

DETECTION OF AKABANE DISEASE IN SHEEP IN IRAQ

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ABSTRACT

Akabane virus is a member of the genus *Orthobunyavirus* in the family *Bunyaviridae*. It is transmitted by arthropod vectors such as *Culicoides*. It is widely distributed in temperate to tropical regions of the world. The virus is well known as a teratogenic pathogen which causes abortions, stillbirths, premature births and congenital abnormalities with arthrogryposis-hydranencephaly syndrome in cattle, sheep and goats.

The aim of this study is to detection of Akabane antibodies in sheep of seven governorates whereby no data was reported before, in Iraq.

This study was performed using 783 randomly sheep serum samples from virology unit in the central veterinary laboratory (CVL) where the tests was carried out during the period of January and February 2017.

The study area was in the five border Iraqi governorate and tow not border, all the collected sera were analyzed using a commercially available Screen® Akabane competition ELISA kit.

The results indicate that the disease was present in a number of governorates in the south of Iraq. Out of 783 randomly sheep sera tested, 30(24%), 25(29%), 3(2%), 23(15%) and 24(19%) of sheep were positive for AKAV antibodies.

As AKAV vaccines are currently not administered to livestock in the country, the detection of seropositive sheep in this study suggests natural exposure of these animals to AKAV.

INTRODUCTION

Akabane virus (AKAV) is an arthropod-borne virus (arbovirus), it is an insect-transmitted virus that causes congenital abnormalities of the CNS in ruminants, which was first isolated from *Aedes vexans* and *Culex tritaeniorhynchus* in Akabane area in Japan in 1959 (from it the name was come), then in Australia, Israel and Korea; antibodies to it have been found in a number of countries in southeast Asia, the Middle East and Africa. The disease affects fetuses of cattle, sheep, and goats. Asymptomatic infection has been demonstrated serologically in horses, buffalo, deer, and pigs (but not in humen) in endemic areas (10).

Etiology, Transmission and Epidemiology:

The causal agent, Akabane virus, is an orthobunyavirus and member of the Simbu serogroup of the family *Bunyaviridae*. It is spread by biting midges (*Culicoides* spp). It causes epizootic and sporadic outbreaks of abortions, premature births, stillbirths, and congenital abnormalities. The virus is transmitted primarily by biting midges of *Culicoides* species (2).

Akabane virus is widely distributed in the tropical and temperate zones between ~35°N and 35°S, in Australia, Southeast Asia, East Asia, the Middle East and Africa (9). The virus was present in the south Turkish coast in 1979 and 1980, here was also evidence of limited virus transmission in the Orontes river valley in Syria in 1979 and less precise evidence to show that occasional infection occurred in the lower Jordan River Valley (11). The herd at the Al-Ahsa oasis

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(Eastern region) of Saudi Arabia showed evidence of Akabane viral activity (1), In Iran the presence of antibody against Akabane virus in suspected sheep and goats was proven (6).

In these endemic areas, herbivores are bitten by the vectors, become infected at an early age, and develop a long-lasting immunity by the time of breeding; thus, congenital abnormalities are seldom seen. However, under favorable environmental conditions such as an extended humid summer, the vector (and hence the virus) may spread beyond its usual range into new areas, and outbreaks of congenital infection may be expected. These outbreaks usually occur at the northern or southern limits of the vector distribution or in areas of higher altitude. Similarly, pregnant ruminants from virus- and vector-free areas moved to virus-infected areas are at risk (9).

Clinical Findings and Lesions

The clinical signs seen at birth depend on the species of animal and the stage of pregnancy at which the dam was bitten by infected insect vectors.

Most strains of Akabane virus infect non-pregnant animals subclinically, but a few can cause encephalomyelitis in calves and adult cattle. Neurological signs that have been reported in these animals include tremors, ataxia, lameness, paralysis, nystagmus, opisthotonos and hypersensitivity. Although some individual animals have been febrile, fever was absent in most cases with CNS signs. More often, Akabane disease is characterized by asymptomatic infections in postnatal animals, and abortions, stillbirths, premature births and congenital defects (congenital malformations of the brain) in fetuses and newborns (8).

Birth complications may cause injuries to the dam that result in infertility or death, particularly when the fetus has malformed joints. Because Akabane virus has different effects at each stage of gestation, an ordered sequence of events tends to be seen. The range of fetal and neonatal defects seen in sheep and goats is similar, but there is more overlap, Arthrogryposis and CNS lesions are seen at the same time during the outbreak, and often occur in the same animals (12).

The virus is able to cross the ruminant placenta and should this happen in early pregnancy, a variety of congenital abnormalities including arthrogryposis and hydranencephaly are seen at parturition. 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. Consequently, the pathogenic effects of Akabane infection are only seen when the virus exceeds the limits of the endemic area and infects susceptible animals in the early stages of pregnancy (11).

