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Original research article

A large-scale serological survey of Akabane virus infection in cattle, yak, sheep and goats in China

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Abstract

Akabane virus (AKAV) is a member of the Simbu serogroup, classified in the genus *Orthobunyavirus*, family *Bunyaviridae*. AKAV infection can cause abortion, stillbirth, and congenital arthrogryposis and hydranencephaly in cattle and sheep. The distribution and prevalence of AKAV infection in China is still unknown. A total of 2731 sera collected from 2006 to 2014 in 24 provinces of China from cattle, sheep, goats and yak were examined by serum neutralization test. The overall seroprevalence rates for AKAV antibodies were 21.3% in cattle (471/2215) and 12.0% (17/142) in sheep or goats, and 0% in yak (0/374). The results indicated widespread AKAV infection in China among cattle and sheep but yak appear to have a low risk of infection. Using a selection of 50 AKAV-positive and 25 AKAV-negative cattle sera, neutralisation tests were also conducted to detect antibodies to several other Simbu serogroup bunyaviruses and closely related Leanyer virus. Although inconclusive, the data suggest that both Aino virus and Peaton virus, which have been reported previously in Japan and Korea, may also be present in cattle in China.

1. Introduction

Akabane virus (AKAV) is a segmented, negative-sense, single-stranded RNA virus. It is classified taxonomically in the genus *Orthobunyavirus*, family *Bunyaviridae* (Plyusnin et al., 2012) and, like Schmallenberg virus (SBV) which emerged in 2011, it is a member of the Simbu serogroup of orthobunyaviruses (Hoffmann et al., 2012; Kinney and Calisher, 1981). AKAV has been isolated on several occasions from mosquitoes but biting midges (*Culicoides* spp.) appear to be the principal vectors (Jennings and Mellor, 1989). AKAV infects a wide range of wild ruminants and livestock including cattle, sheep, goats, buffalo, deer, horses and pigs (Kirkland, 2002; Huang et al., 2003). However, Akabane disease occurs primarily in cattle, and more rarely sheep and goats, manifesting as abortions, stillbirths and congenital abnormalities in newborns. Clinical signs include arthrogryposis and hydranencephaly (A-H syndrome), with the highest incidence and severity of disease when infection occurs during the mid-term of gestation. Post-natal infection of calves with some strains of the virus can also cause encephalomyelitis (Oem et al., 2012a; Oem et al., 2012b). There has been no report of AKAV infection in humans (Kirkland, 2002).

AKAV is known to be widely distributed across tropical and subtropical areas of East Asia as well as Australia, the Middle-East and Africa (Cybinski et al., 1978; Taylor and Mellor, 1994). The virus was first isolated from mosquitoes (*Aedes vexans* and *Culex tritaeniorhynchus*) collected in 1959 in Gumma Prefecture, Japan. Although the collection occurred during an outbreak of disease resulting in congenital malformation in cattle, an etiological link between the virus and this disease was not proposed until much later (Kurogi et al., 1975). A similar serious outbreak in Japan from 1972-1973 resulted in more than 31,000 cases of abortion, stillbirth and congenital A-H syndrome; the outbreak continued through 1974-1975 (Kurogi et al., 1975). AKAV was subsequently isolated from biting

midges (*Culicoides brevitarsis*) in Australia in 1968 and an association between neutralising antibodies to AKAV and A-H syndrome in New South Wales was reported (Doherty et al., 1972; Hartley et al., 1975). AKAV isolations from cattle or biting midges have since been reported from Japan (Kurogi et al., 1987), Australia (St George et al., 1978), Chinese Taipei (Liao et al., 1996) and South Korea (Bak et al., 1980).). Molecular detection of AKAV RNA has also been detected from biting midges and affected animals in Israel (Stram et al., 2004) and Turkey (Oğuzoğlu et al., 2015).

AKAV is also known to occur in China but the distribution, prevalence of infection and impacts on the cattle industry are poorly understood. The periodic and seasonal occurrence of AKAV infections in Japan, Taiwan and Korea suggests that China may play an important role epidemiologically in East Asia but virus distribution and epidemiology have been rarely reported. AKAV was first isolated in China in 1998 from mosquitos collected during a disease outbreak in Shanghai (Qiping and Longtao, 2000). This followed local reports in previous years of Akabane disease outbreaks in Shanghai, Guangdong, Henan, Shandong and Yunnan. A serological survey conducted in cattle and sheep in Xinjiang Province in north-west China in 2010 indicated a seroprevalence of 19 % (Jun et al., 2012). A second virus isolation from mosquitoes in Yunnan Province has also been reported (Feng et al., 2015). However, no detailed data about the epidemiology of AKAV in China have been available for these past 20 years.

In this study, we have surveyed for AKAV neutralising antibodies in sera collected from cattle, yak, sheep and goats across 24 provinces of China during the period August 2006 to September 2015. We also determined the specificity of virus neutralisation among eight Simbu serogroup or closely related orthobunyaviruses previously reported in the Eastern

Hemisphere and screened a selection of Chinese cattle sera for evidence of neutralising antibodies to each of the viruses.

