphoprotein. The human-pathogenic genotypes (GT) HEV-1 to HEV-4 are classified together with additional GT from wild boars and camels into the species *Orthohepevirus A* (Smith et al., 2014). The species *Orthohepevirus B* contains avian HEV strains, whereas mainly strains from rats and ferrets are found in *Orthohepevirus C* and batHEV strains in *Orthohepevirus D* (Smith et al., 2014).

The sources of infection with human-pathogenic HEV are GT-dependent (Johne et al., 2014). HEV-1 and HEV-2 are restricted to humans and mainly transmitted by fecally contaminated water. In contrast, HEV-3 and HEV-4 are zoonotic viruses, with pigs and wild boars representing the main animal reservoirs. These animals do not show any clinical symptoms due to HEV infection. Direct contact between humans and animals and ingestion of virus-containing food are the main transmission routes of these genotypes.

RNA of HEV-3 or HEV-4 as well as HEV-specific antibodies have also been detected in a considerable variety of other wildlife, farmed and pet

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animal species (Spahr et al., 2017b; Doceul et al., 2016; Pavio et al., 2010). To gain knowledge about the distribution of HEV infections in different animal species, zoo-like locations with a large diversity of mammal species represent interesting sites. However, only a few studies analysing zoo animals have been published yet (Spahr et al., 2017a; Li et al., 2015; Zhang et al., 2008).

To analyse HEV infections in zoo animals, a serological survey on HEV-specific antibodies was performed with animals from three zoos in Germany. Animals of taxa showing comparably high seroprevalences were additionally analysed by RT-PCR for the presence of HEV RNA. Follow-up investigations in free-living and feeder Norway rats should identify the source of HEV infections in zoo animals. The results of the investigation should contribute to further clarify the role of zoo animals as susceptible hosts of HEV.

2. Materials and methods

2.1. Sampling

In total, 244 individual sera from 66 mammal species were collected in three zoos (A–C) in Germany (Suppl. Table 1), though most sera were obtained from zoo A. The sera were obtained between 2006 and 2016 during animal immobilizations for different purposes, e.g. routine health checks, and stored at −20 °C. Additionally, liver samples from 12 animals were taken during routine dissections of died zoo animals between 2015 and 2016 and stored at −20 °C. No animal was sampled for the sole profit of this study. All animals in the zoos were routinely checked by their keepers for physical health, which was documented daily. 73 free-living Norway rats were collected between 2009 and 2016 from two zoos (A and D) and stored at −20 °C (Suppl. Table 2). These rats were collected routinely for use in the network "Rodent-borne pathogens" and standard protocols of the network were used for preparation of liver samples and extraction of transudates from the thoracic cavity (Ulrich et al., 2008). Additionally, 20 randomly selected feeder rats from zoo A were killed for internal stock control, using CO_2 inhalation in accordance with animal welfare regulations. All liver and transudate samples were stored at −20 °C until further investigation.

2.2. Serological analysis

The serum samples were analysed for HEV-specific antibodies using the Axiom® HEV-Ab EIA (Axiom Diagnostik, Bürstadt, Germany) and the results were evaluated according to the recommendations of the manufacturer. This assay is based on HEV-1 capsid protein antigens and uses the test principle of a double antigen sandwich ELISA. By this, it is species-independent and can detect all immunoglobulin classes.

2.3. RNA isolation

RNA was extracted from serum samples using the NucleoMag®VET kit (Macherey-Nagel, Düren, Germany) in a King Fisher 96 Flex Workstation (Thermo Fisher Scientific GmbH, Schwerte, Germany), following the manufacturer's instructions. Liver samples were homogenized using a TissueLyser (Qiagen GmbH, Hilden, Germany) and QIAzol®Lysis Reagent (Qiagen GmbH), and RNA was extracted by a modified QIAzol protocol method as described before (Schmidt et al., 2016). The RNA pellets were resolved in 100 µl DEPC-treated water and stored at −80 °C until further use.

2.4. Real-time RT-PCR (RT-qPCR)

RNA samples were tested for the presence of HEV-1 to HEV-4 using a previously described RT-qPCR protocol (Jothikumar et al., 2006). The QuantiTect® Probe RT-PCR Kit (Qiagen GmbH) was used in 20 µl reactions with conditions as previously described (Schielke et al., 2011). The limit of detection of this RT-qPCR as determined by dilution series of in vitro transcribed RNA was seven genome equivalents per PCR reaction (Schielke et al., 2011).

2.5. Nested broad-spectrum RT-PCR (NBS-RT-PCR)

The NBS-RT-PCR was performed according to Johne et al. (2010). This assay amplifies a conserved region within the RNA-dependent RNA polymerase (RdRp)-encoding region of OFR1 and has been demonstrated to be capable of detection of HEV strains from the species *Orthohepevirus A*, *B* and *C* (Johne et al., 2010). The RT-PCR was performed using the One-Step RT-PCR kit (Qiagen GmbH) and the nested PCR using the TaKaRa ExTaq kit (TaKaRa Bio, Japan) as described before (Johne et al., 2010). The nested PCR products were separated by agarose gel electrophoresis and bands according to a length of 331–334 nucleotides (nt) were excised and purified using the QIAquick Gel Extraction Kit® (Qiagen GmbH).

2.6. SW-RT-PCR

The SW-RT-PCR targets a similar genomic region of the HEV genome like the NBS-RT-PCR, but is designed as one-step RT-PCR (Wolf et al., 2013). It has been shown to efficiently detect ratHEV, but should also be able to detect strains of the species *Orthohepevirus A* based on the primer sequences. This RT-PCR was performed using the SuperScriptIII with PlatinumTaq Kit (Invitrogen Life Technologies, Carlsbad, CA, USA) in a 25 µl reaction (Wolf et al., 2013). RT-PCR products with a length of 282 bp were purified using the NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel).

2.7. Sequence analyses

Purified amplification products were either sequenced by a commercial company (Eurofins GmbH, Hamburg, Germany) or sequenced in-house using the BigDye® Terminator version 1.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) in an HITACHI 3130 Genetic Analyser (Applied Biosystems, Darmstadt, Germany). For sequence comparisons and phylogenetic analyses, a sequence fragment of the RdRp-encoding region with a length of 279 nt (nt 4108–4387; numbering according to ratHEV reference strain R63, acc. no. GU345042), derived from the products of the NBS-RT-PCR and/or the SW-RT-PCR, was used. The newly generated HEV sequences were deposited at GenBank (sequence from the Syrian brown bear: acc. no. MF480313, sequences from rats: acc. nos. MF480314–480320). Sequence alignments were performed using BioEdit 7.2.0 (Hall, 1999) and MEGA 7 (Kumar et al., 2016). The GTR + G model was used as it was identified as the best suited substitution model by MEGA 7. The phylogenetic analyses were performed by Bayesian algorithms via the CIPRES online portal (Ronquist et al., 2012) with 8 million generations and by Maximum likelihood algorithm performed via MEGA7 (Kumar et al., 2016) with 1.000 bootstrap replicates and a consensus tree was generated. Reference sequences for phylogenetic reconstructions were taken from Smith et al. (2014).

3. Results

3.1. HEV-specific antibodies are mainly detected in zoo animals of the family Suidae and the order Carnivora

A total of 244 serum samples from mammalian zoo animals, belonging to 66 species, were tested for the presence of HEV-specific antibodies (Table 1 and Suppl. Table 1). In total 28/244 (11.5%) turned out to be anti-HEV antibody-positive. Animals from 16 species in three orders (Artiodactyla, Carnivora, Perissodactyla) were tested positive. The highest seroprevalence was found in animals from the family Suidae with 9/27 (33.3%) positive samples originating from three different species. A high seroprevalence was also recorded for animals of

Table 1
Prevalence of HEV-specific markers in zoo animals from Germany using serological and molecular detection methods.

Order	Family	Axiom® HEV-Ab EIA		RT-qPCR		NBS-RT-PCR		SW-RT-PCR		Sequencing
		pos./total	%	pos./total	%	pos./total	%	pos./total	%	pos./total
Afrosoricida		0/1	N/A	–	–	–	–	–	–	–
	Tenrecidae	0/1	N/A	–	–	–	–	–	–	–
Artiodactyla		**16/167**	**9.6**	0/98	0	–	–	0/8[a]	0	–
	Suidae	**9/27**	**33.3**	0/27	0	–	–	0/2[a]	0	–
	Tayasuidae	0/1	N/A	0/1	N/A	–	–	–	–	–
	Hippopotamidae	0/1	N/A	–	–	–	–	–	–	–
	Camelidae	0/15	0	–	–	–	–	–	–	–
	Cervidae	**2/25**	**8**	0/16	0	–	–	–	–	–
	Giraffidae	0/4	0	–	–	–	–	0/1[a]	N/A	–
	Bovidae	**5/94**	**5.3**	0/54	0	–	–	0/5[a]	0	–
Carnivora		**10/37**	**27**	0/37	0	**3/37**	**8.1**	**2/37**	**2.7**	**1/3**
	Canidae	**2/8**	**25**	0/7	0	**1/7**	0	0/7	0	0/1
	Hyaenidae	**1/1**	**N/A**	0/1	N/A	0/1	0	0/1	N/A	–
	Otariidae	**1/1**	**N/A**	0/1	N/A	0/1	0	0/1	N/A	–
	Phocidae	0/1	N/A	0/1	N/A	0/1	0	0/1	N/A	–
	Ursidae	**1/12**	**8.3**	0/12	0	**2/12**	**16.6**	**2/12**	**8.3**	**1/2**
	Felidae	**5/10**	**50**	0/10	0	0/11	0	0/11	0	–
	Herpestidae	0/4	0	0/3	0	0/4	0	0/4	0	–
Chiroptera		0/4	0	0/4	0	–	–	–	–	–
	Pteropodidae	0/4	0	0/4	0	–	–	–	–	–
Diprotodontia		0/2	0	0/1	N/A	–	–	–	–	–
	Macropodidae	0/2	0	0/1	N/A	–	–	–	–	–
Perissodactyla		**2/24**	**8.3**	0/20	0	–	–	0/2[a]	0	–
	Equidae	**2/20**	**10**	0/20	0	–	–	0/2[a]	0	–
	Rhinocerotidae	0/3	0	–	–	–	–	–	–	–
	Tapiridae	0/1	N/A	–	–	–	–	–	–	–
Proboscidea		0/6	0	–	–	–	–	–	–	–
	Elephantidae	0/6	0	–	–	–	–	–	–	–
Rodentia		0/2	0	0/3	0	–	–	0/2[a]	0	–
	Castoridae	0/2	0	0/2	0	–	–	0/2[a]	0	–
	Chinchillidae	0/1	N/A	0/1	N/A	–	–	–	–	–
	Total	**28/244**	**11.5**	0/161	0	**3/37**	**8.1**	**2/49**	**4.1**	**1/3**

pos., positive; total, total number of individual samples analysed; –, not determined.
N/A, not applicable (only 1 sample analysed).
RT-qPCR, reverse transcription-quantitative polymerase chain reaction; NBS-RT-PCR, nested broad-spectrum RT-PCR; SW-RT-PCR, (rat)HEV-specific RT-PCR.
Results printed in bold are positive results.
[a] Liver samples.

the order Carnivora with 10/37 (27.0%) positive samples originating from six different species. Table 1 gives an overview on the findings, whereas individual data are listed in the Suppl. Table 1.

