

Serological and Molecular Detection of Akabane Virus in Iran

Short Communication

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ABSTRACT

The present study was conducted on sixty healthy female domestic ruminants that suffered from still births or abortion, to find sero-positive cases and also to determine the probable role of uterine tissue in diagnosis of akabane viral infection and viral persistency. Because of this, a serological test (ELISA) and molecular (RT-PCR) diagnostic methods were used to find the seropositive cases and also the presence of the akabane viral genome in uterine samples. Among 2400 head, sixty female animals (20 cattle, 20 sheep and 20 goats) with a history of still births or abortion (suspected group) and sixty healthy ones (control group) were selected and referred to the slaughter house for further evaluations. Blood and uterine body samples were taken and stored at -20 and -70 °C, respectively. At the beginning of the experiment all serum samples were tested for brucellosis and 3 sheep of the suspected group and 1 sheep of the control group were positive. The remaining samples were selected for further study. Results of ELISA showed that among 17 suspected sheep only one of them was positive for akabane virus and among 20 suspected goats only three of them were inconclusive. It's notable that no positive or inconclusive cases were found in suspected cattle and also the control group. The prevalence rate of akabane virus antibody among 57 suspected animals was 1.75 percent. RT-PCR assay was conducted on uterine tissues from the suspected animals and results showed that all samples were free of the akabane genome. According to the obtained results the presence of antibody against akabane virus in suspected sheep and goats was proven or substantiated for the first time in Iran.

KEY WORDS akabane disease, RT-PCR, ruminants, uterus, virus.

INTRODUCTION

Akabane virus is classified into the genus orthobunyavirus in the family bunyaviridae. It is widely distributed in the tropical, subtropical and temperate regions of the world and has been associated with hematophagous arthropods. In general, akabane virus causes sporadic outbreaks of abortions, stillbirths, premature births and congenital malformations with the arthrogryposis-hydranencephaly syndrome (Brenner *et al.* 2004; Lee *et al.* 2002; Liao *et al.* 1996; Taylor and Mellor 1994). Pugh and Baird (2012) suggested that the incidence of infection is linked to vector activity and for

this reason some outbreaks were reported. It must be recognized that genetic sequences of shmallenberg virus (SBV), a novel bunyavirus of the genus orthobunyavirus showed the greatest similarity to viruses of the Simbu serogroup including akabane and shamonda virus (Tarlinton *et al.* 2012). Akabane virus replicates in arthropods and is transmitted by either mosquitoes or midges (Culicoides). Vector species concerned in virus replication and transmission have been intensely studied (Jennings and Mellor, 1989), but introduction of the virus into the bovine uterus by semen causes no developmental defects (Radostits *et al.* 2007). Radostits *et al.* (2007) stated that akabane viremia

occurs in the dam for 2-4 days after exposure and the antibody peaks 4-5 day after that followed by a subsequent secondary rise. They suggested that the dam is unaffected but there is a focal viral persistence in cotyledons and subsequently viremia in the fetus may occur. For these reasons the present study was conducted on 60 domestic ruminants with a history of still births or abortions to find seropositive cases and monitor the viral genome in uterine body tissue to determine the probable role of the infected uterus in the widespread occurrence of akabane virus.

MATERIALS AND METHODS

Animals

Among 100000 head of animal in the Shahrekord district we selected 2400 head, based on their history taken from 26 owners. Finally among 120 head we selected sixty female animals (20 cattle, 20 sheep and 20 goat) with a history of still births or abortion (suspected group) and sixty healthy ones (control group) to refer to the slaughter house for further evaluations.

Samples

During ante mortem examination and after giving an identification number, blood samples were taken from the jugular vein. Additionally, based on owner's information, the age was determined. Inspection was carried out in an abattoir and a uterine tissue sample was taken. Uterine samples were first rinsed quickly with ice-cold saline solution, blotted onto filter paper and then snap-frozen in liquid nitrogen.

Rose Bengal test

To find *Brucella*-free animals (an endemic disease in Iran), the Rose Bengal test was performed on all serum samples and 3 sheep from the suspected group and one sheep from the control group were positive. All remaining *Brucella*-free samples were selected for further studies.

Enzyme-Linked Immunosorbent Assay Method

The ELISA test was performed according to the producer instructions (IDVET France No 357). The optical density (OD) of the samples was measured at 450 nm by dividing the OD value of samples to the OD value of the negative control. An OD less than 30, between 30 to 40 or more than 40 was considered as a positive, inconclusive, or negative results, respectively.