2. Materials and methods

2.1 Collection and analysis of sera and antisera

The study was conducted in 24 provinces of China (**Table 1**). Serum samples were collected from cattle (2215), yak (374), sheep (129) and goats (13) from 2006 and 2014 (June to October). Sera were obtained randomly from adult animals that had no record of AKAV vaccination. All serum samples were collected from animals over 6 months of age. Blood was collected from the jugular vein, placed at 4°C overnight and the serum fraction was then stored at -20°C for further analysis. All sera were gamma-irradiated in order to comply with Australian import requirements and also complement-inactivated at 56°C for 30 min prior to testing.

Tinaroo virus (TINV; strain CSIRO153) mouse immune ascitic fluid (MIAF) was prepared as described previously (Sartorelli et al., 1966). Rabbit antisera to AKAV (strain CSIRO16), Aino virus (AINOV; strain B7974), Peaton virus (PEAV; strain CSIRO110), Thimiri virus (THIV; strain CSIRO1), Douglas virus (DOUV; strain CSIRO150) and Leanyer virus (strain CSIRO2), and mouse antisera to Facey's Paddock virus (FPV; strain Ch16129) were raised using purified viruses as described previously (Lunt et al., 1988). Negative control sera were obtained from Australian cattle located outside the known distribution range of Simbu serogroup viruses. All sera were complement-inactivated at 56°C for 30 min.

2.2 Viruses and cells

Seven viruses assigned to the Simbu serogroup, including THIV (CSIRO1), TINV (strain CSIRO153), AINOV (CSIRO990), DOUV (CSIRO1059), PEAV (CSIRO1210), and LEAV

(CSIRO2) which has been shown to be closely related based on phylogenetic analysis (Huang et al., 2016), were recovered from storage at -80°C at the CSIRO Australian Animal Health Laboratory, Geelong, Victoria. Growth of the viruses in Vero cells has been described previously (Blacksell et al., 1994). AKAV (strain CSIRO1711), collected at Peachester, Queensland, on 6 December 1984, was used for serum neutralisation tests. Vero cells were maintained at 37°C in minimum essential medium (MEM) supplemented with 10% foetal calf serum, 10 nM HEPES, 500 μg/ml fungizone and 6.7 nM $NaHCO_3$.

2.3 Neutralization tests

The serum neutralization test (SNT) was conducted as described previously (Cybinski et al., 1978) according to an accredited method (National Association of Testing Authorities) used routinely for diagnostic testing at the CSIRO Australian Animal Health Laboratory (detailed method available on request). Serum samples were tested in duplicate at 1/4 and 1/8 dilutions in growth medium in a total volume of 50 μl/well of a 96-well plate (screening test). Fifty microlitres of medium containing 100 $TCID_{50}$ AKAV was added to each well and the plate was incubated for 1 h at 37°C. A volume of 100 μl of medium containing 0.15×10^6 Vero cells was then added to each well and incubated for 5 days at 37°C with 5% CO_2. Cells were examined for cytopathic effect (CPE) at 3-5 days. The controls were as follows: AKAV-positive serum diluted from 1/4 to 1/256 (positive control); AKAV-negative serum diluted as for the positive sera (negative control); back titration of virus working stock (virus stock control); untreated cells (cell control); tested serum with cells only (toxicity control). Each control had two replicates. The SNT was repeated when one or more of the following occurred: the titre of the positive control was outside of the required range (1/32 to 1/64); no CPE was observed in the negative control at a titre of 1/4; CPE was observed in the cell control; substantial toxicity was observed; the back titration indicated the virus aliquot was

not approximately 100 $TCID_{50}$. As per the standard accredited procedure, test sera displaying CPE in each replicate at 1/4 dilution were considered to be AKAV-negative; sera showing no evidence of CPE 1/4 dilution were regarded as AKAV-positive; when only one replicate displayed CPE, the results were considered to be inconclusive.

To evaluate the cross-reactivity of antisera to other viruses (endpoint titration) and to detect the presence of antibodies in cattle sera, neutralisation tests were conducted as described above but utilised 100 $TCID_{50}$ of THIV, TINV, AINOV, DOUV, PEAV, FPV and LEAV at serum dilutions of 1/4 to 1/256.

2.4 Statistical analysis

The statistical significance of differences in AKAV seroprevalence between groups were determined by using the χ^2 test (http://www.socscistatistics.com/tests/chisquare).

3. Results

3.1 Prevalence of AKAV neutralising antibodies in cattle, yak, sheep and goats from China

A total of 2731 serum samples obtained from cattle, yak, sheep and goats in China from 2006 to 2014 were tested for the presence of neutralising antibodies to AKAV. There was evidence of AKAV antibodies in cattle in all 24 provinces tested, except for Heilongjiang Province in the far north-east. AKAV seroprevalence varied in different provinces from 4.2% in Shaanxi (collected July 2013) and 8.1% in Qinghai (collected August 2012) to 56.6% in Guangdong Province (collected July 2011) and 50.0% in Hainan Province (collected July 2013). Overall AKAV seroprevalence in cattle was 21.3% (471/2215 tested) and the prevalence exceeded 10% in almost all provinces sampled during this period (**Table 1, Figure 2**). Yak were sampled in Qinghai Province (collected August 2011) and Tibet (collected September 2011);

there was no evidence of AKAV neutralising antibodies in yak (0/374 tested). AKAV neutralising antibodies were detected in sheep and goat sera from all 7 provinces sampled from 2012 to 2015. Overall, the seroprevalence in sheep and goats was 12.0% (17/142 tested), ranging from 6.2% in Guizhou Province (collected October 2013) to 25.0% in Xinjiang Province (collected August 2014). Cattle and sheep were both sampled in only one province (Gansu Province) during the same month and year (July 2012); in this case, AKAV seroprevalence was higher in cattle (23.3%) than in sheep (7.7%) but the difference was not statistically significant ($p < 0.1$).

