The first outbreaks of sheep bluetongue in Khartoum province, Sudan

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Summary: Outbreaks of bluetongue (BT) have occurred for the first time among sheep in Khartoum province, the Sudan. The morbidity rate approached 100% while the mortality rate was 40%. The disease was successfully reproduced by experimentally infecting lambs, and the virus was reisolated from them. Viral antigen was detected directly in the blood plasma and serum from the naturally and experimentally infected lambs by using the complement fixation (CF) and the agar gel immunodiffusion (AGID) tests. The virus was further isolated in chick embryos (CE), baby hamster kidney cells (BHK-21) and chick-embryo fibroblasts (CEF), and it was eventually identified as BT.

It is recommended that blood plasma and serum from BT-suspected or sick sheep be used for the rapid serological diagnosis of the virus.

KEY-WORDS: Bluetongue virus - Experimental infection - Sheep - Sheep diseases - Serological techniques - Sudan.

INTRODUCTION

Bluetongue (BT) is an infectious non-contagious viral disease of domestic and wild ruminants. The causal virus is classified as an orbivirus (5).

In the Sudan, the disease was first identified in 1953 (3), when samples from the Blue Nile province were confirmed by the Veterinary Research Laboratory at Onderstepoort to contain BT virus. Subsequently the virus was not recorded in the country until Eisa et al. (7) reported its isolation from outbreaks among sheep in western Sudan in the mid-seventies.

The present study records the first isolation of BT virus from disease outbreaks involving sheep in Khartoum province.

So far, the existence of BT in cattle in the Sudan has been demonstrated solely by seroconversion (1, 7) and virus isolation from apparently healthy animals (Dr. Jeggo, personal communication; 2). No clinical disease has been seen.

MATERIALS AND METHODS

Investigations of the disease outbreaks

During the second half of 1982, severe outbreaks of a disease involved lambs 3-6 months old at farms in Khartoum province (El-Sileit and Soba). The morbidity
rate approached 100% and the mortality rate was 40%. Affected lambs showed high temperature (105°F), anorexia, panting, salivation, lacrimation and some were lame. Examination of the buccal cavity revealed cyanosis of the tongue, erosion of the dental pads and the dorsum of the tongue. Watery, and in some cases mucopurulent, nasal discharge was also seen.

Reference BT antigen and antiserum

BT reference soluble antigen and antiserum (sheep anti-BT type 4) were kindly supplied by Dr. W. Taylor of the Animal Virus Research Institute, Pirbright, England.

Material for examination

The following were collected from animals with high fever before the onset of the other symptoms: whole blood in ethylenediamine tetra-acetic acid (EDTA), serum, blood films on slides, plasma and faeces.

Sick lambs were killed for post-mortem examination (PM) and the following were collected: spleen, liver, kidneys, lymph nodes, serum, blood plasma and tongue epithelium (to exclude foot and mouth disease). Samples were also sent for bacteriological examination.

The collected tissues were homogenized, separately, in phosphate buffered saline (PBS) pH 7.2, containing 100 IU/ml penicillin and 1 mg/ml streptomycin. The homogenates were clarified by low-speed centrifugation and stored at −70°C until used.

Detection of BT virus antigen in specimens from sick lambs

In an attempt to quickly diagnose BT virus antigen in materials collected from the sick lambs, AGID and CF tests were carried out as described by Della-Porta et al. (6). Samples from healthy lambs were processed as controls in the tests.

Animal inoculation

Two five-month-old lambs, which were free from BT virus antigen and antibodies, were each inoculated intravenously with 3 ml of blood from sick lambs. Inoculated lambs were then kept in an insect-proof room and observed daily for signs of disease. Rectal temperature was recorded daily.

Virus isolation

Chick-embryo fibroblasts (CEF), BHK-21 cells and chick embryos (CE) were inoculated with sonicated blood, homogenates from collected organs, serum and plasma from sick lambs.