3.2. RatHEV RNA is detected in a Syrian brown bear sample by RT-PCR

A subset of 161 serum samples, which were selected according to the availability of sample material, was analysed by the RT-qPCR for detection of RNA of HEV-1 to HEV-4 (Table 1). None of the investigated samples was positive in the assay. To allow the detection of hepeviruses from other species than *Orthohepevirus A*, two broadly reactive RT-PCR assays were applied to 37 individual serum samples belonging to animals of the order Carnivora and to 12 liver samples originating from different animal species, obtained during necropsy (Table 1). Three serum samples originating from a South American coati (*Nasua nasua*), a bush dog (*Speothos venaticus*) and a Syrian brown bear (*Ursus arctos syriacus*) showed bands of the expected lengths in the NBS-RT-PCR. The South American coati and the Syrian brown bear were also positive in the SW-RT-PCR. Attempts to sequence the amplicons were only successful for the products of the NBS-RT-PCR and the SW-RT-PCR of the Syrian brown bear sample, whereas the fainter bands of the other animals could not be sequenced. The analysis of the sequences indicated the closest relationship to ratHEV strain R68 from the species *Orthohepevirus C* (Fig. 1A). The serum sample of the female Syrian brown bear of zoo A was taken in 2011, when the animal was 22 years old. This serum sample was negative for HEV-specific antibodies. However, a second serum sample taken in 2016, before the age-related death of the animal at 27 years, was positive for HEV-specific antibodies (Suppl. Table 1).

3.3. RatHEV RNA sequences from wild rats of the same zoo are closely related to that of the Syrian brown bear

To investigate the source of infection of the bear with ratHEV, rat samples from the same geographic location were analysed. 20 feeder rats from zoo A, hold in 2017, were tested negative in the Axiom® HEV-Ab EIA assay as well as in the SW-RT-PCR (Suppl. Table 2). Additional samples of 73 wild Norway rats, trapped between 2009 and 2016 in zoos A and D (located in a distance of 16 km from each other), were available. HEV-specific antibodies could not be demonstrated in transudates from the thoracic cavity of these rats using the EIA (Suppl. Table 2). HEV-RNA could be detected in 8/73 (10.9%) liver samples using the SW-RT-PCR (Suppl. Table 2). Sequencing of the RT-PCR products revealed the presence of ratHEV in 7/8 of the RNA-positive samples (1 positive sample from zoo D could not be sequenced). A phylogenetic tree was set up for the obtained 279 nt sequences from the RdRp-encoding region of the HEV ORF1 together with other available ratHEV sequences, also including previously published sequences from

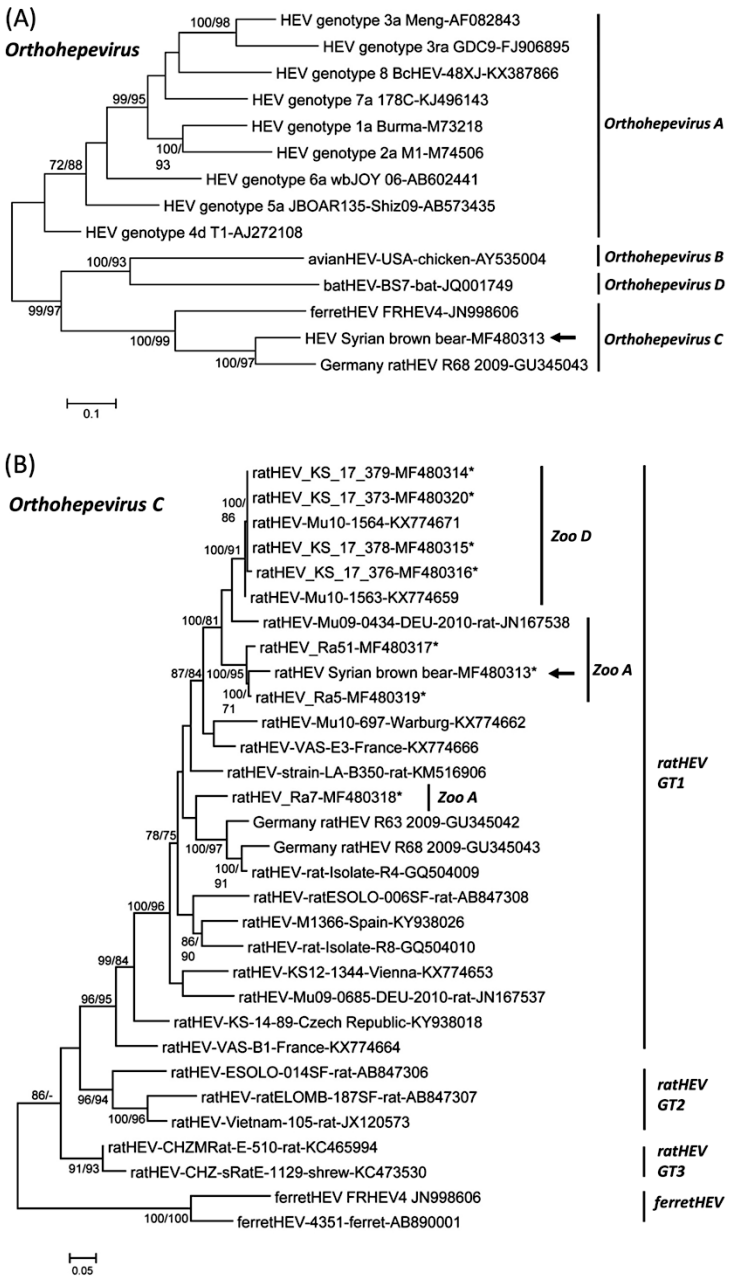

Fig. 1. Phylogenetic relationship between the sequences from animals of zoos A and D and other HEV strains. (A) Comparison of reference sequences from the Genus *Orthohepevirus* with the sequence derived from the Syrian brown bear of zoo A (marked by an arrow). The species *Orthohepevirus A, B, C* and *D* are indicated right and the established genotypes are implemented into the strain designations. (B) Comparison of sequences from ratHEV and ferretHEV strains within species *Orthohepevirus C* with the sequence from the Syrian brown bear of zoo A (marked by an arrow). Sequences newly established in this study are marked by asterisks. The origin of sequences from zoo A or D as well as the proposed genotypes of ratHEV according to Mulyanto et al. (2014) are indicated.

zoo A and D. The sequences of two rats from zoo A and the one of the Syrian brown bear showed nt sequence identities of 94.6% to 97.8% to each other and define a well separated cluster within the ratHEV-clade (Fig. 1B). A sister cluster is formed by the sequence of an already published strain from the same location and published strains from zoo D. One of the newly determined ratHEV sequences (Ra7) from zoo A clusters differently but still within the proposed ratHEV GT1 clade (Mulyanto et al., 2014), which is typical for European ratHEV strains

(Ryll et al., 2017). Asian ratHEV sequences and ferretHEV strains are found in other branches of the tree.

4. Discussion

HEV infections in zoo animals have been investigated scarcely so far. In a previous study, 38 faecal samples belonging to 22 animal species from a zoo-like centre in Eastern China were analysed for the presence of HEV-RNA, revealing a positive result of 28.9% (Zhang et al., 2008). All detected sequences belonged to HEV-4 and were derived from three deer and two carnivore species. A larger study involving 244 sera from 66 mammal species from zoos in Germany was initiated here. HEV-reactive antibodies were detected in 11.5% of the animals belonging to 16 mammalian species out of nine families, indicating that infections with HEV or HEV-like viruses occur in a wide range of different zoo animal species. These data confirm that from other published studies: markers of HEV infection have been identified in suids and cervids, bovids, canids, felids, ursids and equids (Spahr et al., 2017b). In addition, we demonstrated for the first time the presence of HEV-specific antibodies in antelopes, hyenas and otariids. The host spectrum of HEV should be investigated in future studies involving a broader range of animal species and geographical areas.

Domestic pigs and wild boars are well known reservoirs for zoonotic HEV-3 and HEV-4. Therefore, the high seroprevalence of 33.3% obtained for the zoo-housed pigs is in line with the expectations. Reported anti-HEV-IgG seroprevalences in domestic pigs range between 23% (Argentina) and 100% (USA) (Doceul et al., 2016; Pavio et al., 2010) and in wild boars between 3% (USA) and 42.7% (Spain) (De Deus et al., 2008; Dong et al., 2011). Despite the high seroprevalence, we did not detect HEV RNA in the zoo-housed pigs. Productive infection commonly occurs in young pigs and viral excretion decreases with the appearance of antibodies (McCreary et al., 2008). Zoo-housed pigs usually have a long lifetime leading to a high median age of the study group, which could explain the high antibody prevalence and the absence of HEV RNA. In addition, serum may not represent the best sample material for HEV RNA detection as viremia during HEV infection is usually short (Grierson et al., 2015). Studies investigating younger animals and other sample types like faeces or liver should increase the chance to detect HEV RNA and to identify the involved HEV type.

A high seroprevalence of 27% was also identified for zoo-housed carnivores. For mongooses, which are small wild carnivores, seroprevalences of 21% were reported from Japan (Nakamura et al., 2006). Seroprevalences up to 21% for pet dogs (Liang et al., 2014) and 30% for pet cats (Mochizuki et al., 2006) have also been described. The distinct reasons for the high seroprevalence in carnivores are not known yet. However, virus transmission by ingestion of infected animals seems to be a reasonable source of infection.

So far, different HEV GT have been identified in carnivores: HEV-3 in mongooses, HEV-4 in leopards and bears and *Orthohepevirus C* carnivore strains in minks and ferrets (Spahr et al., 2017b). Attempts to detect RNA of HEV-3 and HEV-4 in our carnivore samples failed. In contrast, RNA of ratHEV was identified in a Syrian brown bear. This sample was seronegative for HEV, whereas a second serum sample taken 5 years later was antibody-positive. This might indicate seroconversion due to a ratHEV infection. During this time, no clinical symptoms were reported by the animal keepers performing daily routine checks. The distribution of ratHEV infections in carnivores and its clinical consequences for the animals should be investigated in further studies.