3.2 Neutralisation cross-reactivity of eight viruses

Neutralization tests were conducted using immune ascitic fluid or sera raised in mice or rabbits to determine the serological cross-reactivity between AKAV, six other viruses assigned to the Simbu serogroup and the closely related orthobunyavirus, LEAV. The results of testing indicated moderate homologous neutralisation (titres ranging from 1/16 to 1/64), but no cross-neutralisation between the viruses.

3.3 Survey of cattle sera from China for neutralising antibodies to Simbu serogroup viruses

A random selection of 50 AKAV-positive cattle sera (titres ranging from 1/8 to 1/64) and 25 AKAV-negative Chinese cattle sera of were tested for the presence of neutralising antibodies to THIV, TINV, AINOV, DOUV, PEAV, FPV and LEAV. The sera represented seven provinces (Guangxi, Guizhou, Guangdong, Inner Mongolia, Hainan, Henan, Hubei, Gansu) from the south-eastern tropical region to the northern steppe (**Table 4**). The 25 AKAV-negative sera failed to neutralise any of the other Simbu serogroup viruses. However, of the 50 AKAV-positive sera, ten also neutralised AINOV and eight also neutralised PEAV, with

titres ranging from 1/4 to 1/16. In all but one of these 18 dual-reactive sera, the higher neutralisation titre was against AKAV and in 12 of these sera, the difference in titres was diagnostically significant (>4-fold). One serum reacted to AINOV with a higher neutralisation titre than to AKAV but the difference in titres was not diagnostically significant.

4. Discussion

To our knowledge, this is the first large-scale serological survey for the presence of AKAV antibody in cattle, yak, sheep and goats across China. Sampling was conducted in 24 of the 28 provinces, providing broad coverage of the rural areas of the country. The data indicate that AKAV infection occurs commonly in cattle across most of China, with the possible exception of Heilongjiang Province in the far north-east. Cattle from two locations in Heilongjiang were sampled in the summer of 2012 and showed no evidence of AKAV infection (0/93 samples). Sampling in seven other provinces in that summer indicated high AKAV seroprevalence in central China. We note, however, that there was relatively high AKAV seroprevalence in bordering Jilin Province in 2013, and in Liaoning Province and Inner Mongolia in 2009. As the sampling sites in Jilin and Heilongjiang are separated by less than 250 km of farmland, we cannot exclude the possibility that AKAV infection does occur occasionally in cattle in Heilongjiang but was not detected at the time of our sampling. Jilin Province is regarded as the northern limit of infection in China for bovine ephemeral fever virus (BEFV), another vector-borne pathogen of cattle that has been isolated from both mosquitoes and biting midges (Bai, 1993). Climatic conditions such as the relatively short summer and autumn and very low winter temperatures may limit the distribution of insect vectors in the north-east.

In Xinjiang Province in the far north-west of China, AKAV seroprevalence detected in cattle in August 2013 (23.4%) was similar to the average seroprevalence previously reported for a survey conducted in the same province from April to October 2010 (20.32%) (Jun et al., 2012). Indeed, in that previous study AKAV seroprevalence was observed to peak in July and August when conditions are most suitable to sustain large populations of mosquitoes and biting midges. We also observe that the highest AKAV seroprevalence in cattle occurred in the south-east of China, in Guangdong Province in 2011 (56.6%) and Hainan Province in 2013 (50.0%). The tropical to sub-tropical monsoonal climate in these regions may sustain larger vector populations with the likelihood of year-round transmission. Typically, AKAV endemic regions report lower incidence of A-H syndrome as ruminants become infected at a young age and remain immune to subsequent infection during pregnancy. Despite the higher AKAV seroprevalence, congenital disease risks may therefore be somewhat lower in these provinces. However, neonatal exposure of cattle to infection may well be higher, increasing risks of encephalomyelitis as has been associated with some AKAV strains from East Asia (Oem et al., 2012a).

AKAV seroprevalence in sheep and goats was examined in seven provinces during the four years from the summer of 2012 to summer 2015. The sample sites included a wide span of the country excluding the southeast where sheep and goat numbers are small. The overall seroprevalence in sheep sampled during this period (17/142 or 11.9%) was lower that the seroprevalence in cattle (265/1335 or 19.9%). However, the sheep and cattle samples were collected concurrently in only one province (Gansu), so it was not possible to assess the significance of the difference in overall seroprevalence. Jun et al. (2012) also detected lower AKAV seroprevalence in sheep than in cattle samples in Xinjiang Province but the difference in prevalence was not considered statistically significant.

