Abortions, stillbirths and deformities in sheep at the Al-Ahsa oasis in eastern Saudi Arabia: isolation of a bluetongue serogroup virus from the affected lambs

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Summary
A wave of abortions, stillbirths and deformities in sheep occurred at the Al-Ahsa oasis in the eastern region of Saudi Arabia in the second half of 1999. The abortions were recorded in August and September and stillbirths and deformities in neonates were observed in October. Adult sheep were clinically normal. A virus was isolated in chicken embryos, adapted to Vero cell culture and further identified as bluetongue (BT) virus. The virus isolated was not neutralised by the Akabane virus. Reference hyperimmune serum against antibodies to BT virus detected in the sera of the dams gave positive results for BT but negative results for both Akabane and bovine viral diarrhoea virus. It was concluded that the outbreak was caused by a virus of the BT serogroup. The authors present the clinico-pathological and epidemiological situation of the disease outbreak.

Keywords

Introduction
Abortions, stillbirths and foetal abnormalities manifested by arthrogryposis, hydroencephaly and other brain anomalies, such as hydranencephaly and porencephaly, are usually associated with infection with either bluetongue (BT) or Akabane viruses (7, 14, 15, 17). In the case of the Akabane virus, dams are not affected, while in BT, mild catarrh of the buccal mucosa and coronitis may affect the dams. Although teratogenesis may be associated with other viral diseases, such as Rift Valley fever (RVF), Wesselsbron disease and bovine viral diarrhoea (BVD), clinical effects may also be seen in dams.

Epidemiological and clinico-pathological considerations are vital when making a tentative differential diagnosis of outbreaks in which teratogenesis is a salient feature (19).

Bluetongue antibodies are widespread in the sera of ruminants in Saudi Arabia (12, 13). During the 1980s and before, activity against BT virus serotypes 6, 14, 17, 18 and 19 was detected (13); these serotypes were no longer present during the 1990s. Thereafter, mono-specific antibodies for BT virus serotypes 10, 12, and 15 were detected for the first time in sentinel and other ruminants in Saudi Arabia (1).
Akabane virus infection is not endemic in Saudi Arabia (2, 6). Clinical disease has not been observed and the virus has not been isolated previously (2, 6).

The authors investigated an outbreak of a disease in sheep, manifested by abortions, stillbirths and malformations of full-term foetuses. Adult sheep were not affected. This outbreak constitutes the first of its type in Saudi Arabia. Serotyping of the causative virus and a comparison with other orbiviruses isolated from animals in Saudi Arabia will be reported later.

History of the disease

At the end of September and throughout October 1999, four ewes and their deformed newborn day-old lambs, were presented at the University Veterinary Teaching Hospital of King Faisal University at Al-Ahsa in the eastern Province of Saudi Arabia (23°23′N; 40°51′E). The owner stated that the flock comprised 300 sheep of different age groups and sex. They were kept in the open, at the Al-Ahsa oasis. Clinical signs were first observed in August 1999. Since that date, 13% of the flock had experienced abortions, stillbirths and deformities of newborn lambs. The adult sheep were not affected. The newborn animals had not nursed.

No vaccination was practised in the flock except for sheep pox two years previously.

Materials and methods

Reference antigens and antisera
The reference antigens and antisera for BT and Akabane disease, used in this study, were kindly provided by P.S. Mellor of the Institute for Animal Health in Pirbright, United Kingdom. The BT antiserum against serotype 4 and hyperimmune antiserum against Akabane virus strain JaG Av 39 were produced in sheep.

Radiography
Lateral radiographs were obtained of the head and neck, the thorax and the abdominal and pelvic regions of four deformed lambs and of one normal lamb of the same age and breed obtained from a non-affected herd.

Samples from the lambs and their dams
Blood was collected in ethylenediamine tetra-acetic acid (EDTA) from each affected lamb. Blood for serum was collected from the dams and lambs.

Post-mortem examination
The four deformed lambs were euthanised and a post-mortem examination was conducted. Samples for histopathology were collected in 10% formalin and processed. Paraffin sections were prepared and stained with haematoxylin and eosin, the van Giesen stain and by the periodic acid Schiff stain (10).

Samples of the liver, spleen, brain, spinal cord and lymph nodes of the lambs were collected in sterile flasks for virus isolation and were stored at −86°C prior to use.