Different sources for the ratHEV infection of the bear can be imagined. Infections with ratHEV seem to be common in free-living Norway rats (*Rattus norvegicus*) in Germany, but also in other parts of the world (Ryll et al., 2017; Mulyanto et al., 2014). In addition, ratHEV has been detected in Black rats (*Rattus rattus*), Bandicoot rats (*Bandicota indica*) and Asian musk shrews (*Suncus murinus*) (Spahr et al., 2017b). No HEV-specific antibodies were detected in free-living and feeder Norway rats in our study. However, ratHEV-RNA was detected in free-living Norway rats and the identified ratHEV sequences from zoo A were highly similar to the sequence obtained from the bear. A geographical clustering of ratHEV sequences from different locations in Germany has been previously described (Johne et al., 2012). Taken together, the results indicate that free-living Norway rats might have served as a source of ratHEV infection for this bear. Generally, the distinct host range of ratHEV is largely unknown. The experimental infection of rhesus macaques (*Macaca mulatta*) with ratHEV did not result in seroconversion or virus excretion (Purcell et al., 2011). In contrast, antibodies from healthy German forestry workers showed a higher reactivity to ratHEV than to HEV-3 (Dremsek et al., 2012) and ratHEV-reactive antibodies were recently identified in febrile patients with mild liver dysfunction from Vietnam (Shimizu et al., 2016). The zoonotic potential and the spillover potential of ratHEV therefore deserve more attention in future studies.

5. Conclusion

Our study indicates, that infection of various zoo animals of different mammal species with HEV or HEV-related viruses occurs. The observed seroprevalences were considerably low, except for suids and carnivores, which showed rather high antibody detection rates. Whereas pigs are commonly considered as reservoir animals for HEV, the reason for the high seroprevalence in carnivores remains unclear. The identification of ratHEV in a bear indicates that this virus is also able to infect non-rodent animal species under certain conditions. In the presented case, an accidental spillover infection from the infected wild rats to the bear is most likely. Control of pest animals and feed used for carnivores should be considered in zoos in order to prevent virus transmissions. Further investigations are needed to prove the role of zoo animals and especially carnivores as potential reservoirs for HEV or HEV-related viruses.

Conflicts of interest statement

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vetmic.2017.11.005.

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4 General discussion

4.1 HEV infections in various animal species

Since more than thirty years, attempts have been made to ascertain the animals that are susceptible to HEV infections – either by natural infection, or by experimental infection trials. The results of the studies presented in this thesis further lead to an increase of the number of animal species, which showed serological evidence of HEV infection. Taken together these data with those published by others, HEV and HEV-like viruses or antibodies specific against them have been detected in more than 100 animal species, including 19 avian species (Tab. 2) out of 10 taxonomic orders, about 80 mammal species (Tab. 3 and Tab. 4) out of 9 taxonomic orders, as well as fish so far. The susceptible species include various wild, domestic and zoo animal species.

Order							
Family							
Bird species	**Scientific name**	**Anti-HEV-ab**	**Genome**	**HEV-GT**	**Domestic / pet animals**	**Wild animals**	**Zoo animals**
Accipitriformes							
Accipitridae							
common bussard	*Buteo buteo*	n.d.	**+**	**avian HEV**	–	ZHANG et al. 2017	–
Himalayan griffon	*Gyps himalayensis*	n.d.	**+**	**HEV-3**	–	–	LI et al. 2015
Anseriformes							
Anatidae							
duck	*Anas plathyrhynchos domesticus*	**+**	n.d.	unknown	ZHANG et al. 2008b	–	–
Columbiformes							
Columbidae							
feral pidgeon	*Columba livia*	n.d.	**+**	**avian HEV**	–	ZHANG et al. 2017	–
pidgeon	*Columba livia domestica*	**+**	n.d.	unknown	ZHANG et al. 2008b	–	–
Falconiformes							
common kestrel	*Falco tinnunculus*	n.d.	**+**	**novel**	–	REUTER et al. 2016b	–
red-footed falcon	*Falco vespertinus*	n.d.	**+**	**novel**	–	REUTER et al. 2016b	–
Galliformes							
Phasianidae							
chicken	*Gallus gallus domesticus*	n.d.	**+**	**avian HEV**	HAQSHENAS et al. 2001	–	–
		n.d.	**+**	**avian HEV**	AGUNOS et al. 2006	–	–
		+	**+**	**avian HEV**	HUANG et al. 2002	–	–
		+	**+**	**avian HEV**	PERALTA et al. 2009	–	–
		+	**+**	**avian HEV**	HSU and TSAI 2014	–	–
silver pheasant	*Lophura nycthermera*	n.d.	**+**	**HEV-4**	–	–	ZHANG et al. 2008a
turkey	*Meleagris gallopavo*	**+**	**+**	**avian HEV**	SUN et al. 2004*	–	–
Gruiformes							
Gruidae							
crowned crane	*Balearica regulorum*	n.d.	**+**	**HEV-4**	–	–	ZHANG et al. 2008a

Order							
Family							
Bird species	**Scientific name**	**Anti-HEV-ab**	**Genome**	**HEV-GT**	**Domestic / pet animals**	**Wild animals**	**Zoo animals**
Passeriformes							
Alaudidae							
oriental skylark	*Alauda gulgula*	+	n.d.	unknown	CONG et al. 2014	–	–
Fringillidae							
black-tailed grosbeak	*Coccothraustes migratorius*	+	n.d.	unknown	CONG et al. 2014	–	–
Eurasian siskin	*Carduelis spinus*	+	n.d.	unknown	CONG et al. 2014	–	–
Turdidae							
song thrush	*Turdus philomelos*	n.d.	+	**avian HEV**	–	ZHANG et al. 2017	–
Pelecaniformes							
Ardeidae							
little egret	*Egretta garzetta*	n.d.	+	**novel**	–	REUTER et al. 2016a	–
Psittaciformes							
Psittacidae							
Alexandrine parakeet	*Psittacula eupatria*	+	n.d.	unknown	ZHANG et al. 2014	–	–
budgerigar	*Melopsittacus undulates*	+	n.d.	unknown	ZHANG et al. 2014	–	–
Strigiformes							
Strigidae							
little owl	*Athene noctua*	n.d.	+	**avian HEV**	–	ZHANG et al. 2017	–

+, positive; n.d., not determined; *, laboratory animals.

Table 2: Natural HEV infections in birds.

Domestic pig (HEV-3 / HEV-4), wild boar (HEV-3 / HEV-4), rabbit (HEV-3), deer (HEV-3) and dromedary camel (HEV-7) are well known to serve as reservoir animals that can be infected with the human-pathogenic HEV types (HUANG et al. 2016; LI et al. 2005; RUTJES et al. 2010; SRIDHAR et al. 2017). However, infections with zoonotic HEV-3 and HEV-4 are not restricted to these reservoir animals and humans only (PAVIO et al. 2015; YAN et al. 2016). They have been reported for several avian and mammal species including ungulates (cow, goat), non-human primates (rhesus macaque, chimpanzee), rodents, predators, wales and avian species (LI et al. 2015; MONTALVO VILLALBA et al. 2017; YAMAMOTO et al. 2012; ZHANG et al. 2008a). Especially in low income countries, many people are housing several kinds of free-ranging livestock to nourish their families. Therefore, different livestock species could come in close contact to each other or to wild animals. In these countries, for traditional reasons, intimate human animal contact and the consumption of raw milk and raw meat together with only basal hygienic standards may enforce the transmission of zoonotic HEV types to humans (HUANG et al. 2016; LEE et al. 2016; LONG et al. 2017).

Additional HEVs have been described in further reservoir animals: mongoose (HEV-3), moose (moose HEV), wild boar (HEV-5 and HEV-6), Bactrian camel (HEV-8), rodents (HEV-C1), red fox, ferret and mink (all HEV-C2), lagomorphs (rabbit HEV), bat (bat HEV), birds (avian HEV) and fish (fish HEV) (SPAHR et al. 2018b). New and

undefined HEVs have been reported in chimpanzee, falcons, and little egret (*Egretta garzetta*) (REUTER et al. 2016a,b; ZHOU et al. 2014).

In most animal species, HEV is detected sparsely. On the one hand, this may be due to low sample numbers investigated for each of these species. On the other hand, “spillover infections” from reservoir species to host species resulting in only a few successful virus transmissions seem to be possible. In other animal species, HEV infections are more often described and/or with higher prevalence. Based on the data from this thesis and other published results, predators and hoofed animals show higher prevalences than other groups of animals and therefore seem to be predisposed for HEV infections (DÄHNERT et al. 2018; SPAHR et al. 2018a; SPAHR et al. 2017). The next chapters should give a more detailed overview and discussion on the prevalences of HEV infections in different groups of animal species.

4.2 Prevalence of natural HEV infections in non-human primates

Reports about natural HEV-infections in non-human primates are scarcely available, so far (Tab. 3). Besides, the detection rates of anti-HEV-ab are varying quite a lot between the different species and different studies (SPAHR et al. 2018a). For example, the reported IgG-seroprevalences in monkeys from India and China ranged from 2% (1/50) in langur monkeys (*Semnopithecus entellus*) up to 35.87% (33/92) or 36.7% (36/98) in rhesus macaques from a breeding facility in Japan (ARANKALLE et al. 1994; HUANG et al. 2011). The IgG-seroprevalence in bonnet macaques was reported to be 19.1% (9/47) (ARANKALLE et al. 1994). Anti-HEV-IgM-ab were reported for 3/33 rhesus macaques from China and a small number of rhesus macaques in a breeding facility at the Primate Research Institute of the University of Kyoto, Japan, where seroconversion after HEV-3 infection was observed (HUANG et al. 2011; YAMAMOTO et al. 2012). The highest IgG-seroprevalence of 78.5% (96/121) was reported for rhesus macaques (YAMAMOTO et al. 2012). In contrast, only one out of nine lower primate species (1.2%, 1/86), showed HEV-reactive antibodies in the study presented in this thesis (SPAHR et al. 2018a).

To date, the investigation of lower non-human primates is standing to the forth, as certain species are housed in laboratories and are therefore easily available for sampling. To widen the range of species, we investigated 259 zoo-housed individuals, belonging to 15 species, for the presence of anti-HEV-ab, which also included 172 great ape samples (*Hominidae*) (SPAHR et al. 2018a). As a result, 3.9% (10/259) of the animals (7 gorillas, 1 bonobo, 1 lar gibbon, and 1 drill) were anti-HEV-IgG-ab

positive. Surprisingly, most seropositive animals were gorillas (15.2%, 7/46), followed by lar gibbons (9.1%, 1/11) and bonobos (4%, 1/25). There is only one additional report about natural HEV infection in chimpanzees from a Chinese zoo (ZHOU et al. 2014). A novel HEV-RNA was detected in the faeces of 7/24 (29.2%) of these chimpanzees (ZHOU et al. 2014). In contrast, anti-HEV-ab could not be detected in 70 chimpanzees' sera from European zoos in the study described in this thesis (SPAHR et al. 2018a).