We found no evidence of AKAV infection in yak (0/374) sampled in August and September 2011 at two sites on the Qinghai-Tibetan Plateau, which is the primary location of yak herds in China. This contrasts with a recent study of BEFV in yak from the same region of China from 2012 to 2015 in which overall seroprevalence was estimated to be 40.4% (Liu et al., 2016). Bluetongue virus (BTV) antibodies have also been reported in yak from Tibet in 2012 but with a much lower seroprevalence of 4.89% (Li et al., 2015). In many regions of the world, BTV and AKAV are transmitted by the same species of biting midges that associate with ruminants (Mellor et al., 2000). As AKAV seroprevalence in cattle from Qinghai Province tested in August 2012 was moderately high (8.1%), the apparent absence of AKAV infection in yak suggests either that yak may not be susceptible to AKAV infection, that they are not preferred hosts for the midge vectors or that the harsh climatic conditions in this region may impose an unfavourable habitat for competent vector species. However, we cannot exclude the possibility that AKAV transmission was not active at the sampling sites prior to the collections in summer of 2011. To our knowledge, there have been no previous reports of AKAV infection in yak.

The results of neutralisation tests that we conducted to evaluate serological cross-reactions amongst several Simbu serogroup bunyaviruses were in agreement with similar tests reported previously for smaller sets of viruses. When comparing the cross-reactivity of rabbit antisera to Australian isolates of AKAV, AINOV, PEAV, DOUV and TINV, Cybinski (1984) reported very weak cross-neutralisation only between AKAV and TINV. Similarly, Matsumori et al. (2002) reported no cross-neutralisation between Japanese isolates of AKAV, AINOV and PEAV. Kinney and Calisher (1981) also reported that AKAV antiserum cross-neutralised TINV but homologous titre to AKAV was not determined. Although no cross-neutralization was observed among the eight viruses (AKAV, THIV, TINV, AINOV, DOUV,

PEAV, FPV, LEAV) in our testing panel (**Table 3**), the homologous titres of our rabbit and mouse antisera were also lower than those observed in previous reports.

The detection of neutralising antibodies to AKAV, AINOV and PEAV in our samples is perhaps unsurprising as these virus have been reported previously in Asia (Kato et al., 2016; Kim et al., 2015; Lim et al., 2007; Matsumori et al., 2002). The absence of neutralising antibodies to THIV in our samples is not completely unexpected as this virus has previously only been reported to be associated with birds and macropods (Standfast et al., 1982). It is interesting, however, that neutralising antibodies to AINOV and PEAV were detected in 18 of the 50 cattle sera that were AKAV-seropositive and in none of the 25 cattle sera that were AKAV-seronegative (**Table 4**). It is also interesting that individual AKAV-seropositive sera neutralised either AINOV or PEAV, but not both viruses. Overall, the data suggest that AINOV and PEAV may be present in China. However, as neutralising antibodies to AINOV and PEAV were detected only in AKAV-seropositive cattle, we cannot exclude the possibility of serological cross-reactions or non-specific reactions. In addition, as the virus used to test the sera originated from Australia, variations between Asian and Australian strains may be responsible for the low titres observed. A similar survey for neutralising antibodies to 10 Simbu serogroup bunyaviruses (AKAV, AINOV, DOUV, PEAV, TINV, SBV, Sathuperi virus, Shamonda virus, Sabo virus and Simbu virus) in cattle sera from Tanzania indicated multiple reactivity for all but one of 45 sera and evidence of infection in cattle with all except Simbu virus (Mathew et al., 2015). A more limited set of Simbu serogroup viruses appears to be present in China.

In conclusion, China is a large country with great differences in land use and vegetation in different provinces and regions. There are also extreme variations in climatic conditions from southern to the northern latitudes and between eastern and western regions, ranging from

tropical and subtropical to temperate and semi-arid subarctic zones. Despite the likelihood that these variations could significantly influence vector-borne disease transmission, we have detected evidence that AKAV infections occur commonly in most regions of the country. We have also detected evidence that AINOV and PEAV may be present in China but their clinical significance is presently unclear. The Simbu serogroup also includes Schmallenberg virus, which causes an Akabane-like disease syndrome in sheep and cattle in Europe (Hoffmann et al., 2012), and more than 20 other viruses that have been isolated in Africa, southern Asia or the Americas (Kinney and Calisher, 1981; Saeed et al., 2001). The relatively recent detection in Japan of Sathuperi virus (Yanase et al., 2004), which was previously only known to be present in Africa and India, suggests that the distribution of Simbu serogroup viruses may be expanding under influences of factors such as climate change and increased travel and trade. It has also been established that genome segment reassortment between Simbu serogroup bunyaviruses occurs commonly and this may lead to new variants (Akashi et al., 1997; Briese et al., 2013). As livestock production represents an important sector of the economy in China, further investigations of the epidemiology and socio-economic impacts of AKAV and other Simbu serogroup viruses should be undertaken.

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6. References

Akashi, H., Kaku, Y., Kong, X., Pang, H., 1997. Antigenic and genetic comparisons of Japanese and Australian Simbu serogroup viruses: evidence for the recovery of natural virus reassortants. Virus Res. 50, 205-213.