Virus isolation

Inoculation of chicken embryos
Red blood cells from each deformed lamb were washed three times in phosphate-buffered saline (PBS) pH 7.4 and sonicated. They were diluted to a 10% (v/v) suspension in PBS and inoculated into the yolk sac of six-day-old chicken embryos as described by Cunningham (8); and via the vascular route into eleven-day-old chicken embryos as described by Goldsmid and Barzilai (11).

Inoculation of cell culture
Tissues from dead chicken embryos, inoculated with blood and tissues from the affected lambs, were used to inoculate monolayers of baby hamster (BHK-21) and Vero cells in ten 25 ml flasks, as described by Liendo and Castro (16). The inoculated cell monolayers were maintained with F-12 medium without serum and incubated at 37°C. Five of each type of cell monolayer were left stationary, while the other five were placed in a rocker (16). Control Vero and BHK-21 cell monolayers were inoculated with PBS, pH 7.4, and incubated in the same conditions. The monolayers were examined daily for the presence of a cytopathic effect (CPE).

Virus identification

Agar gel immunodiffusion test
The dead chicken embryos were homogenised to a 50% suspension (w/v) in PBS, pH 7.4, and used in the micro agar gel immunodiffusion test (AGID) as described by Abu Elzein et al. (1). Reference BT antiserum and a 50% suspension of unoincubated control chicken embryo were included in the test. The slides were placed in a humid chamber and left overnight at room temperature (26°C) and a reading was taken after 24 h. A further reading was made after 48 h.

Fluorescent antibody test
The method advocated by Liendo and Castro for the indirect fluorescent antibody test (FAT) was followed (16),
using protein A conjugated to fluorescein, to detect BT or Akabane virus in sections of the inoculated chicken embryos. All diluents used in the test procedure contained 2% egg albumin to minimise non-specific binding. Control slides without anti-BT or Akabane virus specific serum were included. The slides were examined under a fluorescent microscope.

Serology

Serum neutralisation test to detect antibodies to Akabane virus

The micro serum neutralisation test, as described by Cybinski et al. (9) and Sellers and Herniman (18), and modified by Abu Elzein et al. (2), was used to detect Akabane antibodies in the serum of affected lambs and their dams.

Agar gel immunodiffusion test to detect bluetongue antibodies

The AGID test as described by Abu Elzein et al. (1) was used to detect BT antibodies in the sera of the affected ewes and a further 45 adult sheep in the same flock.

Virus neutralisation test to detect Akabane virus

The same procedure as described above was employed in a virus neutralisation test to examine the virus isolated in Vero cell culture against the Akabane reference hyperimmune serum (9).

Enzyme-linked immunosorbent assay to detect bovine viral diarrhoea antigen

A commercial kit for the detection of BVD virus (BVDV) antigen was used to check for the presence of BVDV antigen in brain, spleen, lymph node and other tissues from the affected lambs, and in cell culture inoculated with infected material from the lambs.

Results

Clinical examination

The four affected lambs were born recumbent and they showed malformation of the head. In one lamb, the lower jaw was shorter than the upper jaw (brachygnathism) (Fig. 1). The neck was short in two lambs. One animal showed slight arthrogryposis (Fig. 2) and one lamb presented straight legs (Fig. 3a).

Radiography

There were variations in the appearance of the cranial bones in the four lambs (Fig. 4). One lamb had brachygnathism (Fig. 4a). The lower jaw was shorter and the maxilla protruded slightly beyond the mandibles. Two lambs had smaller rounded frontal bones (Figs 4b and 4c). In another lamb, the frontal bones showed anterior protrusion (Fig. 4c), while in another, the occipital condyles protruded caudally (Fig. 4d).

Measurements of the cervical vertebrae indicated shortening of the vertebrae in two lambs (Figs 4a and 4b) and incomplete development of the end plates (Figs 4a and 4b).

Figure 5 shows a normal lamb of the same age and breed as the affected lambs but from a non-affected herd.

![Fig. 1](image1.png)
Enlarged head of the lamb with brachygnathism

![Fig. 2](image2.png)
The lamb with kyphosis and arthrogryposis
Post-mortem findings

The lambs appeared to be suffering from intra-uterine growth retardation. The lungs showed diffuse haemorrhages and many dark red areas of consolidation in all lobes. The hearts were flaccid. The livers and kidneys were congested and, in one animal, clotted blood was seen on the visceral surface of the dorsal lobe of the liver and also around the spleen. The brain exhibited marked congestion of the meningeal vessels. Dilation of the lateral ventricles was seen in one animal.