Taken together, the study presented in this thesis was able to show that non-human primates including great apes can show markers of HEV infection. However, the determined antibody prevalences were generally lower as compared to most other published studies. In addition, it showed a higher proportion of anti-HEV-IgG positive great apes (3.5%, 6/172) compared to the very lower prevalence in non-human primates (1.1%, 1/87). As the samples from the different studies originate from different areas, general differences due to the geographical origin of the samples may explain these findings. In addition, different assays have been used for analysis, which therefore cannot be compared directly. The IgG assay used in the study described in this thesis is originally adapted for human sera and may therefore be more sensitive for the closely related great apes than for lower non-human primate species. An additional problem in studying non-human primates is, that blood samples for HEV monitoring are mostly not available spontaneously. Therefore, many zoos established their own serum banks, storing sera from non-human primates, collected during immobilisations for different purposes. Long-term storage and frequent freeze-thaw cycles may have led to a decrease of HEV-ab amounts in the investigated sera.

It can be concluded that non-human primates including great apes seem to be susceptible to HEV infections. However, the determined prevalences were low thus arguing against a role of these animal species as a reservoir for HEV. It cannot be decided if HEV infections lead to clinical disease in these species or not, because at the time of sampling, all investigated animals were HEV-RNA- and anti-HEV-IgM-negative, indicating that no ongoing infections could be observed in this study. However, as no history of hepatitis was recorded in these animals, the presence of subclinical infections is likely.

Animal species	Scientific name	Anti-HEV-ab	Genome	HEV-GT	Wild animals	Zoo-housed animals	Experimental infection
Great apes							
bonobo	*Pan paniscus*	**4.0%**	0%	**unknown**	–	SPAHR et al. 2018a	–
chimpanzee	*Pan troglodytes*	**+**	**+**	**HEV-1**	–	–	YU et al. 2010
		n.d.	**VLPs**	**HEV-2**	–	–	ARANKALLE et al. 1988
		+	**+**	**HEV-3**	–	–	MENG et al. 1998
		n.d.	**+**	**HEV-4**	–	–	YUGO et al. 2014
		0%	0%	n.d.	–	SPAHR et al. 2018a	–
		n.d.	**29.2%**	**novel**	–	ZHOU et al. 2014	–
Western lowland gorilla	*Gorilla gorilla gorilla*	**15.2%**	0%	**unknown**	–	SPAHR et al. 2018a	–
lar gibbon	*Hylobates lar*	**9.1%**	0%	**unknown**	–	SPAHR et al. 2018a	–
Bornean orangutan	*Pongo pygmaeus*	0%	0%	n.d.	–	SPAHR et al. 2018a	–
Sumatran orangutan	*Pongo abelii*	0%	0%	n.d.	–	SPAHR et al. 2018a	–
Lower primates							
African green monkey	*Chlorocebus sabaeus*	**+**	n.d.	**HEV-1**	–	–	DOCEUL et al. 2016
		+	n.d.	**HEV-2**	–	–	
black capped squirrel monkey	*Saimiri boliviensis*	0%	0%	n.d.	–	SPAHR et al. 2018a	–
black howler monkey	*Alouatta caraya*	0%	0%	n.d.	–	SPAHR et al. 2018a	–
bonnet macaque	*Macaca radiata*	**19.1%**	n.d.	**unknown**	ARANKALLE et al. 1994	–	–
cynomolgus macaque	*Macaca fasciularis*	n.d.	**+**	**HEV-1**	–	–	BALAYAN et al. 1983
		n.d.	**+**	**HEV-1**	–	–	BRADLEY et al. 1987
		+	**+**	**HEV-1**	–	–	TSAREV et al. 1994
		n.d.	**+**	**HEV-2**	–	–	BALAYAN et al. 1983
		+	**+**	**HEV-2**	–	–	BRADLEY et al. 1987
		n.d.	**+**	**HEV-2**	–	–	TICEHURST et al. 1992
		+	**+**	**HEV-2**	–	–	AGGARWAL et al. 2001
		+	**+**	**HEV-3**	–	–	ERKER et al. 1999
		+	**+**	**HEV-3**	–	–	DE CARVALHO et al. 2013
		+	**+**	**rabbit HEV**	–	–	LIU et al. 2013
drill	*Mandrillus leucophaeus*	**14.3%**	0%	**unknown**	–	SPAHR et al. 2018a	–
Eastern owl monkey	*Aotus trivirgatus*	n.d.	**+**	**HEV-1**	–	–	YUGO et al. 2014
		+	**+**	**HEV-1**	–	–	TICEHURST et al. 1992
gelada baboon	*Theropithecus gelada*	0%	0%	n.d.	–	SPAHR et al. 2018a	–
grey langur	*Semnopithecus entellus*	**2%**	n.d.	**unknown**	ARANKALLE et al. 1994	–	–
Japanese macaque	*Macaca fuscata*	0%	0%	n.d.	–	SPAHR et al. 2018a	–
		78.5%	n.d.	n.d.	YAMAMOTO et al. 2012	–	–
		n.d.	**+**	**HEV-3**		–	–
Javan silvered leaf monkey	*Trachypithecus auratus*	0%	0%	n.d.	–	SPAHR et al. 2018a	–
moustached tamarin	*Saguinus mystax mystax*	+	+	**HEV-1**	–	–	BRADLEY et al. 1987
		+	+	**HEV-2**	–	–	
patas monkey	*Erythrocebus patas*	n.d.	+	**HEV-1**	–	–	YUGO et al. 2014
		n.d.	+	**HEV-2**	–	–	

Animal species	Scientific name	Anti-HEV-ab	Genome	HEV-GT	Wild animals	Zoo-housed animals	Experimental infection
rhesus macaque	*Macaca mulatta*	**36.7%**	n.d.	**unknown**	ARANKALLE et al. 1994	–	–
		+	n.d.	n.d.		–	–
		35.8%	0%	n.d.	HUANG et al. 2011	–	–
		n.d.	0%	-		–	–
		+	+	**HEV-3**	–	–	MENG et al. 1998
		+	+	**HEV-3**	YAMAMOTO et al. 2012*	–	–
		n.d.	+	**HEV-3**		–	–
		0%	0%	**rat HEV**	–	–	PURCELL et al. 2011
		0%	0%	**avian HEV**	–	–	HUANG et al. 2004
squirrel monkey	Saimiri sciureus	n.d.	+	**HEV-1**	–	–	TSAREV et al. 1994
		n.d.	+	**HEV-2**	–	–	
vervet monkey	*Chlorocebus sabaeus*	n.d.	+	**HEV-1**	–	–	TSAREV et al. 1994
		n.d.	+	**HEV-2**	–	–	
white crowned mangabey	*Cercocebus atys lunulatus*	0%	0%	n.d.	–	SPAHR et al. 2018a	–
white-faced saki	*Pithecia pithecia*	0%	0%	n.d.	–	SPAHR et al. 2018a	–
white-fronted spider monkey	*Ateles hybridus*	0%	0%	n.d.	–	SPAHR et al. 2018a	–

+, positive; n.d., not determined; 0%, negative; %, positive in %; *, natural infection in laboratory monkeys.

Table 3: HEV infections in non-human primates.

4.3 Prevalence of natural HEV infections in other zoo-housed mammals

In general, reports about HEV infections in zoo-housed animals (birds and mammals) are scarcely available so far (LI et al. 2015; ZHANG et al. 2008a; ZHOU et al. 2014). To better assess the role of zoo-housed mammals as reservoir or host species, we investigated 244 serum samples from 66 clinically healthy mammal species in the study described in this thesis (SPAHR et al. 2017). As a result, anti-HEV-specific-ab were found in the sera of 16 mammal species from European zoos. Besides the well-known reservoir species, swine (33.3%) and deer (8%), two species of goat and donkey, one antelope species and five carnivore species were anti-HEV-IgG-positive (Tab. 4, zoo animals).

Particularly, the high seroprevalence in carnivores (27.0%) was remarkable, thus justifying further investigations in this animal group. In summary, five out of seven analysed carnivore species were tested seropositive, including maned wolf, California sea lion (*Zalophus californianus*), Syrian brown bear (*Ursus arctos syriacus*), Persian leopard (*Panthera pardus saxicolor*) and snow leopard (*Unica unica*). Interestingly, rat HEV-RNA was detected in the serum of a female Syrian brown bear and seroconversion was demonstrated in the same animal (SPAHR et al. 2017). Conclusively, wild-ranging pest rats may have been the source of HEV infection for this animal.

By summarising the findings of the study presented in this thesis and other published data, infections with HEV-3, HEV-4, HEV-C2 or rat HEV are reported for the

following carnivore species: Asiatic black bear (*Ursus thibetanus*), cat (*Felis catus silvestris*), California sea lion, clouded leopard (*Neofelis nebulosa*), dog, ferret (*Mustela putorius*), maned wolf (*Chrysocyon brachyurus*), mink (*Mustela lutreola*), mongoose (*Herpestes javanicus*), Persian leopard, raccoon (*Procyon lotor*), raccoon dog (*Nyctereutes procyonoides*), red fox, snow leopard and Syrian brown bear (Tab. 4). HEV infections in cats and dogs have been described before (LIANG et al. 2014; LIU et al. 2009; MOCHIZUKI et al. 2006). ARANKALLE et al. (2001) provided an indication that carnivores living in HEV-endemic areas are more susceptible to HEV infections. He ascertained an anti-HEV-IgG seroprevalence of 22.7% (10/44) in dogs from India (ARANKALLE et al. 2001). The high seroprevalence may be explained by the feeding behaviour of the straying dogs and the bad hygiene conditions in India. ZHANG et al. (2008a) first detected mammalian HEV-4-RNA in one clouded leopard and one Asiatic black bear. All animals were clinically healthy. The findings were explained by “spillover infection” from other animal species in this zoo, which were also tested positive in this study (ZHANG et al. 2008a).

It can be concluded, that the available data show, that many mammalian species are susceptible to HEV infections. Most of these species show only low prevalences, which are suggestive for “spillover infections”. Porcine species clearly show high prevalences in congruence with their role as reservoir animals. Remarkably, carnivore species turn out more and more to also show higher seroprevalences. Very recently, DÄHNERT et al. (2018) reported high seroprevalences in raccoons (53.8%, 43/80), raccoon dogs (34.3%, 25/73), pet dogs (56.6%, 47/83) and pet cats (32.3% (21/65) from Brandenburg, Germany. These recent findings underline the notion of carnivores being natural HEV hosts (DÄHNERT et al. 2018; SPAHR et al. 2017). The reasons for the indicated high seroprevalences are not known so far. Infection by ingestion of infected prey animals may be speculated. However, as mostly only (cross-reacting) antibodies have been demonstrated, the HEV types responsible for the antibody production are not known in most cases. In addition, productive infection and transmission of virus needs to be demonstrated in future studies in order to clarify the role of carnivores in HEV epidemiology.