Diagnosis

A presumptive diagnosis can be made on the gross CNS lesions, but the disease must be differentiated from other infectious and genetic conditions. Infection can be confirmed by testing sera or body fluids (e.g. pleural fluid) from unsuckled, affected offspring and their dams for antibodies against Akabane virus. While the detection of antibody in maternal serum does not confirm Akabane as an etiologic agent, its absence is definitive for exclusion other viruses (such as BVDV) (8).

Tests available

Many diagnostic tests are available.

Table 1: Tests available for Akabane virus ⁽⁷⁾.

Test	Samples required	Turnaround time
Akabane virus antibody ELISA	Clotted blood or foetal fluid	Up to 1 week
Akabane virus PCR	Fresh tissue	2-3 working days
Akabane VNT ²	Clotted blood (red top tube)	1-2 weeks
Histopathology examination	Fixed tissues	Up to 2 weeks for brain samples, 5 days for other samples.

Treatment and Control

There is no specific treatment for animals affected with Akabane virus infection. Measures should be directed at the prevention of infection of susceptible animals with Akabane virus during pregnancy, the movement of herds from nonendemic to endemic areas should be done well before first breeding. Effective vaccines are available in Japan (9).

Materials and Methods

Sample

This study was performed using 783 randomly sheep serum samples from virology unit in the central veterinary laboratory (CVL) where the tests was carried out during the period of January and February 2017.

Study Area

The study area was in the seven Iraqi governorate (Muthanna, Basrah, Missan, Diwaniyah, Najaf, Wasit and Dhi Qar).

Laboratory Technology used

All the collected sera were analyzed using a commercially available Screen® Akabane competition ELISA kit (ID.vet/ France) that detects anti-G1 antibodies directed against AKAV in ruminant serum and plasma. The test, which was reported by the manufacturer to lack cross-reactivity with other viruses in the Bunyaviridae family, such as Schmallerberg virus (SBV), Rift Valley fever virus (RVFV), and Aino virus, was performed according to the kit protocol. For each sample, results were expressed as sample/negative percentage (S/N%) using the optical densities (OD) from the ELISA reader: S/N% = Samples that presented S/N% less than 30%, between 30% and 40%, and >40% were considered positive, doubtful, and negative, respectively (4).

MATERIAL AND TOOLS

Material and tools that used in the study (Table 2).

Table 2: Material and tools

No.	Materials and tools	quantity
1	Competitive ELISA Test Kit	1
2	Multichannel micropipette 300µl	1
3	Single micropipette 100µl	1
4	Single micropipette 10µl	1
5	Tips 1-10µl ,10-200µl, 100-1000µl	1Bag for each
6	Gloves, Mask	4 Box, 1 Box
7	Overall	4
8	ELISA Device	1
9	Printer	1

RESULTS AND DISCUSSION

The results indicate that the virus was present in a number of provinces on the south of Iraq (Table 3 and Fig. 1), the persist of Akabane virus in southern Iraqi indicates that this area is open to epidemic rather than endemic infection. The results are according to the formula adopted by the producing company.

Table 3: Percentage of infected sheep of AKAV in the study area.

No. of Provinces	Number of examined samples	Number of positive cases	Number of doubtful cases	Number of negative cases	Percentage of infection
1-Muthanna	122	30	3	89	24%
2-Basrah	22	-	1	21	-
3-Missan	85	25	11	49	29%
4-Diwaniyah	114	-	5	109	-
5-Najaf	127	3	12	112	2%
6-Wasit	147	23	8	116	15%
7-Dhi Qar	121	24	2	95	19%
Total	783	105	42	591	