Histopathology

The lungs showed marked congestion, alveolar haemorrhaging and wide areas of collapse. Occasionally, neutrophils were seen in some alveoli.

Hypercellular red pulp were visible in the spleen and distinct lymphoid nodules. Pericapsular haemorrhage was observed in one case.

The liver sections exhibited congestion of the portal vessels, endothelial swelling and mononuclear cell infiltration in portal areas. Random hepatocyte degeneration was seen. One case showed hepatocyte necrosis and subcapsular haemorrhage that extended into the parenchyma.

The heart muscles showed slight hyalinisation. Muscle cells were thin with enlarged nuclei, thus appearing hypercellular.

The kidneys showed congestion, tubular dilation, and thickened capsular epithelium. Glomeruli appeared above normal and hyper-cellular.

The brains and spinal cords exhibited marked vascular engorgement, capillary proliferation and swelling of the vascular endothelium. Clusters of glial cells, mostly microglia, were seen in sub-ependymal and deeper parts of the cerebrum; these were clearer near blood vessels (Fig 6). Haemorrhages were also observed. Some vacuoles were seen in the granular layer of the cerebellum. Spinal cord sections showed dilation of the central canal and thickening of the ependymal lining.

The tongue had slight swelling and vacuolation of the cells of the stratum spinosum and granular layer.
**Virus isolation**

**Chicken embryos**
Deaths occurred in the chicken embryos inoculated with the sonicated washed red blood cells three to five days following inoculation. The dead embryos showed severe haemorrhages and oedema and were cherry-coloured in appearance (Fig. 7), while the control embryos were pale.

**Cell culture**
The Vero cell monolayers inoculated with the chicken embryo homogenates, showed cytopathic changes, characterised by cell rounding on the third day post-inoculation. The cell monolayers were completely destroyed eight days post inoculation. Two further passages were made. The isolates were stored at –86°C. The BHK-21 cells were slower to develop CPE, so the cell culture work was pursued with the Vero cells.

**Virus identification**

**Fluorescent antibody test**
Slides from the infected chicken embryos showed fluorescence when tested in the indirect FAT using BT antiserum, but sections treated with Akabane reference hyperimmune antiserum did not. Sections from uninoculated chicken embryos gave negative results with both antisera.

**Akabane virus neutralisation test**
Virus isolated in the Vero cells was not neutralised by the Akabane reference antiserum.

**Agar gel immunodiffusion test**
A discernible line of precipitation was produced between the BT antiserum and chicken embryo suspensions. This line merged with a precipitation line produced between the reference BT antigen and antiserum to make a line of complete identity. No precipitation line was produced between the non-inoculated chicken embryo suspensions and the BT reference antiserum.

**Enzyme-linked immunosorbent assay to detect bovine viral diarrhoea virus antigen**
No BVDV antigen was detected in any of the materials examined from the affected lambs or from the inoculated cell culture.

**Serology**

**Detection of antibody against Akabane virus**
No neutralising antibody against Akabane virus was detected in the sera of the affected lambs or their dams.

**Agar gel immunodiffusion test to detect antibody against bluetongue virus**
Precipitating antibodies were detected in the sera of the four affected ewes which were presented at the clinic, and in 33 of 45 (73%) of the examined sheep sera collected randomly from the affected sheep flock.
Discussion

Clinical investigations and radiography in the present study indicated that the newborn recumbent lambs had deformation of the skull and cervical vertebrae. Gross pathological effects in the brain manifested by dilation of the lateral ventricles were evident.

The histopathological findings indicated marked vascular engorgement, capillary proliferation and swelling of the vascular endothelium which was observed in the brain and spinal cord, as well as in tissue sections from other organs. Haemorrhaging was also observed in the cerebrum, lung and liver tissue. These findings conform with those occurring in BT infection (17). It is well known that following infection, BT virus multiplies in haematopoietic cells and subsequently replicates in endothelial cells throughout the body. Endothelial swelling and damage causes oedema, haemorrhages and other circulatory changes (17).

Transplacental infection of sheep with BT virus may result in severe necrotising encephalopathy or focal necrotic changes characterised by mononuclear cell infiltration, which appear grossly as hydranencephaly and porencephaly, respectively (17). Typical necrotic changes were not detected in the present investigation. However, dilation of the lateral ventricles, marked brain congestion, haemorrhages and glial cell proliferation in the cerebrum, as well as the circulatory changes described previously in other organs, strongly suggest a reaction to BT virus infection.