Bai, W.B., 1993. Epidemiology and control of bovine ephemeral fever in China. In: St. George, T.D., Uren, M.F., Young, P.L., Hoffmann, D. (Eds.), Bovine Ephemeral Fever and Related Rhabdoviruses, ACIAR, Canberra, pp. 20-22.

Bak, U.-B., Lim, C., Cheong, C., Hwang, W., Cho, M., 1980. Outbreaks of Akabane disease of cattle in Korea. Korean J. Vet. Res. 20, 65-78.

Blacksell, S.D., Lunt, R.A., White, J.R., 1994. A rapid indirect ELISA for the serogrouping of Australian orbiviruses. J. Virol. Meth. 49, 67-78.

Briese, T., Calisher, C.H., Higgs, S., 2013. Viruses of the family *Bunyaviridae*: are all available isolates reassortants? Virology 446, 207-216.

Cybinski, D.H., 1984. Douglas and Tinaroo viruses: two Simbu group arboviruses infecting *Culicoides brevitarsis* and livestock in Australia. Aust. J. Biol. Sci. 37, 91-97.

Cybinski, D.H., St. George, T.D., Paull, N.I., 1978. Antibodies to Akabane virus in Australia. Aust. Vet. J. 54, 1-3.

Doherty, R.L., Carley, J.G., Standfast, H.A., Dyce, A.L., Snowdon, W.A., 1972. Virus strains isolated from arthropods during an epizootic of bovine ephemeral fever in Queensland. Aust. Vet. J. 48, 81-86.

Feng, Y., He, B., Fu, S., Yang, W., Zhang, Y., Tu, C., Liang, G., Zhang, H., 2015. Isolation and identification of the Akabane virus from mosquitoes in Yunnan Province, China. Chin. J. Virol. 31, 51-57 (in Chinese).

Hartley, W.J., Wanner, R.A., Della-Porta, A.J., Snowdon, W.A., 1975. Serological evidence for the association of Akabane virus with epizootic bovine congenital arthrogryposis and hydranencephaly syndromes in New South Wales. Aust. Vet. J. 51, 103-104.

Hoffmann, B., Scheuch, M., Hoper, D., Jungblut, R., Holsteg, M., Schirrmeier, H., Eschbaumer, M., Goller, K.V., Wernike, K., Fischer, M., Breithaupt, A., Mettenleiter,

T.C., Beer, M., 2012. Novel orthobunyavirus in cattle, Europe, 2011. Emerg. Infect. Dis. 18, 469-472.

Huang. B.X., Allock. R., Warrilow. D., 2016. Newly characterized arboviruses of northern Australia. Virol. Rep. 6, 11-17.

Huang, C.C.,Huang, T.S., Deng, M.C., Jong, M.H., Lin, S.Y., 2003. Natural infections of pigs with Akabane virus. Vet. Microbiol. 94, 1-11.

Jennings, M., Mellor, P.S., 1989. *Culicoides*: biological vectors of Akabane virus. Vet. Microbiol. 21, 125-131.

Jun, Q., Qingling, M., Zaichao, Z., Kuojun, C., Jingsheng, Z., Minxing, M., Chuangfu, C., 2012. A serological survey of Akabane virus infection in cattle and sheep in northwest China. Trop. Anim. Hlth. Prod. 44, 1817-1820.

Kato, T., Yanase, T., Suzuki, M., Katagiri, Y., Ikemiyagi, K., Takayoshi, K., Shirafuji, H., Ohashi, S., Yoshida, K., Yamakawa, M., Tsuda, T., 2016. Monitoring for bovine arboviruses in the most southwestern islands in Japan between 1994 and 2014. BMC Vet. Res. 12, e125.

Kim, Y.H., Oem, J.K., Lee, E.Y., Lee, K.K., Kim, S.H., Lee, M.H., Park, S.C., 2015. Seroprevalence of five arboviruses in sentinel cattle as part of nationwide surveillance in South Korea, 2009-2012. J. Vet. Med. Sci. 77, 247-250.

Kinney, R.M., Calisher, C.H., 1981. Antigenic relationships among Simbu serogroup (*Bunyaviridae*) viruses. Amer. J. Trop. Med. Hyg. 30, 1307-1318.

Kirkland, P.D., 2002. Akabane and bovine ephemeral fever virus infections. Vet. Clin. Nth. Amer.: Food Anim. Pract. 18, 501-514.

Kurogi, H., Akiba, K., Inaba, Y., Matumoto, M., 1987. Isolation of Akabane virus from the biting midge *Culicoides oxystoma* in Japan. Vet. Microbiol. 15, 243-248.

Kurogi, H., Inaba, Y., Goto, Y., Miura, Y., Takahashi, H., Sato, K., Omori, T., Matumoto, M., 1975. Serologic evidence for etiologic role of Akabane virus in epizootic abortion-arthrogryposis-hydranencephaly in cattle in Japan, 1972–1974. Arch. Virol. 47.