Eleven-day-old CE were inoculated intravascularly as described by Goldsmit and Barzilai (9). Inoculated eggs were incubated at 33.5°C for seven days and candled twice daily. Dead embryos were removed aseptically and processed as described by Stott et al. (14).

BHK cell cultures maintained in Eagles medium with 2% foetal calf serum were inoculated as described by Liendo and Castro (10). CEF cultures were prepared as
described by Mascoli and Burrell (12). Cultures were incubated at 37°C and examined daily for cytopathic effect (CPE).

Virus identification

Tests for BT virus antigen were conducted on dead chick embryos and cell cultures by using agar gel immunodiffusion (AGID) and complement fixation (CF) tests as described by Della-Porta et al. (6). Uninfected tissue culture and CE suspensions were used as controls.

Screening for BT antibodies in sheep serum

Screening of sheep of different age groups, from the affected farms, for the presence of serum antibodies against BT was carried out using the AGID test as described by Della-Porta et al. (6).

RESULTS

Post-mortem (PM) findings on naturally-infected lambs

Necropsy showed petechial haemorrhages in the skeletal muscles, severe conjunctivitis, erosion of the gum epithelium, cyanosis of the tongue, and hyperaemia of the upper respiratory tract epithelium. The carcases were pale and emaciated.

Experimentally-infected lambs

Both animals showed a sudden increase in temperature on the 11th day after inoculation which persisted for 8 days before returning to normal. During fever the lambs were dull, anorexic and showed lacrimation, followed by nasal discharge, laboured respiration and severe conjunctivitis. Examination of the buccal cavity revealed extensive cyanosis of the tongue and hyperaemia of the gums.

Generally speaking, the PM findings for the experimentally-infected lambs were similar to those of the naturally-infected ones.

Tongue epithelium samples were negative for FMD virus.

Virus isolation

Inoculated chick embryos died within 4-6 days of inoculation. They showed severe haemorrhages and congestion. None of the control embryos died.

A cytopathic effect (CPE), characterized by rounding of cells, was observed in BHK and CEF cultures four days after inoculation. Supernatant fluid was collected from cultures showing a complete CPE. It was centrifuged at 3000 rpm for 15 min and then concentrated as described by Liendo and Castro (10).

Virus identification

Specific BT virus precipitating and complement fixing antigens were detected in blood plasma, serum and liver from the naturally and experimentally-infected sheep, and in the concentrated suspensions from inoculated cell cultures and CE. No BT virus antigen was detected in serum, plasma or liver from uninoculated sheep nor in uninoculated cell culture or CE (Table I).
Results of the AGID and CF tests on specimens from naturally and experimentally infected lambs for the presence of BT virus antigen

<table>
<thead>
<tr>
<th>No. tested</th>
<th>AGID</th>
<th>CF</th>
</tr>
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<tbody>
<tr>
<td>Samples from naturally-infected lambs:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood plasma</td>
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<tr>
<td>Serum</td>
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<tr>
<td>Kidney</td>
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<td>Lymph node</td>
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<td>Liver</td>
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<td>Spleen</td>
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<tr>
<td>Samples from experimentally-infected lambs:</td>
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<tr>
<td>Blood plasma</td>
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<td>Lymph node</td>
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<tr>
<td>Kidney</td>
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<tr>
<td>Samples from normal sheep:</td>
<td></td>
<td></td>
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<tr>
<td>Plasma</td>
<td></td>
<td></td>
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<tr>
<td>Serum</td>
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</tbody>
</table>

Results of screening sheep sera for BT antibodies

Table II shows the results of testing serum samples from the affected farms for BT antibodies. Lambs 4 months old were completely free from BT antibody (i.e. were fully susceptible). The proportion of positive results increased with age.