Order							
Family							
Mammal species	**Scientific name**	**Anti-HEV-ab**	**Genome**	**HEV-GT**	**Domestic / pet animals**	**Wild animals**	**Zoo animals**
Artiodactyla							
Bovidae							
American bison	*Bison bison*	+	-	unknown	DONG et al. 2011	–	–
cape buffalo	*Syncerus caffer*	+	n.d.	unknown	EL-TRAS et al. 2013	–	–
Congo dwarf goat	*Capra hircus domestic congo dwarf*	**25%**	n.d.	unknown	–	–	SPAHR et al. 2017
dairy cattle	*Bos taurus primigenius*	+	n.d.	unknown	EL-TRAS et al. 2013	–	–
goat	*Capra hircus aegagrus*	+	n.d.	unknown	EL-TRAS et al. 2013	–	–
		+	n.d.	unknown	SANFORD et al. 2013	–	–
		n.d.	+	**HEV-3**	DI MARTINA et al. 2016	–	–
		+	n.d.	unknown	PERALTA et al. 2009	–	–
		+	+	**HEV-4**	LONG et al. 2017	–	–
Holstein Frisian cattle	*Bos taurus primigenius*	-	+	**HEV-4**	HUANG et al. 2016	–	–
Lesser Kudu	*Tragelaphus imberis*	**7%**	n.d.	unknown	–	–	SPAHR et al. 2017
Rocky mountain goat	*Oreamnos americanus*	**40%**	n.d.	unknown	–	–	SPAHR et al. 2017
sheep	*Ovis aries orientalis*	+	n.d.	unknown	EL-TRAS et al. 2013	–	–
yak	*Bos grunniens*	-	+	**HEV-4**	XU et al. 2014	–	–
yellow cattle	*Bos taurus primigenius*	+	+	**HEV-4**	YAN et al. 2016	–	–
Camelidae							
Bactrian camel	*Camelus bactrianus*	n.d.	+	**HEV-8**	WOO et al. 2016	–	–
dromedary	*Camelus dromedarius*	n.d.	+	**HEV-7**	WOO et al. 2014	–	–
		+	+	**HEV-7**	RASCHE et al. 2016	–	–
Cervidae							
Mesopotamian fallow deer	*Dama mesopotamica*	**8%**	n.d.	unknown	–	–	SPAHR et al. 2017
red deer	*Cervus elaphus*	n.d.	+	**HEV-3**	–	FORGÁCH et al. 2010	–
		-	+	**HEV-3**	–	ANHEYER-BEHMENBURG et al. 2017	–
reeves' muntjac	*Muntiacus reevesi*	n.d.	+	**HEV-4**	–	–	ZHANG et al. 2008a
roe deer	*Capreolus capreolus*	-	+	**HEV-3**	–	REUTER et al. 2009	–
		n.d.	+	**HEV-3**	–	FORGÁCH et al. 2010	–
		-	+	**HEV-3**	–	ANHEYER-BEHMENBURG et al. 2017	–
Sika deer	*Cervus nippon nippon*	+	n.d.	unknown	SONODA et al. 2004	–	–
		n.d.	+	**HEV-4**	–	–	ZHANG et al. 2008a
Swedish moose	*Alces alces*	-	+	**moose HEV**	LIN et al. 2014	–	–
		+	+	**moose HEV**	LIN et al. 2015	–	–
tufted deer	*Elaphodus cephalophus*	n.d.	+	**HEV-4**	–	–	ZHANG et al. 2008a
yezo deer	*Cervus nippon yesoensis*	+	n.d.	unknown	–	TOMYAMA et al. 2009	–
		+	-	unknown	–	SONODA et al. 2004	–

Order							
Family							
Mammal species	**Scientific name**	**Anti-HEV-ab**	**Genome**	**HEV-GT**	**Domestic / pet animals**	**Wild animals**	**Zoo animals**
Suidae							
domestic pig	*Sus scrofa domestica*	+	+	**HEV-3**	–	BREUM et al. 2010	–
		+	+	**HEV-4**	–	BREUM et al. 2010	–
		45.5	n.d.	unknown	–	–	SPAHR et al. 2017
wild boar	*Sus scrofa*	+	+	**HEV-3**	–	SONODA et al. 2004	–
		+	+	**HEV-3**	–	ADLHOCH et al. 2009	–
		n.d.	+	**HEV-3**	–	MARTELLI et al. 2008	–
		+	+	**HEV-3**	–	DE DEUS et al. 2008	–
		n.d.	+	**HEV-3**	–	WIRATSUDUKAL et al. 2012	–
		n.d.	+	**HEV-3**	–	KACI et al. 2008	–
		+	+	**HEV-3**	–	DONG et al. 2011	–
		+	+	**HEV-3**	–	ANHEYER-BEHMENBURG et al. 2017	–
		+	+	**HEV-3**	–	TAKAHASHI et al. 2014	–
		+	+	**HEV-4**	–		–
		n.d.	+	**HEV-5**	–	TAKAHASHI et al. 2011	–
				HEV-6	–		–
		+	n.d.	unknown	–	LARSKA et al. 2015	–
		+	n.d.	unknown	–	CARPENTIER et al. 2012	–
		+	n.d.	unknown	–	CHANDLER et al. 1999	–
Carnivora							
Canidae							
dog	*Canis lupus familiaris*	+	-	unknown	LIU et al. 2009	–	–
		+	n.d.	unknown	LIANG et al. 2014	–	–
		+	-	unknown	MC ELROY et al. 2015	–	–
		+	n.d.	unknown	ARANKALLE et al. 2001	–	–
Maned wolf	*Chrysocyon brachyurus*	**66.7%**	n.d.	unknown	–	–	SPAHR et al. 2017
red fox	*Vulpes vulpes*	n.d.	+	**HEV-C2**	–	BODEWES et al. 2013	–
Felidae							
cat	*Felis catus silvestris*	+	n.d.	unknown	LIANG et al. 2014	–	–
		+	-	unknown	MOCHIZUKI et al. 2006	–	–
clouded leopard	*Neofelis nebulosa*	n.d.	+	**HEV-4**	–	–	ZHANG et al. 2008a
Persian leopard	*Panthera pardus saxicolor*	**66.7%**	n.d.	unknown	–	–	SPAHR et al. 2017
Snow leopard	*Unica unica*	**60%**	n.d.	unknown	–	–	SPAHR et al. 2017
Herpestidae							
Javan mongoose	*Herpestes javanicus*	+	+	**HEV-3**	–	NAKAMURA et al. 2006	–
		+	-	unknown	–	LI et al. 2006	–
		n.d.	+	**HEV-3**	–	NIDAIRA et al. 2012	–
Mustelidae							
European ferret	*Mustela putorius*	+	+	**HEV-C2**	–	RAJ et al. 2012	–
European mink	*Mustela lutreola*	n.d.	+	**HEV-C2**	KROG et al. 2013	–	–
Otariidae							
California sea lion	*Zalophus californianus*	**+ / N/A**	n.d.	unknown	–	–	SPAHR et al. 2017
Ursidae							
Asiatic black bear	*Ursus thibetanus*	n.d.	+	**HEV-4**	–	–	ZHANG et al. 2008a
Syrian brown bear	*Ursus arctos syriacus*	**25%**	+	**rat HEV**	–	–	SPAHR et al. 2017

Order / Family	Scientific name	Anti-HEV-ab	Genome	HEV-GT	Domestic / pet animals	Wild animals	Zoo animals
Mammal species							
Cetacea							
Bottlenose dolphin	*Tursiops truncatus*	+	+	**HEV-3**	–	MONTALVO VILLALBA et al. 2017	–
Chioptera							
Aba roundleaf bat	*Hipposideros abae*	n.d.	+	**bat HEV**	–	DREXLER et al. 2012	–
Bechstein's bat	*Myotis bechsteinii*	n.d.	+	**bat HEV**	–	DREXLER et al. 2012	–
Daubenton's bat	*Myotis daubentonii*	n.d.	+	**bat HEV**	–	DREXLER et al. 2012	–
Great stripe- faced bat	*Vampyrodes caraccioli*	n.d.	+	**bat HEV**	–	DREXLER et al. 2012	–
Serotine bat	*Eptesicus serotinus*	n.d.	+	**bat HEV**	–	DREXLER et al. 2012	–
Eulipotyphla							
Asian musk shrew	*Suncus murinus*	+	+	**HEV-1**	–	GUAN et al. 2013	–
Lagomorpha							
European brown hare	*Lepus europaeus*	+	-	unknown	–	HAMMERSCHMIDT et al. 2017	–
Japanese White rabbit	*Oryctolagus cuniculus domesticus*	+	n.d.	unknown	XIA et al. 2015	–	–
New Zealand White rabbit	*Oryctolagus cuniculus domesticus*	+	n.d.	unknown	BIRKE et al. 2014	–	–
Rabbit	*Oryctolagus cuniculus*	n.d.	+	**rabbit HEV**	IZOPET et al. 2012	–	–
		+	+	**rabbit HEV**	COSSABOOM et al. 2011	–	–
		n.d.	+	**rabbit HEV**	IZOPET et al. 2012	–	–
		n.d.	+	**rabbit HEV**	CARUSO et al. 2015	–	–
		+	+	**HEV-3**	HAMMERSCHMIDT et al. 2017	–	–
		+	+	**rabbit HEV**	HAMMERSCHMIDT et al. 2017	–	–
Rex rabbit	*Oryctolagus cuniculus domesticus*	+	+	**rabbit HEV**	ZHAO et al. 2009	–	–
		+	+	**rabbit HEV**	GENG et al. 2011	–	–
		+	n.d.	unknown	XIA et al. 2015	–	–
Perisodactyla							
horse	*Equus caballus ferus*	+	+	**HEV-1**	SAAD et al. 2006	–	–
		+	+	**HEV-3**	ZHANG et al. 2008b	–	–
Poitou donkey	*Equus asinus domestic Poitou*	**33.3%**	n.d.	unknown	–	–	SPAHR et al. 2017
Somali wild ass	*Equus africanus somaliensis*	**33.3%**	n.d.	unknown	–	–	SPAHR et al. 2017
Rodentia							
black rat	*Rattus rattus hainanus*	+	-	**HEV-C1**	–	LI et al. 2013	–
black rat	*Rattus rattus*	+	+	**HEV-C1**	–	MULYANTO et al. 2014	–
		n.d.	+	**HEV-C1**	–	RYLL et al. 2017	–
greater bandicoot rat	*Bandicota indica*	+	+	**HEV-C1**	–	LI et al. 2013	–
Norway rat	*Rattus norvegicus*	+	n.d.	**HEV-C1**	–	EASTERBROOK et al. 2007	–
		n.d.	+	**HEV-C1**	–	JOHNE et al. 2010a,b	–
		-	+	**HEV-C1**	–	JOHNE et al. 2010a,b	–
		+	+	**HEV-C1**	–	JOHNE et al. 2012	–
		+	n.d.	unknown	–	KABRANE-LAZIZI et al. 1999	–
		+	+	**HEV-C1**	–	LI et al. 2013	–
		+	+	**HEV-C1**	–	PURCELL et al. 2011	–

Order / Family							
Mammal species	Scientific name	Anti-HEV-ab	Genome	HEV-GT	Domestic / pet animals	Wild animals	Zoo animals
		n.d.	+	HEV-C1	–	WIDÉN et al. 2014	–
		+	+	HEV-3	–	KANAI et al. 2012	–
		n.d.	+	HEV-3	–	LACK et al. 2012	–
		n.d.	+	HEV-C1	–	RYLL et al. 2017	–
		n.d.	+	rabbit HEV	–	RYLL et al. 2017	–
		6.8%	+	rat HEV	–	SPAHR et al. 2017	–
yellow-breasted rat	*Rattus flavipectus*	+	+	HEV-C1	–	LI et al. 2013	–
Taiwan rat	*Rattus rattoides losea*	+	+	HEV-C1	–	LI et al. 2013	–

+, positive; -, negative; n.d., not determined; *, laboratory animals. This table does not include all references to the individual animal species. There are numerous publications on pigs in particular (PAVIO et al. 2017).