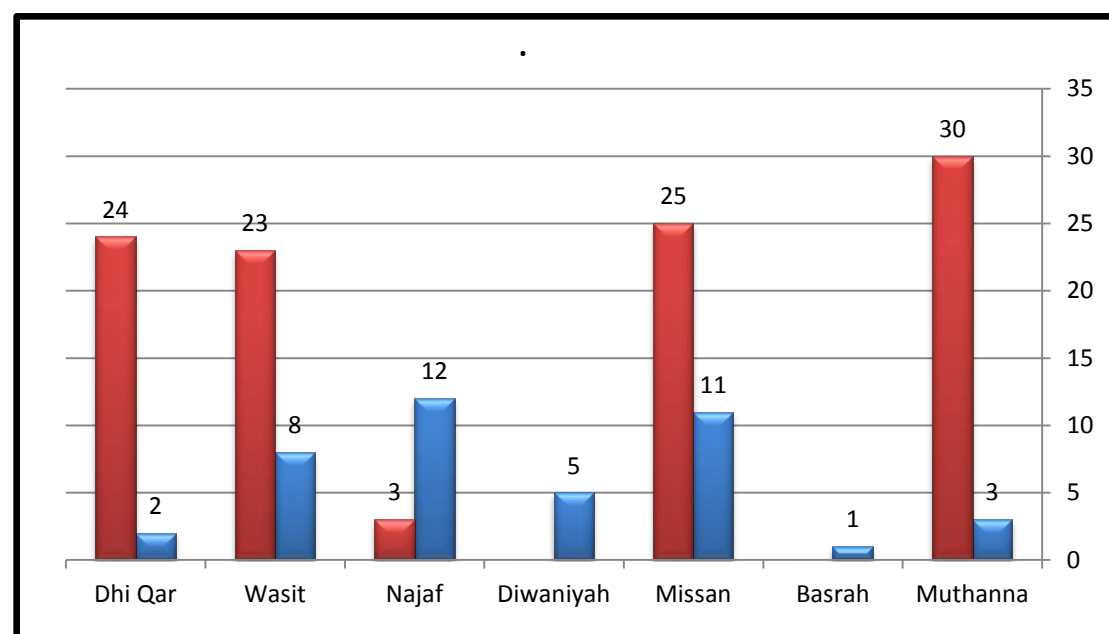


Fig. 1: Infected and doubtful sheep of AKAV.

Akabane virus has been shown to be an important pathogen causing abortions and congenital malformations in ruminants thus causing economic losses. Antibodies to this, Simbu serogroup virus have been found in ruminants (3). However, this is the first report of AKAV antibody-positive of animals in Iraq.

In the present study, infected cases of AKAV present in a number of provinces on the south of Iraq, as AKAV vaccines are currently not administered to livestock in the country, the detection of seropositive sheep in this study suggests natural exposure of these animals to AKAV. This possibility is buttressed by the knowledge that sheep are allowed to graze more extensively,

thus making them more exposed to the *Culicoides* vectors of AKAV. Furthermore, the climate of the southern provinces in terms of temperature and the presence of marshland, making it a suitable environment for the vector (5). So as there is unregulated transborder movement of ruminants from neighboring Iraqi countries, Turkey, Syria, Iran and Saudi Arabia which the virus had presence, the presence of neutralizing antibodies in the eastern Turkish provinces of Gaziantep and Diyarbakir suggests that this might be the route whereby Akabane virus occasionally invades the Middle East region (11). The herd at the Al-Ahsa oasis (Eastern region) showed evidence of Akabane viral activity, as reflected by the presence of maternal (colostral) antibody (1). In Iran the antibody against Akabane virus of suspected sheep and goats was proven or substantiated but the akabane virus had not received enough attention in ruminants in southwest of Iran (6). It is possible that some of the seropositive sheep could have introduced the disease into the country.

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التحري عن مرض الأكابانا في الأغنام في العراق

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الملخص

فيروس الأكابانا هو من جنس أورثوبونيا فيروس من عائلة بونيا فيريداي، ينتقل عن طريق ناقلات المفصليات مثل الكوليكوئادس. ويتوزع الفيروس على نطاق واسع في المناطق المعتدلة إلى المناطق الاستوائية في العالم. أن الفيروس معروف بأنه مرض مسبب للتشوهات في الأجنة مما يؤدي إلى الإجهاض، ولادة جنين ميت، والولادات المبكرة والتشوهات الخلقية مع متلازمة أرثروغريوسيس-هدرانانسيفالي في الماشية (الأغنام والماعز). تهدف هذه الدراسة إلى الكشف عن مرض الأكابانا في الأغنام في سبع محافظات في العراق، إذ لم يتم الإبلاغ عن أية بيانات عن المرض من قبل.

أجريت هذه الدراسة باستخدام 783 عينة عشوائية من مصل الأغنام من وحدة الفيروسات في قسم المختبرات والبحوث البيطرية المركزي، إذ تم إجراء الاختبار أثناء مدة كانون ثاني وشباط من سنة 2017 وكانت منطقة الدراسة في خمس محافظات حدودية ومحافظتين غير حدودية في العراق، وتم تحليل المصل جميعها التي تم جمعها بتقنية الأنزيم المناعي المتمز وباستخدام عدة تشخيصية تجارية.

تشير النتائج إلى أن فيروس الأكابانا موجود في عدد من المحافظات في جنوب العراق. فمن أصل 783 نموذج لمصل الأغنام، كانت نسبة 30 (24٪)، 25 (29٪)، 3 (2٪)، 23 (15٪) و 24 (19٪) من الأغنام إيجابية للأجسام المضادة لفيروس الأكابانا.

تبين هذه الدراسة وجود حالات أصابه بقطعان الأغنام في بعض المحافظات الجنوبية، ونظرا لأنها بيئة مناسبة للوسط الناقل من حيث درجات الحرارة ووجود الأهوار فيها والتنقل غير نظامي لقطعان الماشية من الدول المجاورة وبالإضافة لعدم وجود تلقيح سابق ضد المرض، لذا فإن كل هذه الدلائل تشير إلى التعرض الطبيعي لهذه القطعان للمرض.