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BRIEF COMMUNICATION

A serological survey of Akabane virus infection in cattle and sheep in northwest China

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Abstract
Purpose Akabane disease characterized mainly by fetal damage is a ruminant disease caused by insect-transmitted Akabane virus infection.
Methods We investigated Akabane disease using serum neutralization tests in 446 blood samples collected from 187 cattle and 259 sheep of Xinjiang province, northwest China.
Results (1) The overall prevalence rate of neutralizing antibody was 19.06 % (85/446), (2) the prevalence rates of Akabane disease in cattle and sheep were 20.32 % (38/187) and 18.15 % (47/259), respectively, (3) the disease prevalence rates were not significantly different between cattle and sheep, but significantly different among samples collected from different sampling months, (4) the disease was most prevalent in July when mosquitoes and culicoides were most active, and (5) the disease prevalence rates were significantly different between individuals with abortion experience and without abortion experience ($P<0.05$), suggesting that Akabane virus infection may significantly increase abortion risk in cattle and sheep.
Conclusions To our knowledge, this is the first report confirming that Akabane virus infection is common in cattle and sheep of Xinjiang province, northwest China and providing useful epidemiological information for cattle and sheep abortion prevention and control.

Keywords Serological survey · Akabane virus · Cattle and sheep · Northwest China

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Introduction

Akabane disease is a disease in cattle, sheep, and goats caused by mosquitoes- and biting midges-transmitted Akabane virus infection (Charles 1994; Al-Afaleq et al. 1998). Inapparent Akabane disease in adults can lead to abortion, stillbirth, and congenital defects in newborns (Coverdale et al. 1978; Kirkland et al. 1988). During 1972 to 1975, this disease had led to the birth of more than 42,000 abnormal calves in Japan, causing significant economic loss in cattle industry (Kurogi et al. 1975, 1977). Moreover, some Akabane virus strains can cause outbreaks of encephalomyelitis in calves and adult cattle (Uchida et al. 2000; Kamata et al. 2009). To date, Akabane virus has been found in Australia, Saudi Arabia, Israel, Japan, and Korea (Coverdale et al. 1978; Abu Elzein et al. 1998; Kirkland 2002; Lee et al. 2002; Stram et al. 2004; Kono et al. 2008; Yang et al. 2008). Its antibodies have been found in cattle from many countries in Southeast Asia, Middle East, and Africa (Kurogi et al. 1975; Metselaar and Robin 1976; Taylor and Mellor 1994; An et al. 2010). Asymptomatic infection of Akabane virus has been demonstrated in free-

living wild animals such as buffalo greater kudu, impala, blue wildebeest, warthog, and elephant (Al-Busaidy et al. 1987).

In recent years, with the importation of cattle and sheep from abroad, the incidence of many diseases, especially abortion diseases, has increased in northwest China, posing serious threats to cattle and sheep industry. Surveys on abortion diseases in domestic cattle and sheep have been carried out, but most were restricted to brucellosis, campylobacteriosis, neosporosis, and other diseases. Being one of important pathogenic microorganisms for abortion diseases, Akabane virus has not received enough attention in ruminants in northwest China. So far, little has been known about Akabane virus infection in cattle and sheep (Brenner et al. 2004; Lim et al. 2007). In order to develop an effective prevention and control program to fight against abortion diseases in ruminants, it is important to obtain epidemiological information on the prevalence of Akabane disease in cattle and sheep. To this end, we characterized the specific antibody against Akabane virus and studied the prevalence rate of the abortion disease caused by Akabane virus in cattle and sheep from Xinjiang province, northwest China.

Materials and methods

Collection of blood samples

A total of 446 blood samples were collected during April–October 2010 from 187 cattle and 259 sheep from 12 herds and 8 flocks in Aletai, Tacheng, Yining, Shihezi, Urumqi, Akesu, Kashi in Xinjiang province, northwest China. Aletai, Tacheng, Yining, Shihezi, and Urumqid are located in the north Xinjiang province and belong to the moderate temperate zone. These areas have temperature of 23.1– °C in July and windy spring with most wind toward northwest and northeast. Akesu and Kashi are located in south Xinjiang and belong to warm temperate zone. These areas have temperature of 25.5–33.7 °C in July and four windy seasons with most wind toward northwest and northeast. The herds and flocks were selected randomly and tagged individually. The blood samples were stored on ice while being transferred to the laboratories where sera were separated and kept at −20 °C until required.

Viruses and cells

Akabane virus strain JaGAR 39/MB19 was originally supplied by Dr. Inaba of the National Institute of Animal Health, Japan. BHK-21 cells were maintained in minimum essential medium (Invitrogen, Carlsbad, CA, USA) containing 10 % fetal bovine serum (Biofluid, Richmond, VA, USA).