Table 4: Natural HEV infections in mammals.

4.4 Transmission pathways of HEV in a zoo-setting

In a zoo-setting, different animal species live together in a comparatively confined space and wild animals mostly could come in contact with these. Therefore, various transmission pathways of HEV seem to be possible. Some of these transmission pathways are illustrated in Figure 9, using the example of the Syrian brown bear. In general, HEV transmission via direct (bold arrows) or indirect (dotted arrows) contact is delineated.

Direct contact between reservoir species (e.g. swine, wild boar, rabbit, rat, fox, dromedary, human) and other species (e.g. brown bear, carnivores, non-human primates) can lead to transmissions of HEV. This transmission pathway seems to be very likely in a zoo-setting as contacts between neighboured animals and with wild animals (e.g. rats) cannot be excluded. As an evidence, we detected rat HEV-RNA in the Syrian brown bear and demonstrated seroconversion in the same animal (SPAHR et al. 2017). The additional PCR-screening of 73 free-ranging Norway rats from two German zoos (including the zoo, housing the Syrian brown bear) resulted in the detection of eight rat HEV-positive animals (11%). Sequence analysis revealed that all detected rat HEV sequences were closely related to each other and showed a high nucleotide sequence identity (94.6% – 97.8%) to the isolate from the bear. These results indicate that “spillover infection” from free-ranging pest rats (delineated in green), representing a known reservoir for rat HEV, is most likely in the case of the Syrian brown bear (SPAHR et al. 2017). Although pest control programmes are enforced, free-ranging pest rats or rabbits may enter outdoor enclosures from carnivores, that are able to catch and gorge them.

Additional transmission pathways may be theoretically possible within zoo settings. Direct transmission of HEV between fellow species (delineated in blue), e.g. via social behaviour or mating, seems to be one more possible pathway for HEV infection. On the basis of preservation measures for many zoo animal species, the pan-European and worldwide exchange of these animals between zoos involves the danger of HEV transmissions. Infected animals, that were captured in the wild, transferred into a zoo and socialised with the zoo population, portray a possible risk for HEV infection in the housed animal stock (SPAHR et al. 2018a). However, also the shipment of zoo-borne animal stock reveals a potential risk of HEV transmission between fellow species.

Zoo animals, especially pigs and carnivores, may circulate and amplify the virus leading to a high virus release to the environment, which can thereafter be transmitted to other animal species by oronasal contact (SPAHR et al. 2017). Consumption of contaminated water resources may lead to HEV infections in animals, too (SPAHR et al. 2018b). Even feed (e.g. hay, straw, vegetables, fruits, grains, pelleted feed) could be contaminated with HEV by excretions of pest animals, dependent on storage conditions. Feeder animals (e.g. rat, rabbit), zoo animals killed for feeding purposes (e.g. deer, swine, camel, dromedary, antelopes, goat, sheep) or contaminated meat may also be a source of HEV infection in carnivorous zoo animal species.

Staff may also play a part contributing to indirect HEV transmission. If keepers are changing between animal enclosures or using the very same dunghill for disposing excretions of swine, carnivores and non-human primates, HEV transmission via contaminated material (e.g. shoes, wheel barrow, bugs, pitchfork, dustpan, brush, rake, vehicles) seems to be possible and may even portray a risk for the transmission of zoonotic HEVs from reservoir species (delineated in red) as swine, wild boar, rabbit, deer, mongoose or dromedary camel.

In conclusion, there are manifold possibilities for transmission of HEV and HEV-related viruses in a zoo-like setting. Attempts should be done to minimize the degree of virus transmission by applying several hygienic measures and control of wild animals in order to prevent infections of zoo animals and – in specific situations – also of the staff.

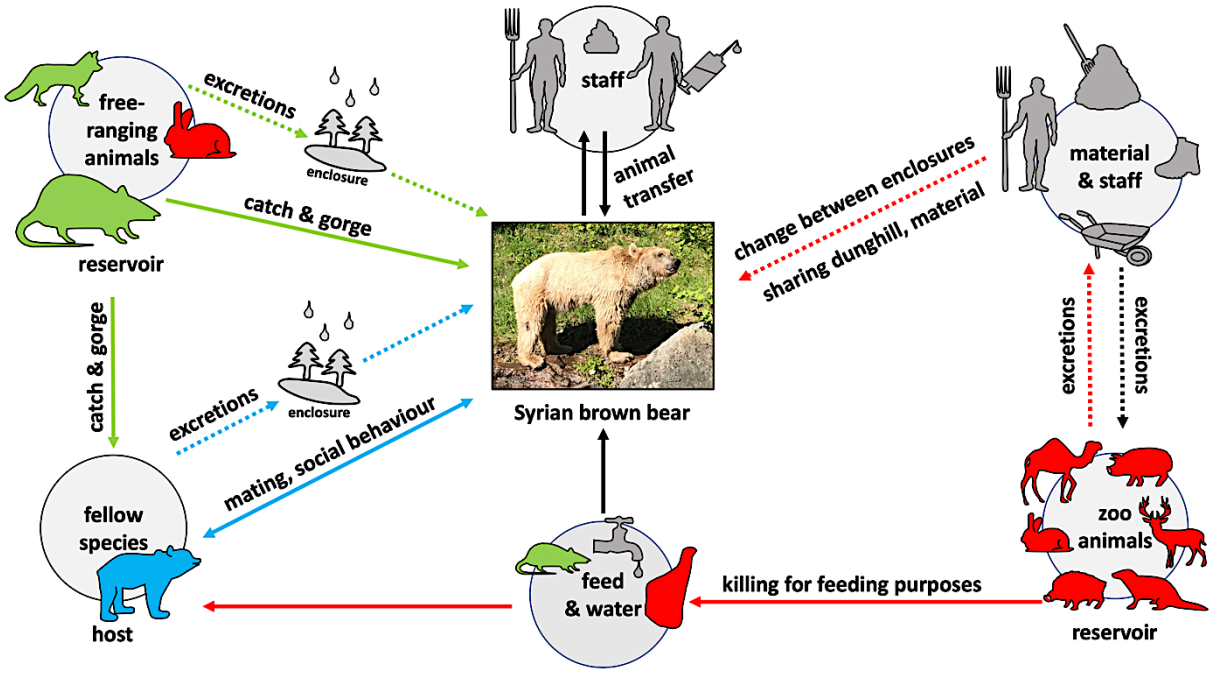

Figure 9: Possible transmission pathways of HEV in a zoo-setting.

4.5 Risk of virus transmission from zoo animals to humans

Non-human primates, especially great apes, are closely related to human beings and are known to be susceptible to diverse human pathogens, including HEV (SPAHR et al. 2018a). Due to medical training and treatment, non-human primates kept in zoos are usually in close contact with their keepers. Although hygiene standards in the holding institutions are high and the staff is encouraged to use face masks and gloves, direct contact to infected animals cannot be ruled out as possible way of vertical HEV transmission (BUITENDIJK et al. 2014; FICKENSCHER and FLECKENSTEIN 2001; MEALS et al. 2016). The relatively low seroprevalence rates in our field study with 3.9% (10/259) seropositivity in non-human primates argue against a high risk of HEV transmission to humans. Additionally, there was no direct evidence for an infection with zoonotic HEV-1 to HEV-4 in the animals in the study presented in this thesis as only antibodies were detected (SPAHR et al. 2018a).

However, YAMAMOTO et al. (2012) reported on an HEV outbreak in a Japanese monkey breeding facility were anti-HEV-3-IgG-seroprevalences from the staff simultaneously increased with the decrease of anti-HEV-3-IgM-ab and anti-HEV-3-IgG-ab from the animals. Anti-HEV-3-IgG-seroprevalence in humans was 6.9% (2007), 9.7% (2008) and 11.8% (2009) in comparison to the anti-HEV-3-IgM-seroprevalence in the animals with 1.1% (2007) and 0% (2008, 2009). The authors

assumed, that the staff got infected by direct contact to the monkeys or their blood or excretions (YAMAMOTO et al. 2012).

ZHANG et al. (2008a) detected mammalian HEV-4-RNA in carnivores, deer and birds from a Chinese zoo-like location. In parallel, seven workers including a veterinarian and six feeders, were tested for anti-HEV-ab (IgM and IgG) plus HEV-RNA (ZHANG et al. 2008a). All sera were HEV-RNA negative, but one person was positive for IgM, 3 persons for IgG and another was positive for both IgM and IgG (ZHANG et al. 2008a).

Infection of humans via direct contact to animals (e.g. wild boar), faeces, blood or aerosols from animals has previously been discussed as a possible transmission route (DREMSEK et al. 2013; DREMSEK et al. 2012). It is known, that people occupationally working with animals and animal products are at higher risk for HEV infections with zoonotic HEV-3 and HEV-4 (BfR FAQs 2016; PAVIO et al. 2017). In principle, staff working in a zoo-like setting has also to be considered to be exposed to HEV by contact to the animals and to their excretions. However, as the HEV prevalences seem to be lower in most zoo animal species as compared to domestic pigs, the risk of virus transmission has to be considered to be lower in general. In line with this, zoo animals of porcine species origin and carnivores should be considered of higher risk for HEV transmission.

Another factor limiting the assessment of the virus transmission to humans is the missing information on the specific virus types present in the zoo animals. In fact, only rat HEV was convincingly demonstrated in the study presented in this thesis. Generally, the zoonotic potential of rat HEV for transmission to humans has been considered low for a long time due to its divergent phylogenetic relationship to the other human-pathogenic HEVs (JOHNE et al. 2014). However, very recently, a rat HEV infection leading to chronic hepatitis has been described in an immunosuppressed human patient from Hong Kong (SRIDHAR et al. 2018). Further studies are necessary to clarify if this represents only a single exception, or if rat HEV indeed represents a human pathogen.

5 Conclusion and perspectives

Our results significantly increase the number of animal species with indications of natural infections with HEV or HEV-related viruses. In particular, predators and hoofed animals seem to be predisposed for HEV infections.