Li, J., Li, K., Shahzad, M., Han, Z., Nabi, F., Gao, J., Han, J., 2015. Seroprevalence of bluetongue virus in domestic yaks (*Bos grunniens*) in Tibetan regions of China based on circulating antibodies. Trop. Anim. Hlth. Prod. 47, 1221-1223.

Liao, Y., Lu, Y., Goto, Y., Inaba, Y., 1996. The isolation of Akabane virus (Iriki strain) from calves in Taiwan. J. Basic Microbiol. 36, 33-39.

Lim, S.I., Kweon, C.H., Tark, D.S., Kim, S.H., Yang, D.K., 2007. Sero-survey on Aino, Akabane, Chuzan, bovine ephemeral fever and Japanese encephalitis virus of cattle and swine in Korea. J. Vet. Sci. 8, 45-49.

Liu, D., Li, K., Zhang, L., Lan, Y., Wang, X., Zhang, H., Wang, L., Gui, R., Han, Z., Jang, W., Sizhu, S., Li, J., 2016. Seroprevalence investigation of bovine ephemeral fever in yaks in Tibetan Plateau of China from 2012 to 2015. Trop. Anim. Hlth. Prod. 49, 227-230.

Lunt, R., White, J., Della-Porta, A., 1988. Studies with enzyme-linked immunosorbent assays for the serodiagnosis of bluetongue and epizootic haemorrhagic disease of deer. Vet. Microbiol. 16, 323-338.

Mathew, C., Klevar, S., Elbers, A.R., van der Poel, W.H., Kirkland, P.D., Godfroid, J., Mdegela, R.H., Mwamengele, G., Stokstad, M., 2015. Detection of serum neutralizing antibodies to Simbu sero-group viruses in cattle in Tanzania. BMC Vet. Res. 11, e208.

Matsumori, Y., Inai, K., Yanase, T., Ohashi, S., Kato, T., Yoshida, K., Tsuda, T., 2002. Serological and genetic characterization of newly isolated Peaton virus in Japan. Arch. Virol. 147, 401-410.

354 Mellor, P.S., Boorman, J., Baylis, M., 2000. Culicoides biting midges: their role as arbovirus
355 vectors. Ann. Rev. Entomol. 45, 307-340.
356 Oem, J.K., Lee, K.H., Kim, H.R., Bae, Y.C., Chung, J.Y., Lee, O.S., Roh, I.S., 2012a. Bovine
357 epizootic encephalomyelitis caused by Akabane virus infection in Korea. J. Comp.
358 Pathol. 147, 101-105.
359 Oem, J.K., Yoon, H.J., Kim, H.R., Roh, I.S., Lee, K.H., Lee, O.S., Bae, Y.C., 2012b. Genetic
360 and pathogenic characterization of Akabane viruses isolated from cattle with
361 encephalomyelitis in Korea. Vet. Microbiol. 158, 259-266.
362 Oğuzoğlu, T., Toplu, N., Koç, B., Doğan, F., Epikmen, E., İpek, E., Akkoç, A., 2015. First
363 molecular detection and characterization of Akabane virus in small ruminants in
364 Turkey. Arch. Virol. 160, 2623-2627.
365 Oya, A., Okuno, T., Ogata, T., Kobayashii, Matsuyama, T., 1961. Akabane, a new arbor virus
366 isolated in Japan. Jap. J. Med. Sci. Biol. 14, 101-108.
367 Plyusnin, A., Beaty, B.J., Elliott, R.M., Goldbach, R., Kormelink, R., Lundkvist, A.,
368 Schmaljohn, C.S., Tesh, R.B. 2012. Family *Bunyaviridae*. In: King, A.M.Q., Adams,
369 M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy: Ninth Report of the
370 International Committee on Taxonomy of Viruses, Elsevier Academic Press,
371 Amsterdam, pp. 725-741.
372 Qiping, L., Longtao, Y., 2000. Isolation of Akabane virus in China. Chin.Anim. Quarant.17,
373 27-29 (in Chinese).
374 Saeed, M.F., Li, L., Wang, H., Weaver, S.C., Barrett, A.D., 2001. Phylogeny of the Simbu
375 serogroup of the genus *Bunyavirus*. J. Gen. Virol. 82, 2173-2181.
376 Sartorelli, A.C., Fischer, D.S., Downs, W.G., 1966. Use of sarcoma 180/TG to prepare
377 hyperimmune ascitic fluid in the mouse. J. Immunol. 96, 676-682.

St George, T.D., Standfast, H.A., Cybinski, D.H., 1978. Isolations of akabane virus from sentinel cattle and *Culicoides brevitarsis*. Australian Veterinary Journal 54, 558-561.

Stram, Y., Brenner, J., Braverman, Y., Banet-Noach, C., Kuznetzova, L., Ginni, M., 2004. Akabane virus in Israel: a new virus lineage. Virus Res. 104, 93-97.

Standfast , H.A., A. L. Dyce. 1982. Isolation of Thimiri virus from *Culicoides histrio* (Diptera: Ceratopogonidae) collected in northern Australia. J. Med. Entomol. 19, 212.

Taylor, W., Mellor, P., 1994. The distribution of Akabane virus in the Middle East. Epidemiol. Infect. 113, 175-185.

