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Susceptibility of Sudanese sheep to a bluetongue virus isolated from apparently healthy cattle in the Sudan

FAYZA (A.O.), ABU ELZEIN (E.M.E.), TAG ELDIN (M.H.), HAJER (I.E.). Sensibilité des moutons soudaniens au virus de la fièvre catarrhale isolé sur des bovins apparemment sains au Soudan. *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (3) : 313-316

L'étude tente de clarifier le rôle des bovins apparemment sains comme réservoir du virus de la fièvre catarrhale à l'égard des moutons au Soudan. Elle confirme les travaux antérieurs et établit que les bovins peuvent héberger le virus auquel sont sensibles les ovins de ce pays. Les conditions de transmission expérimentale du virus entre les deux espèces suggèrent que le meilleur indicateur pour déterminer la virémie sur des bovins apparemment sains consiste à inoculer des moutons sensibles avec le virus bovin suspecté. Les conditions de la virémie et la survie du virus dans la nature font l'objet d'une discussion. *Mots clés* : Bovin - Ovin - Hôte - Fièvre catarrhale du mouton - Virus - Sensibilité aux maladies - Soudan.

INTRODUCTION

Cattle can harbour the bluetongue virus (BTV) in their blood without showing symptoms and thus can act as a reservoir of infection towards animals including sheep (25).

In the Sudan, it is quite evident that BTV antibodies are widespread in the domestic species of livestock examined so far (1, 3, 5, 10, 20). On the other hand, BTV was isolated from BT outbreaks in sheep (6, 11), from apparently healthy cattle (4), and from Culicoides midges (18) in this country.

With the exception of one report of clinical BT in a calf (21), no information is so far available regarding pathogenicity of BT in cattle in the Sudan. Again, the role of cattle as a reservoir of infectious BTV towards animals has not been elucidated.

In the present study the pathogenicity of a BTV isolated from apparently healthy cattle was explored in Sudanese sheep in order to determine the role of cattle in the epidemiology of BT in this country.

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MATERIALS AND METHODS

The virus (BT/CS/83)

The BTV was isolated from apparently healthy cattle in the Khartoum Province (4). It was first passaged twice by intravascular inoculation in 11-day old chick embryos. It was then further passaged twice in bovine kidney cell cultures (BKC) incubated at 37 °C as described by LEINDO and CASTRO (15) and examined daily for cytotoxic effects (CTE). The virus was titrated in BK cell cultures and the $TCID_{50}$ was calculated according to REED and MUENCH (24). The virus was concentrated 25 times using polyethylene glycol (PEG) (BDH England), and, when necessary, serological identification was performed using the agar gel immunodiffusion test (AGID) (2).

Pathogenicity in susceptible sheep

Five BT antibody-seronegative indigenous 6-12 month old sheeps from the Sudanese desert were used in this study. Four of them were intravenously inoculated with 2 ml of a 10^7 $TCID_{50}$/ml virus suspension and one animal was used as a uninoculated control. All animals were kept in insect-proof isolation units, and supplied with water and food *ad libitum*. Rectal temperature was taken daily in the morning, and all the animals were bled weekly for detection of antibodies against BTV. During the febrile stage blood for virus reisolation, was collected in ethylene-diamine-tetracetic acid (EDTA).

Post-mortem

Dead and sacrified animals were examined for *post mortem* (PM) findings. Representative samples from lung, heart, kidney, intestine, lymph nodes, spleen, skeletal muscles and tongue were collected in 10 % formal saline for histopathological examination.

AGID for antibody detection

This was performed as described (2) to detect antibodies against BTV in the post-inoculation sera using

reference BTV soluble antigen and antiserum, kindly provided by Dr. W. TAYLOR, AVRI, Pirbright.

Virus re-isolation from inoculated animals

Whole blood from the experimental viraemic animals was intravascularly inoculated into 11-day old chicken embryos, as described by GOLDSMIT and BRAZILAI (13). Isolated virus was further adapted to BK cell cultures, concentrated 25 times using PEG as described above and identified using the AGID tests (2).