Zoo-housed non-human primates, particularly gorillas, showed serological markers of HEV infection, although with low prevalences. This leads to the assumption, that non-human primates are accidental hosts, but not important reservoir animals for HEV. To clarify the variation in seroprevalences between bonobos, chimpanzees, orangutans and gorillas, human ELISAs should be assessed for their specificity and sensitivity in detecting anti-HEV-ab in sera from non-human primates.

Based on the study presented in this thesis, the range of susceptible zoo-housed mammals, which show HEV seroprevalences, contains canids, felids, otariids, ursids, suids, bovids, cervids and equines. Whereas the high seroprevalences were expected for the porcine-related species, they were somewhat surprising for the carnivores. However, only a few studies have analysed HEV-infections in carnivores so far. The high prevalences may indicate a role as reservoirs or transmitters for these animal species. However, especially in the carnivores, the high seroprevalences may also be explained by a reaction on frequent ingestions of HEV-infected animals, which not necessarily has to result in high virus replication and excretion. The identified rat HEV infection in the Syrian brown bear may reflect such an infection gained by ingestion of infected wild rats. Further studies should attempt to elucidate the HEV infection of carnivores in more detail including studies on the route of infection, the involved HEV types and the amount of excreted virus.

The absence of the detection of any human-pathogenic HEV-GT-RNA in the animals investigated in the study presented in this thesis may suggest, that anthropozoonotic transmission is unlikely. However, as HEV-specific antibodies are known to cross-react between human-pathogenic and HEV-like viruses, the virus types originally infecting the animals are not known. People occupationally working with animals have been shown in several studies to be at higher risk for zoonotic HEV infections. Therefore, staff working in a zoo-like setting has also to be considered to be exposed to HEV by contact to the animals and to their excretions. However, the low HEV prevalences in most zoo animal species may indicate a lower risk for zoo workers as compared to workers in contact with domestic pigs. Future studies comparing the seroprevalences of zoo workers with that of non-exposed persons will be necessary to

clarify the risk of HEV transmission in zoo-like locations. In addition, in light of the recent publication of a human rat HEV infection, the zoonotic potential of rat HEV should be investigated in more detail as this virus was detected by us in a zoo animal.

Future HEV monitoring in carnivores, hoof stock and non-human primates is highly recommended to prove their role as potential reservoirs for HEV or HEV-related viruses, to evaluate potential differences in the susceptibility of certain animal species, to identify potential transmission routes and to assess possible veterinary and public health risk consequences. This should also include known reservoir species living in zoos, such as pigs, deer, rabbits and rats, in order to identify the virus sources. To prevent virus transmissions, the control of pest animals and feeder animals used for carnivores and improved feeding hygiene should be considered in zoos. Additionally, specific material for cleaning work should be assigned separately to each species to prevent virus transmissions by the staff.

6 Summary

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Title: Investigations on the occurrence of infections with hepatitis E virus and related viruses in zoo animals

Institute / Faculty: Institute of Virology, Faculty of Veterinary Medicine, University of Leipzig

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Bibliography: 78 pages (till end of summary), 9 figures, 4 tables, 188 references, 3 appendices

Keywords: hepatitis E virus, zoonosis, reservoir animals, carnivores, non-human primates, rats

Introduction

Hepatitis E is a worldwide distributed disease, which is caused by the hepatitis E virus (HEV). In addition to humans, domestic pigs, wild boars, rabbits and dromedaries can be subclinically infected as reservoir animals with the zoonotic HEV genotypes 3, 4 and 7. In addition, HEV and HEV-like viruses have been described sporadically in other mammals, as well as in birds and fish, although their distinct role as reservoirs or carriers of the virus is still unclear.

Aims

The aim of the study was therefore to analyse in more detail the importance of different mammalian species, which do not belong to the known HEV reservoirs, for the epidemiology of HEV infections, thus enabling a better assessment of the risk of virus transmission by these animal species.

Material and Methods

Fourteen non-human primate species and 66 other mammal species, as well as Norway rats (*Rattus norvegicus*) and feeder rats (*Rattus norvegicus forma domestica*) from German zoos were selected for the investigations. In total 259 individual non-

human primate sera and 244 individual mammalian sera of clinically healthy zoo animals were analysed for the presence of HEV-specific antibodies (ab) using a species-independent double-antigen sandwich ELISA. The non-human primate sera were additionally examined using a commercial human ELISA. Real-time reverse-transcription (RT)-PCR, nested broad-spectrum RT-PCR and a rat HEV-specific RT-PCR were used to detect the HEV genome in sera of mammals and rat liver samples. A commercial and an in-house method were used for the DNA sequencing.

Results

HEV-specific ab were detected in 3.9% (10/259) of the non-human primate sera (4 species) and 11.5% (28/244) of the mammalian sera (16 species). The highest detection rates were recorded with 33.3% (9/27) in porcines and with 27.0% (10/37) in carnivores. HEV-RNA was detected in a clinically healthy female Syrian brown bear (*Ursus arctos syriacus*) and in 8 of the investigated Norway rats. Sequence analysis identified the virus as rat HEV; the viruses from the bear and the free-ranging rats from the same zoo showed a high nucleotide sequence identity (94.6%–97.8%). Because of the small number of samples due to the small populations within the individual zoos, further statistical evaluations were not carried out.

Conclusions

The results show that non-human primates in zoos may be infected with HEV or HEV-like viruses; however, the low ab detection rates together with the negative genome detection argue against a high risk of virus transmission to humans. The study in other zoo-housed mammalian species was able to significantly increase the number of animal species with indications of HEV infections. In most animal species, only rare evidence and low detection rates were available, which can best be explained by “spillover-infections”. In addition to the expected high detection rate in porcine species, the high percentage of HEV antibody-positive carnivores is remarkable. Their role as possible HEV reservoir animals should therefore be clarified in further investigations. The detection of rat HEV in the serum of the bear and its high nucleotide sequence identity with the HEVs of the pest rodents provides first evidence of transmission of this virus species between rodents and carnivores.

7 Zusammenfassung

Verfasser: Carina Spahr

Titel: Untersuchungen zum Vorkommen von Infektionen mit Hepatitis E-Virus und verwandten Viren in Zootieren

Institut / Klinik: Institut für Virologie, der Veterinärmedizinischen Fakultät, der Universität Leipzig

Eingereicht im Februar 2019

Bibliographische Angaben: 78 Seiten (bis Ende Zusammenfassung), 9 Abbildungen, 4 Tabellen, 188 Literaturangaben, 3 Anhänge

Schlüsselwörter: Hepatitis E-Virus, Zoonose, Reservoirtiere, Zootiere, Karnivoren, Affen, Ratten

Einleitung

Hepatitis E ist eine durch das Hepatitis E-Virus (HEV) verursachte, weltweit verbreitete Erkrankung. Neben dem Menschen können Hausschwein, Wildschwein, Kaninchen und Dromedar als Reservoirtiere subklinisch mit den zoonotischen HEV-Genotypen 3, 4 und 7 infiziert werden. Darüber hinaus wurden HEV und HEV-ähnliche Viren vereinzelt bei weiteren Säugetieren, sowie Vögeln und Fischen beschrieben, wobei deren genaue Rolle als Reservoir oder Überträger des Virus bislang unklar ist.

Ziele

Ziel der Arbeit war es deshalb, die Bedeutung verschiedener Säugetierarten, die nicht zu den bekannten HEV-Reservoiren gehören, für die Epidemiologie der HEV-Infektionen besser zu erfassen und dadurch das Risiko einer Virusübertragung durch diese Tierarten besser abzuschätzen.

Material und Methoden

Vierzehn Affenarten und 66 weitere Säugetierarten, sowie Wanderratten (*Rattus norvegicus*) und Futterratten (*Rattus norvegicus forma domestica*) aus deutschen Zoos wurden für die Untersuchungen ausgewählt. Insgesamt wurden 259 individuelle

Affenseren und 244 individuelle Säugerseren klinisch gesunder Zootiere mittels eines Spezies-unabhängigen Doppel-Antigen-Sandwich-ELISAs auf das Vorhandensein von HEV-spezifischen Antikörpern (AK) untersucht. Die Affenseren wurden zusätzlich mittels eines kommerziellen humanen ELISAs untersucht. Real-time reverse-transcription (RT)-PCR, nested broad-spectrum RT-PCR sowie eine Ratten-HEV-spezifische RT-PCR wurden für den HEV-Genomnachweis in Seren der Säuger und in Ratten-Lebern verwendet. Für die DNA-Sequenzierungen wurden eine kommerzielle und eine In-house-Methode verwendet.

Ergebnisse

In 3,9% (10/259) der Affenseren (4 Arten) und 11,5% (28/244) der Säugerseren (16 Arten) wurden HEV-spezifische AK nachgewiesen. Die höchsten Nachweisraten wurden mit 33,3% (9/27) in Schweineartigen und 27,0% (10/37) in Fleischfressern ermittelt. HEV-RNA wurde in einer klinisch gesunden Syrischen Braunbärin (*Ursus arctos syriacus*), sowie in 8 der untersuchten Wanderratten nachgewiesen. Die Sequenzanalyse identifizierte das Virus als Ratten-HEV; die Viren aus der Bärin und aus den wildlebenden Ratten desselben Zoos zeigten eine hohe Nukleotidsequenz-Identität (94,6%–97,8%). Weitergehende statistische Auswertungen wurden wegen der geringen Probenzahlen aufgrund der kleinen Populationen innerhalb der einzelnen Zoos nicht durchgeführt.

Schlussfolgerungen

Die Ergebnisse zeigen, dass Affen in Zoos mit HEV oder HEV-ähnlichen Viren infiziert sein können, jedoch sprechen die geringen AK-Nachweisraten zusammen mit den negativen Genomnachweisen gegen ein hohes Übertragungsrisiko auf den Menschen. Die Studie an anderen Säugetierarten in Zoos konnte die Zahl der Tierarten mit Hinweisen auf HEV-Infektionen deutlich erhöhen. Bei den meisten Tierarten lagen nur seltene Nachweise und niedrige Detektionsraten vor, die am besten durch „Spillover-Infektionen“ erklärt werden können. Neben der erwarteten hohen Nachweisrate bei Schweineartigen ist der hohe Prozentsatz an HEV AK-positiven Fleischfressern bemerkenswert, weshalb deren Rolle als mögliche HEV-Reservoirtiere in weiteren Untersuchungen geklärt werden sollte. Der Ratten-HEV-Nachweis im Serum der Bärin, sowie dessen hohe Nukleotidsequenz-Identität zu den HEVs der Schadnager geben erstmals Hinweise auf eine Übertragung dieser Virusart zwischen Nagern und Fleischfressern.

