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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 2015 in 24 provinces of China from cattle, sheep, goats and yak were examined by serum neutralisation 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,b). 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 (Oya et al., 1961). 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 to 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

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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 2015 (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,

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