RT-PCR

Uterine specimens (approximately 5 grams) from each animal were ground under liquid nitrogen. Total RNA was isolated using the RNX plus solution (Cinnagen, Iran) according to the manufacturer's instructions. Briefly, RNX

solution (1 mL) was added to the tube containing the 100 mg sample and incubated at room temperature for 5 min after briefly vortexing. Chloroform was added to the solution and centrifuged for 12000 rpm at 4 °C for 15 min. The upper phase was then transferred to another sterile tube and an equal volume of isopropanol was added. The mixture was centrifuged for 15 min at 12000 rpm and the precipitated RNA was then washed with ethanol, followed by another round of centrifugation. The extracted and pelleted RNA was dissolved in DEPC-treated water and stored at -70 °C. In the next step, cDNA was synthesized for each RNA sample using specific reverse primer and reverse transcriptase. The reaction was started with the incubation of a mixture containing total RNA (10 µL), primers (20 pmol/L) and sterile DEPC water (1 µL) at 65 °C for 5 min. The mixture was then briefly quenched on ice and added to a mixture containing 4 µL RT reaction buffer, 2 µL dNTP and 1 µL of M-MuLv reverse transcriptase (Cinnagen Co, Iran). cDNA synthesis was carried out at 42 °C for 1 h and terminated by incubation at 70 °C for 10 min. The produced cDNA was used for the next PCR reaction. A multiplex PCR reaction was performed with forward and reverse primers for the akabane genome (forward primer F2, 5'-ACCAGAAGAAGGCCAAGATG-3' and reverse primer R2, 5'-CACACGGTGCATGTCGATAA-3', respectively) (Lee *et al.* 2002). Polymerase chain reaction amplifications were performed using 3 µL of cDNA in 1u Taq DNA polymerases, 0.2 mM MgCl₂, 5 µL of 10X PCR buffer, 200 µM dNTP, 1 µmol of each primer in a 50 µL final volume reaction. The PCR reaction was conducted under the following conditions: an initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s and a final extension at 72 °C for 7 min. The amplified PCR products were run on a 1.2% agarose gel (Amplicon 347 bp).

RESULTS AND DISCUSSION

According to ELISA results, among seventeen sheep, one of them had a positive result, among twenty goats three of them had inconclusive results. There was no evidence of infection with akabane virus in the 20 serum samples from suspected cattle or from the sixty control healthy animals. In summation, of the 57 selected animals, the rate of akabane virus seropositive cases was 1.75 percent (Table 1).

According to the RT-PCR method, all uterine samples were tested for the genome of the akabane virus. It must be noted that all animals with positive or inconclusive ELISA results were negative in RT-PCR assay (Table 1). Being an important pathogenic microorganism for still births or abortion diseases, the akabane virus has not received enough attention in ruminants in southwest of Iran.

Table 1 Results of some serological and molecular detection tests of akabane virus

Animals	Number	Rose Bengal Test (Serum samples)		ELISA (Serum samples)			RT-PCR (Uterine tissue)	
		Positive	Negative	Positive	Negative	Inconclusive	Positive	Negative
Cattle	20	0	20	0	20	0	0	20
Sheep	20	3 (15%)	17	1 (5.88%)	16	0	0	20
Goats	20	0	20	0	17	3 (15%)	0	20
Total	60	3 (5%)	57	1 (1.75)	53	3 (5.26%)	0	60

Previously bovine congenital arthrogryposis due to the akabane virus was reported in Iran (Ahourai *et al.* 1992) while the incidence of viral infection in sheep or goat has not been considered yet.

Based on results the sero-positive rate of akabane infection was 5.88 percent for selected sheep and in selected goats 3% of the results were inconclusive which confirmed the presence of antibody against akabane virus (although not seropositive) in the small ruminant population in Iran. In adult animals, however, infection appears to be entirely subclinical and in endemic areas most breeding-age animals will have acquired an active immunity sufficient to prevent the virus from reaching the developing foetus (Jun *et al.* 2012; Radostits *et al.* 2007; Taylor and Mellor 1994). Yoshida and Tsuda (1998) and Tsuda *et al.* (2004) explained that the competitive ELISA is acceptable as a rapid and specific method for detecting antibodies to akabane virus and is a potential alternative to the serum neutralization test.

According to our results the prevalence rates of akabane virus infection were lower than those in the survey mentioned above because the prevalence rate may be related to local climatic conditions and distribution of insects and also due to the type of animal selection.

Consequently, the pathogenic effects of akabane infection are only seen when the virus exceeds the limits of the endemic area and also at the edges of the endemic area and may be due to the movement of either infected hosts or infected vectors (Inaba and Matsumoto 1990; Taylor and Mellor 1994).

Radostits *et al.* (2007) suggested that a focal viral infection may occur in cotyledons, while our RT-PCR results did not confirm the presence of viral genome in seropositive and inconclusive animals. These findings are similar to other studies where antibody could be detected but the akabane genome was not (Tsuda *et al.* 2004). For this reason the use of uterine tissue for detecting viral genome and its persistency is questionable.

CONCLUSION

According to the obtained results the presence of antibody against *Akabane virus* in suspected sheep and goats was proven or substantiated for the first time in Iran. While the

use of uterine tissue for detecting viral genome and its persistency is questionable.

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