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List of animals investigated in the study

Family	Animal Species	Scientific name
Atelidae		
	Black howler monkey	*Alouatta caraya*
	White-fronted spider monkey	*Ateles hybridus*
Bovidae		
	Addax	*Addax nasomaculatus*
	African buffalo	*Syncerus caffer nanus*
	Alpine ibex	*Capra ibex*
	Anoa	*Bubalus depressicornis*
	Barbary sheep	*Ammotragus lervia*
	Bezoar goat	*Capra hircus domestic bezoar*
	Bongo	Tragelaphus eurycercus
	Common waterbuck	*Kobus ellipsiprymnus*
	Congo dwarf goat	*Capra hircus domestic congo dwarf*
	Domestic cattle (Hinterwald cow)	*Bos taurus Taurus hinterwald*
	Domestic cattle (Limpurger cow)	*Bos taurus Taurus limpurger*
	Domestic goat (Damara goat)	*Capra hircus domestic damara*
	Domestic sheep (Cameroon sheep)	*Ovis aries aries cameroon*
	Domestic sheep (Skudde sheep)	*Ovis aries aries skudde*
	Dorcas gazelle	*Gazella dorcas*
	European wisent	*Bison bonasus bonasus*
	Greater kudu	*Tragelaphus strepsiceros*
	Lesser kudu	*Tragelaphus imberis*
	Markhor	*Capra falconeri*

	Mishmi takin	*Budorcas taxicolor taxicolor*
	Rocky Mountain goat	*Oreamnos americanus*
Canidae		
	Bush dog	*Speothos venaticus*
	European grey wolf	*Canis lupus lupus*
	Fennec fox	*Vulpes zerda*
	Maned wolf	*Chrysocyon brachyurus*
	Red fox	*Vulpes vulpes*
Camelidae		
	Alpaca	*Lama pacos domesticus*
	Bactrian camel	*Camelus bactrianus*
	Vicugna	*Vicugna vicugna*
Castordiae		
	Amercian beever	*Castor canadensis*
	Eurasian red squirrel	*Sciurus vulgaris*
Cebidae		
	Black-capped squirrel monkey	*Saimiri boliviensis*
Cercopithecidae		
	Drill	*Mandrillus leucophaeus*
	Gelada baboon	*Theropithecus gelada*
	Javan silvered leaf monkey	*Trachypithecus auratus*
	Japanese macaque	*Macaca fuscata*
	White-crowned mangabey	*Cercocebus atys lunulatus*
Cervidae		
	Mesopotamian fallow deer	*Dama mesopotamica*
Chinchillidae		
	Plains viscacha	*Lagostomus maximus*
Elephantidae		
	African elephant	*Loxodonta africana*
	Indian elephant	*Elephas maximus indicus*
Equidae		
	Dulmen pony	*Equus caballus caballus dulmen*
	Grevy’s zebra	*Equus grevyi*

	Persian onager	*Equus hemionus onager*
	Poitou donkey	*Equus asinus domestic Poitou*
	Przewalski's wild horse	*Equus caballus przewalskii*
	Somali wild ass	*Equus africanus somaliensis*
Felidae		
	Jaguar	*Panthera onca*
	Persian leopard	*Panthera pardus saxicolor*
	Serval	*Leptailurus serval*
	Snow leopard	*Uncia uncia*
	Sumatran tiger	*Panthera tigris sumatrae*
Giraffidae		
	Okapi	*Okapi johnstoni*
	Reticulated giraffe	*Giraffa camelopardalis reticulata*
Herpestidae		
	Banded mongoose	*Mungos mungo*
	Slender-tailed meerkat	*Suricata suricatta*
Hippopotamidae		
	Pigmy hippopotamus	*Choeropsis liberiensis liberiensis*
Hominidae		
	Bonobo	*Pan paniscus*
	Bornean orangutan	*Pongo pygmaeus*
	Chimpanzee	*Pan troglodytes*
	Lar gibbon	*Hylobates lar*
	Sumatran orangutan	*Pongo abelii*
	Western lowland gorilla	*Gorilla gorilla gorilla*
Hyaenidae		
	Spotted hyena	*Crocuta crocuta*
Hylobatidae		
	Lar gibbon	*Hylobates lar*
Macropodidae		
	Red kangaroo	*Macropus rufus*
Muridae		
	Norway rat	*Rattus norvegicus*

	Norway rat	*Rattus norvegicus forma domestica*
Otariidae		
	California sea lion	*Zalophus californianus*
Phocidae		
	Harbor seal	*Phoca vitulina*
Pitheciidae		
	White-faced saki	*Pithecia pithecia*
Pteropus		
	Indian flying fox	*Pteropus giganteus*
Rhinocerotidae		
	One-horned rhinoceros	*Rhinoceros unicornis*
Suidae		
	African bush pig	Potamochoerus porcus pictus
	Babirusa	Babyrousa babyrussa
	Common warthog	Phacochoerus africanus
	Domestic pig (Kunekune pig)	Sus scrofa scrofa kunekune
	Domestic pig (Schwäbisch-Hall)	*Sus scrofa forma domestica*
	European wild boar	*Sus scrofa*
Tapiridae		
	Malayan tapir	*Tapirus indicus*
Tayasuidae		
	Collared peccary	*Pecari tajacu*
Tenrecidae		
	Common tenrec	*Tenrec ecaudatus*
Ursidae		
	Polar bear	*Ursus maritimus*
	South American coati	*Nasua nasua*
	Spectacled bear	*Tremarctos ornatus*
	Syrian brown bear	*Ursus arctos syriacus*

List of publications

Publications part of this thesis

Spahr C, Knauf-Witzens T, Vahlenkamp TW, Ulrich RG, Johne R. Hepatitis E virus and related viruses in wild, domestic and zoo animals: A review. ZPH. **2018** Feb.;65(1):11–29, Epub 2017 Sep. 24, <https://dx.doi.org/10.1111/zph.12405>.

Spahr C, Knauf-Witzens T, Dähnert L, Enders M, Müller M, Johne R, Ulrich RG. Detection of HEV-specific antibodies in four non-human primate species, including great apes, from different zoos in Germany. Epidemiol Inf. **2018** Jan.;146(1):119–124, Epub 2017 Nov. 23, <https://dx.doi.org/10.1017/S0950268817002606>.

Spahr C, Ryll R, Knauf-Witzens T, Vahlenkamp TW, Ulrich RG, Johne R. Serological evidence of hepatitis E virus infection in zoo animals and identification of a rodent-borne strain in a Syrian brown bear. Vet Mic. **2017**;212:87–92, Epub 2017 Nov. 9, <https://dx.doi.org/10.1016/j.vetmic.2017.11.005>.

Additional publications

Heuser E, Fischer S, Ryll R, Mayer-Scholl A, Hoffmann D, Spahr C, Imholt C, Murni Alfa D, Fröhlich A, Lüschow D, Johne R, Ehlers B, Essbauer S, Nöckler K, Ulrich RG. Survey for zoonotic pathogens in Norway rat populations from Europe. PMS. **2017**;73(2):341–348, <https://dx.doi.org/10.1002/ps.4339>.

Ryll R, Bernstein S, Heuser E, Schlegel M, Dremsek P, Zumpe M, Wolf S, Pépin M, Bajomi D, Müller G, Heiberg AC, Spahr C, Lang J, Groschup MH, Ansorge H, Freise J, Guenther S, Baert K, Ruiz-Fons F, Pikula J, Knap N, Tsakmakidis I, Dovas C, Zanet S, Imholt C, Heckel G, Johne R, Ulrich RG. Detection of rat hepatitis E virus in wild Norway rats (*Rattus norvegicus*) and Black rats (*Rattus rattus*) from 11 European countries. Vet Mic. **2017**;208(2017):58–68, <https://dx.doi.org/10.1016/j.vetmic.2017.07.001>.

Miscellaneous publications and abstracts

Spahr C, Ryll R, Knauf-Witzens T, Groschup MH, Ulrich RG, Johne R. Screening of zoo animals for markers of hepatitis E virus infection. National Symposium on Zoonosis Research. 12th – 13th October **2017**, Berlin, Germany. Abstract publication.

Heuser E, Fischer S, Ryll R, Mayer-Scholl A, Hoffmann D, Spahr C, Imholt C, Johne R, Ehlers B, Essbauer S, Nöckler K, Ulrich RG. Network „Rat-borne pathogens“: Searching for pathogen coinfections. German Center for Infection Research (DZIF). 24th – 26th November **2016**, Cologne, Germany. Abstract publication.

Fischer S, Heuser E, Ryll, R, Mayer-Scholl A, Hoffmann D, Spahr C, Essbauer S, Nöckler K, Ulrich RG. Viral and bacterial agents in Norway rat populations from European zoos. Mammalian Biology. 81S (**2016**):6. Abstract publication.

Presentations at scientific meetings

Spahr C, Knauf-Witzens T, Ryll R, Dähnert L, Vahlenkamp TW, Ulrich RG, Johne R. Ratten Hepatitis E-Virus bei einem Syrischen Braunbären (*Ursus arctos syriacus*). 38. Jahrestagung des Verbandes der Zootierärzte (VZT). 10th – 13th May **2018**, Stuttgart, Germany. Poster and abstract publication.

Spahr C, Knauf-Witzens T, Vahlenkamp TW, Ulrich RG, Johne R. Hepatitis E virus infections in zoo animals. 28th Annual Meeting of the Society for Virology (GfV). 14th – 17th March **2018**, Würzburg, Germany. Poster.

Spahr C, Knauf-Witzens T, Dähnert L, Enders M, Müller M, Johne R, Ulrich RG. Hepatitis E virus in zoo-housed primates. 91st Annual Meeting of the German Society of Mammalogy (DGS). 18th – 21st September **2017**, Greifswald, Germany. Oral presentation and abstract publication.

Spahr C, Knauf-Witzens T, Ulrich RG, Johne R. Hepatitis E-Viren – Gibt es das auch bei Zootieren? Stuttgarter Tierärztliche Gesellschaft (STG). 8th and 9th March **2017**, Fellbach und Aulendorf, Germany. Oral presentation.

Spahr C, Johne R, Knauf-Witzens T, Ulrich RG, Johne R. Hepatitis E-Viren – ein Update. 36. Arbeitstagung der Zootierärzte im deutschsprachigen Raum. 03rd – 06th November **2016**, Krefeld, Germany. Oral presentation and abstract publication.

Spahr C, Johne R, Knauf-Witzens T, Ulrich RG. Hepatitis E-virus and HEV-related viruses – do they also occur in zoo animals? Joint AAZV/EAZWV/Leibniz-IZW Conference. 16th – 22nd July **2016**, Atlanta, Georgia, USA. Poster and abstract publication.

Spahr C, Johne R, Knauf-Witzens T, Johne R, Ulrich RG. Hepatitis E-Viren – Gibt es das auch bei Zootieren? 35. Arbeitstagung der Zootierärzte im deutschsprachigen Raum. 29th October – 1st November **2015**, Rapperswil, Switzerland. Oral presentation and abstract publication.

Knauf-Witzens T, Spahr C, Weigold A. Erfahrungen mit zwei geriatrischen Elefanten. 35. Arbeitstagung der Zootierärzte im deutschsprachigen Raum. 29th October – 1st November **2015**, Rapperswil, Switzerland. Abstract publication.

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