Yanase, T., Fukutomi, T., Yoshida, K., Kato, T., Ohashi, S., Yamakawa, M., Tsuda, T., 2004. The emergence in Japan of Sathuperi virus, a tropical Simbu serogroup virus of the genus *Orthobunyavirus*. Arch. Virol. 149, 1007-1013.

Figure legends

Figure 1. Map of China showing sites of sample collection (cattle, sheep, goats and yak) and the prevalence of AKAV-neutralising antibodies in cattle in each province. Provinces and municipalities shaded in grey were not sampled.

Highlights

- Cattle, sheep, goat and yak sera collected from across China were tested for neutralising antibody to Akabane virus (AKAV).
- AKAV neutralising antibody was detected in 23 of 24 provinces sampled (2006-2014).
- Overall seroprevalence rates were 21.3% in cattle (471/2215) and 12.0% in sheep/ goats (17/142); there was no evidence of AKAV infection in yak (0/374).
- Neutralising antibodies to Aino virus and Peaton virus (but not five other Simbu group bunayviruses or closely related Leanyer virus) were also detected but only in a selection of AKAV-positive sera.

Table 1. Prevalence of AKAV neutralising antibodies in bovine sera collected in China, 2006-14.

Province	Region	Date collected	Species	Total samples	Number positive	Prevalence (%)
Heilongjiang	Harbin	July 2012	cattle	45	0	0
	Qiqihaer	July 2012	cattle	48	0	0
Jilin	Changchun	October 2013	cattle	72	15	20.8
Liaoning	Dalian	August 2009	cattle	53	8	15.1
	Panjin	August 2009	cattle	67	14	20.9
Shandong	Jinan	September 2014	cattle	92	34	37.0
	Yuncheng	June 2014	cattle	19	9	47.4

Zhejiang	Jinhua	August 2012	cattle	62	8	12.9
Hebei	Xingtai	July 2012	cattle	47	12	25.5
	Shijiazhuang	June 2012	cattle	31	8	25.8
	Hengshui	July 2012	cattle	36	9	25.0
Shanxi	Taiyuan	July 2012	cattle	104	30	28.8
Shaanxi	Yanan	July 2013	cattle	71	3	4.2
Henan	Luoyang	July 2012	cattle	39	5	12.8
	Zhumadian	July 2009	cattle	25	2	8.0
	Kaifeng	August 2006	cattle	23	5	21.7
Hubei	Suizhou	September 2012	cattle	141	38	27.0
Hunan	Hengyang	June 2011	cattle	29	9	31.0
	Zhangjiajie	August 2008	cattle	36	8	22.2
Jiangxi	Gaoan	June 2013	cattle	31	6	19.3
Guangdong	Guangzhou	July 2011	cattle	83	47	56.6
Guangxi	Nanning	September 2013	cattle	42	4	9.5
	Yulin	October 2010	cattle	119	30	25.2
	Liuzhou	July 2008	cattle	141	13	9.2
Hainan	Haikou	July 2013	cattle	34	17	50.0
Xinjiang	Hetian	August 2013	cattle	37	11	29.7
	Kashgar	August 2013	cattle	70	14	20.0
Inner Mongolia	Chifeng	June 2010	cattle	82	15	18.3
	Baotou	August 2006	cattle	65	8	12.3
Gansu	Longxi	September 2013	cattle	45	7	15.6
	Zhangye	July 2011	cattle	30	9	30.0
	Tianshui	July 2012	cattle	30	7	23.3
Qinghai	Xining	August 2011	yak	152	0	0
	Haibei	August 2012	cattle	74	6	8.1
Ningxia	Zhongwei	June 2013	cattle	67	8	11.9
Tibet	Nagqu	September 2011	yak	222	0	0
Sichuan	Deyang	August 2010	cattle	34	11	32.2
Guizhou	Guiyang	August 2010	cattle	93	27	29.0
Yunnan	Kunming	September 2013	cattle	98	14	14.2

Table 2. Prevalence of AKAV neutralising antibodies in sheep and goat sera collected in China, 2012-15.

Province	Region	Date collected	Species	Total samples	Number positive	Prevalence (%)
Jilin	Kunan	August 2012	sheep	13	2	15.0
Henan	Luoyang	August 2013	sheep	25	2	8.0
Xinjiang	Yili	August 2014	sheep	24	6	25.0
Gansu	Gannan	July 2012	goat	13	1	7.7
Ningxia	Jingyuan	July 2015	sheep	18	2	11.1

Guizhou	Yulin	October 2013	sheep	32	2	6.2
Yunnan	Kunming	June 2013	sheep	17	2	11.8

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421 **Table 3. Virus neutralization test to determine serological cross-reactivity of Simbu serogroup**
422 **viruses.**
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Virus	Virus strain	Antiserum							
		Akabane	Thimiri	Tinaroo	Aino	Douglas	Peaton	Facey's Paddock	Leanyer
AKAV	CSIRO1711	1/16	-	-	-	-	-	-	-
THIV	CSIRO1	-	1/64	-	-	-	-	-	-
TINV	CSIRO153	-	-	1/32	-	-	-	-	-
AINOV	CSIRO990	-	-	-	1/64	-	-	-	-
DOUV	CSIRO1059	-	-	-	-	1/16	-	-	-
PEAV	CSIRO1210	-	-	-	-	-	1/32	-	-
FPV	CSIRO264	-	-	-	-	-	-	1/64	-
LEAV	CSIRO2	-	-	-	-	-	-	-	1/64