Gross or histopathological changes suggestive of other teratogenic viruses, such as RVF or Wesselsbron disease, were not observed.

The virological findings in this study indicated the presence of BT virus in the affected lambs. No Akabane virus was isolated or detected by the FAT. Furthermore, no Akabane antibodies were detected in the sera of the lambs or their dams. The presence of Akabane antibodies in the sera of colostrum-deprived newly-born lambs indicates in utero infection with Akabane virus (6, 14). Akabane infection was excluded as a cause of the deformities.

Bovine viral diarrhoea virus was not detected in the inoculated cell culture or in the tissues of the affected lambs.

Bluetongue antibodies are widespread in ruminants in Saudi Arabia (1, 3, 4, 12, 13). However, virus serotypes 6, 14, 17, 18 and 19 known to prevail during the 1980s, and possibly before (3, 12, 13), were replaced by newly introduced serotypes during the 1990s. This was reported earlier in a collaborative study between the King Faisal University Laboratory in Al-Ahsa and the OIE (World Organisation for Animal Health) Reference Laboratory for BT in Onderstepoort, South Africa (1). The Reference Laboratory confirmed the absence of antibodies against serotypes 6, 14, 17, 18 and 19 and the presence of antibodies against serotypes 11, 13 and 16 which had not been recorded previously in Saudi Arabia (13).

The affected lambs examined in the present study were born in October 1999. Bearing in mind that the gestation period in sheep is five months, the animals had most likely been infected between May and October 1999. This timeframe fits well with the activity of BT virus at the Al-Ahsa oasis (1, 5) which shows that maximum abundance of Culicoides midges occurs in summer between May and October (1, 5). It must be stressed that no abortion or stillbirth was recorded in this flock before May 1999.

The authors are of the opinion that further studies are required to elucidate the epidemiological situation of the newly introduced BT serotypes in Saudi Arabia. Such introductions are expected in Saudi Arabia as the country imports sheep, goats, cattle and semen from BT-endemic countries.

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Avortements, mortinatalités et difformités chez le mouton à l’oasis d’Al-Ahsa en Arabie saoudite orientale : isolement d’un serogroupe de virus de la fièvre catarrhale du mouton des agneaux atteints


Résumé
Les ovins de l’oasis d’Al-Ahsa, dans l’est de l’Arabie saoudite, ont été victimes d’une vague d’avortement, de mortinatalité et de difformité au cours du second semestre 1999. Les avortements ont été constatés en août et en septembre ; les cas de mortinatalité et les difformités chez les nouveau-nés ont été observés en octobre. Les animaux adultes ne présentaient aucun signe clinique. Le virus isolé sur des œufs de poule embryonnés et, après adaptation, sur des cultures de cellules Vero a été identifié comme le virus de la fièvre catarrhale du mouton. Le virus isolé n’a pas été neutralisé par le virus Akabane. Le sérum hyperimmun de référence dirigé contre les anticorps au virus de la fièvre catarrhale dépistés dans le sérum des brebis a réagi positivement avec le virus de la fièvre catarrhale, alors qu’aucune réaction n’a été obtenue avec le virus Akabane ou le virus de la diarrhée virale bovine. L’origine du foyer a donc été attribuée à une infection par un virus appartenant au groupe sérologique de la fièvre catarrhale. Les auteurs présentent les données clinico-pathologiques et épidémiologiques relatives à ce foyer.

Mots-clés

Abortos, mortalidad perinatal y malformaciones en ganado ovino del oasis de Al-Ahsa, en Arabia Saudí oriental: aislamiento de un serogrupo del virus de la lengua azul en corderos afectados


Resumen
En el oasis Al Ahsa, situado al este de Arabia Saudí, el ganado sufrió una oleada de abortos, partos de crías muertas y malformaciones durante la segunda mitad de 1999. Los abortos se registraron en agosto y septiembre, y los partos de crías muertas y las malformaciones en octubre. El estado clínico de los ovinos adultos era normal. Se identificó el virus de la lengua azul previo aislamiento en huevos embrionados de gallina y cultivo en células Vero. El virus Akabane no neutralizó al virus aislado. El suero hiperinmune de referencia contra los anticuerpos de la
References