<table>
<thead>
<tr>
<th>Age</th>
<th>No. tested</th>
<th>% positive</th>
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</thead>
<tbody>
<tr>
<td>Four months</td>
<td>20</td>
<td>0%</td>
</tr>
<tr>
<td>One year</td>
<td>40</td>
<td>27.5%</td>
</tr>
<tr>
<td>Two years</td>
<td>20</td>
<td>35%</td>
</tr>
<tr>
<td>Above 3 years</td>
<td>22</td>
<td>40.9%</td>
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<tr>
<td>Convalescent lambs</td>
<td>80</td>
<td>93.8%</td>
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DISCUSSION

The present study records the first BT outbreaks in sheep in Khartoum province, the Sudan. The disease was reproduced successfully in experimental lambs
and the virus was reisolated and identified from them. Serum from lambs which recovered from experimental infection contained specific precipitating antibodies against BT virus whereas, before infection, the samples were negative for BT antibodies.

It is recognized that blood is the best material for BT virus isolation when sampling suspect animals (11, 14). However, it cannot be used directly in the AGID test for rapid detection of BT virus antigen, because haemolysis interferes with reading of the results. In the present study it has been shown that blood plasma and serum from viraemic animals are equally rich in BT virus, and have an advantage over whole blood because they can be used successfully for the rapid detection of BT virus antigen. We therefore recommend the use of blood plasma and serum from living BT-suspected or sick sheep for the prompt detection of BT virus antigen by using the AGID test.

Results of a serological survey of sheep from the affected farms indicated that the number of positives was directly related to the animals' age. Young lambs are the most susceptible to infection, and it seems that maternal immunity in lambs in the Sudan probably wanes within 4 months or less after birth. Since none of the animals had been vaccinated against BT, the antibodies must have been acquired as a result of exposure to the virus. Such a situation could explain why the young lambs were the primary target of the disease in the outbreaks reported in this article.

The reservoir of BT virus in the Sudan is not yet known. However, Boorman and Mellor (4) have shown that potential insect vectors of BT exist in the country, and that *Culicoides kingi* and *C. imicola* are the species most likely to be involved in BT virus transmission. Although the examination of several thousands of *C. kingi* and hundreds of other *Culicoides* species by these authors failed to yield BT virus, we feel that research in that direction should continue in order to pin-point the insect vectors involved in BT transmission.

The emergence of BT in sheep in Khartoum province is of great concern, because intensive sheep farming is expanding, and an appreciable amount of money is being invested in the industry. The occurrence of BT in sheep has to be taken seriously and a vaccine should be prepared from the BT serotypes present. Otherwise the annual lamb crop will be at risk, and intensive sheep farming could eventually be handicapped.

ACKNOWLEDGEMENTS

Our thanks are due to Dr. W. Taylor of the Animal Virus Research Institute, Pirbright, for the reference BT antigens and antisera. The skilful technical assistance of Mr. Nag Eldin Babiker, Mr. Mohamed Elhassan Mirgani and Mr. Mirgani Elgillani of the CVRL, Soba, Khartoum, is gratefully acknowledged.


Résumé : Des foyers de fièvre catarrhale du mouton sont apparus pour la première fois chez des ovins dans la province de Khartoum, au Soudan. Le taux
La maladie a pu être reproduite en infectant expérimentalement des agneaux chez lesquels le virus a été réisolé. Les techniques de fixation du complément (FC) et d’immunodiffusion en gélose (IDG) ont permis de détecter directement l’antigène viral dans le plasma et le sérum sanguin des agneaux infectés par voies naturelle et expérimentale.


Resumen : Se presentaron por primera vez focos de lengua azul en ovinos de la provincia de Jartum, en Sudán. La tasa de morbilidad fue de casi el 100%, mientras que la tasa de mortalidad era del 40%. Se pudo reproducir la enfermedad infectando experimentalmente corderos en los que se volvió a aislar el virus. Con las técnicas de fijación del complemento (FC) y de inmunodifusión en agar (IDA) se detectó directamente el antígeno vírico en el plasma y suero sanguíneo de los corderos infectados por vías natural y experimental.

PALABRAS CLAVE : Enfermedades de los ovinos - Infección experimental - Ovinos - Sudán - Técnicas serológicas - Virus de la lengua azul.

REFERENCES