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427 **Table 4. Results of serum neutralization test using 50 AKAV-positive and 25 AKAV-negative**
428 **cattle sera.**
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Cattle sera	Number of samples
Positive to AKAV only	32
Positive to AKAV and AINOV	10
Positive to AKAV and PEAV	8
Negative to all viruses	25

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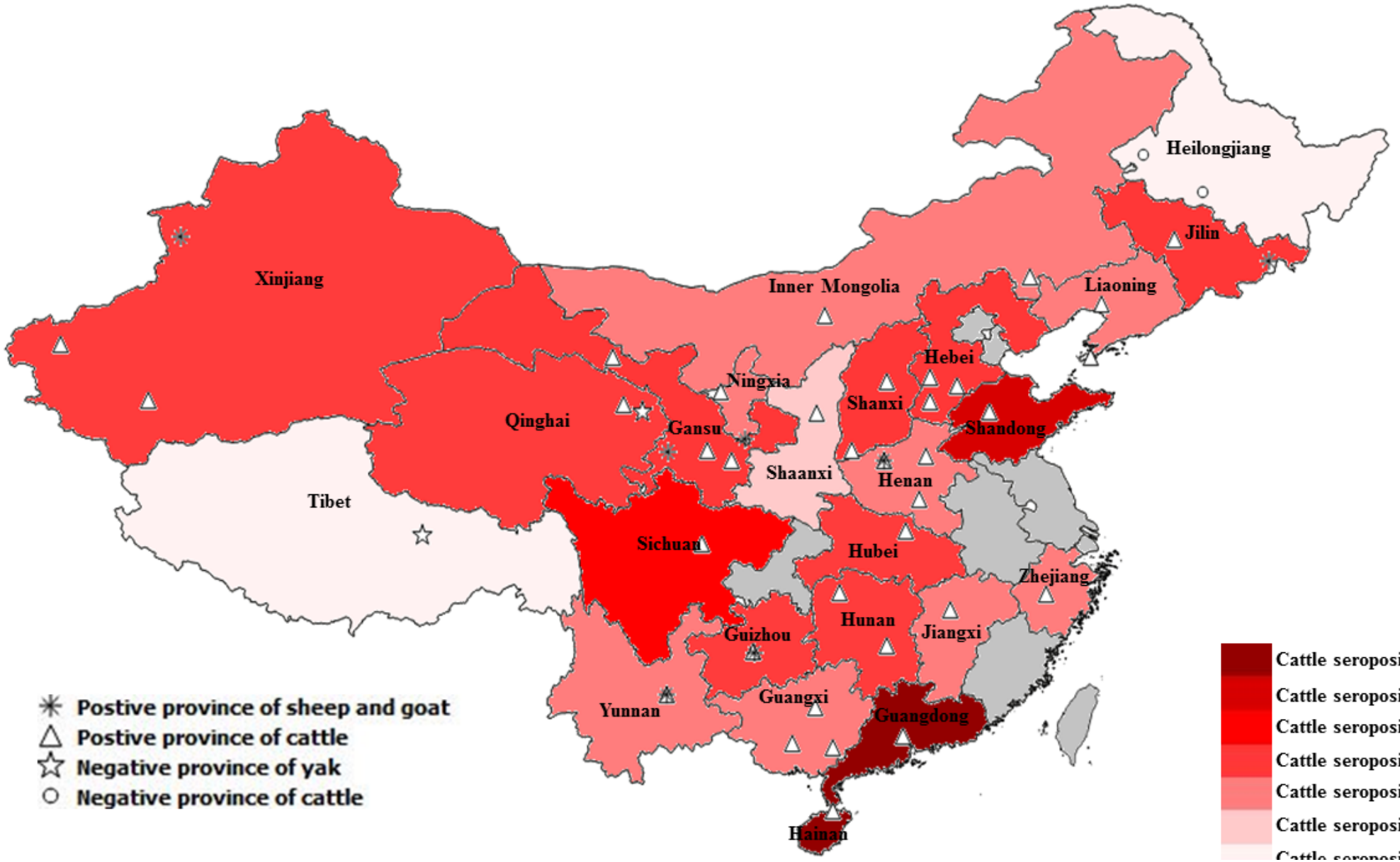
Heilongjiang
Jilin
Xinjiang
Inner Mongolia
Liaoning
Hebei
Ningxia
Shanxi
Qinghai
Gansu
Shandong
Shaanxi
Henan
Tibet
Sichuan
Hubei
Zhejiang
Hunan
Jiangxi
Guizhou
Guangxi
Guangdong
Yunnan
Hainan
Postive province of sheep and goat
Postive province of cattle
Negative province of yak
Negative province of cattle
Cattle seropositive rate 50%
Cattle seropositive rate 40%
Cattle seropositive rate 30%
Cattle seropositive rate 20%
Cattle seropositive rate 10%
Cattle seropositive rate 1%-