Table 1 Prevalence rates (percentage) measured by serum neutralization test of Akabane virus in blood samples collected from a total 187 cattle and 259 sheep from Xinjiang province, northwest China and the prevalence rates of abortion among the seropositive animals

Species	Overall prevalence rate (%)	With abortion history	Without abortion history
Cattle	38/187 (20.3)*	12/38 (31.6)*	26/38 (68.4)**
Sheep	47/259 (18.2)*	10/47 (21.3)*	37/47 (78.7)**
Total	85/446 (19.1)*	22/85 (25.9)*	63/85 (74.1)**

*$p>0.05$, indicates no significant differences between these columns; **$p<0.05$, significant difference in the same row

Serum neutralization tests in duplicate rows

Serum neutralization test was performed in flat-bottomed 96-well plates using a specific antibody against Akabane virus. Briefly, after heat-inactivated at 56 °C for 30 min, 50 μl of twofold serially diluted sample was mixed with 50 μl of culture medium containing Akabane virus with concentration of 100 $TCID_{50}$/100 μl in a well of 96-well plates in duplicate. After incubation for 1 h at 37 °C, 100 μl of BHK-21 cell suspension containing 5×10^8/ml cells was added to each well. The plates were then incubated at 37 °C in a humidified incubator supplemented with 5 % CO_2. Plates were examined microscopically after 3 and 5 days for evidence of virus-specific cytopathic effects (CPE); wells showing no evidence of CPE were considered to be protected by the sera. Antibody titers were expressed as the reciprocal of the highest serum dilution at which the CPE was inhibited. A titer of 1:4 or greater was considered to be positive.

Statistical analysis

The statistical analyses of prevalence of Akabane disease in cattle and sheep from northwestern China were performed

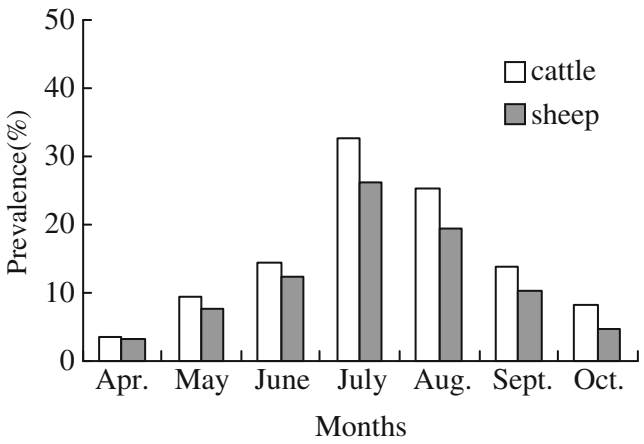

Fig. 1 Prevalence rates of Akabane virus infection in cattle and sheep from April to October 2010

by using the χ^2 test with Excel (Microsoft® Excel 2003). Differences with a p value of <0.05 were considered significant.

Results

Out of the 446 blood samples tested in this study, 85 blood samples were classified as seropositive for Akabane virus. The overall prevalence of Akabane disease was 19.06 % (85/446). The average prevalence was 20.32 % (38/187) in cattle and 18.15 % (47/259) in sheep, showing no significant difference ($p>0.05$). However, the prevalence rates were significantly different among samples from different sampling months (Table 1). The highest prevalence rates were 32.47 % in cattle and 26.22 % in sheep examined in July (Fig. 1). In addition, the prevalence rate between individuals with abortion experience and without abortion experience was significantly different ($p<0.05$; Table 1).

Discussion

The prevalence rates of Akabane virus infection in cattle and sheep have been reported in surveys conducted in many countries or areas and vary among different geographical areas (Hartley et al. 1975; Kurogi et al. 1975; Metselaar and Robin 1976; Brenner et al. 2004; Liao et al. 1996; An et al. 2010; Al-Busaidy et al. 1988; Uchida et al. 2000; Stram et al. 2004; Kono et al. 2008). An et al. (2010) investigated and assessed the prevalence rates of Akabane virus antibodies in domestic ruminants from different ecological zones of Sudan and found that the highest prevalence rate was 27, 36, and 47 % in sheep, goats, and cattle, respectively. In this study, the average prevalence rates of Akabane virus infection were 20.32 % (38/187) in cattle and 18.15 % (47/259) in sheep, lower than those in the survey mentioned above because the prevalence rate may be related to local climatic conditions and distribution of insects.

A significant difference in prevalence rate was seen in animals examined at different months. Akabane disease in cattle and sheep has been proved to be associated with the presence of haematophagous insects. Mosquito and biting midges occur in summer in most of their habitats worldwide. They are abundant haematophagous insects transmitting lots of pathogens to human, and domestic and wild animals. Therefore, these insects play important roles in the epidemiology of many important arboviral diseases. In this survey, all the seven sampling sites are located in agricultural oasis in Xinjiang province, where cattle and sheep are raised in combination of captive and grazing ways. Although the climates of theses seven sites are different, their average temperature between June and August is over 20 °C and humidity in rice plantation is very high, which are suitable for proliferation of haematophagous insects including mosquito and biting midges. High density of these insects in pens and pastures significantly increases the probability of insect-mediated viral transmission among individual cattle and sheep. As a result, the prevalence rates fluctuate with the activity of haematophagous insects; the highest prevalence rates were found in July in cattle (32.47 %) and sheep (26.22 %), which further confirmed that the epidemic of Akabane is closely associated with seasonal conditions in favor of haematophagous insect activity. Moreover, our results also found the prevalence rate in individuals with abortion experience was significantly higher than that of individuals without abortion experience, suggesting that the infection of Akabane virus may significantly increase the risk of abortion in cattle and sheep.

To our knowledge, this is the first serological survey on Akabane disease in northwest China, which provides evidence that Akabane virus infection in cattle and sheep in Xinjiang province is common. Therefore, comprehensive strategies and measures, such as vaccination, quarantine, and summer control of haematophagous insects, should be taken in cattle and sheep. Moreover, the infected animals from endemic areas should not be imported to non-endemic areas.

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