RESULTS

Experimental animals

The temperature of the four inoculated sheep rose from day 17 post-inoculation, reached a peak of 105 °F and extended on an average period of 7 days. This was followed by lachrymation, mild salivation and nasal discharge, hyperaemia of the buccal and nasal cavities, cyanosis of the tongue, weakness of the hind limbs and coronitis which led to lameness. One animal died and the remaining animals were sacrificed.

Post-mortem findings

The most prominent gross lesions were : congestion of the buccal cavity, heart and kidneys. The lungs were congested and the trachea contained frothy exudate. The thigh and back muscles were pale and showed peticheal haemorrhages.

Histopathological lesions

Lungs of the infected sheep exhibited interlobular oedema. Their alveolar capillaries were filed with red blood cells.

The kidneys displayed necrosis of tubular epithelial cells and mononuclear cell infiltration. Some fibres of skeletal, tongue and cardiac muscles showed degeneration with loss of striations. The liver showed congestion and focal necrosis. Macrophages containing haemosiderin were seen in the spleen.

Virus isolation and identification

Chicken embryos inoculated with original virus or virus from experimental animals, died within 2-7 days post-inoculation. Harvested embryos showed a cherry red colour, and were haemorrhagic, oedematous and congested.

The BK cell cultures inoculated with the original virus or blood from the experimental sheep during the febrile stage depicted a CTE which was characterized by rounding of the cells in 4-7 days post-inoculation.

The concentrated virus isolate from chicken embryo homogenates or BKC suspension gave complete line of identity with the reference BT antiserum in the AGID tests. No lines were obtained from concentrated BKC or chicken embryos inoculated with blood from the control sheep.

Antibody detection

BT-specific precipitin lines were obtained when 3-4 week post inoculation sera from experimental sheep were reacted against the BTV soluble antigen in the AGID tests. Sera from the control sheep did not give lines against the BTV soluble antigen in the AGID tests.

DISCUSSION

It is well understood that epidemiology and pathogenicity of blue tongue disease vary with geographical regions and breeds of animals. In BT enzootic area, most bovine infections are inapparent : viraemia and seroconversion may thus be the only evidence of infection (19, 23). BT viraemia in cattle has been extensively reviewed by HOURRIGAN and KLINGSPORN (14). In certain circumstances it was reported that viraemia could extend up to 3 years (14). Thus, cattle can be considered as important and long-term BT virus reservoirs. Viraemia in infected cattle is often prolonged to allow the virus to persist even when the vector is absent (7, 16). So, this phenomenon is involved in the virus maintenance in the field.

On the other hand, it has also been reported that Culicoides midges prefer to feed on cattle rather than on sheep (22) and that the monthly average forage ratio for *C. imicola* was 1.3 for cattle and only 0.2 for sheep. Based on this information, NEVILL (22) proposed the use of cattle to protect sheep from BT infection by keeping both species in the same pens.

Experimental transmission of BT virus from cattle to cattle and sheep, and from sheep to cattle and sheep has been successful (9). HOURRIGAN and KLINGSPORN (14) suggested that the best indicator to deter-

mine a possible viraemia in apparently healthy cattle, is to inoculate susceptible sheep with blood from suspected cattle.

Although BT virus has been isolated from apparently healthy cattle (1) from the Sudan, no studies have been undertaken to examine whether cattle could act as reservoirs of infection for sheep. The present study proves that the virus isolated from apparently healthy cattle can cause clinical disease in sheep. This information agrees with previous studies (8, 9, 12) and shows that cattle can harbour infectious BT virus for sheep in the Sudan. It adds a new dimension to our knowledge of epidemiology of BTV infection in the Sudan.

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FAYZA (A.O.), ABU ELZEIN (E.M.E.), TAG ELDIN (M.H.), HAJER (I.E.). Susceptibility of Sudanese sheep to a bluetongue virus isolated from apparently healthy cattle in the Sudan. *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (3) : 313-316

This study intends to clarify the role of apparently healthy cattle as a reservoir of bluetongue (BT) virus to sheep in the Sudan. It confirms earlier work and establishes that cattle can harbour bluetongue virus to which sheep are susceptible in the country. Experimental transmission of BT virus between the two species suggests that the best indicator to determine viraemia in apparently healthy cattle is to inoculate susceptible sheep with suspected cattle virus. The condition of the viraemia and the virus survival in the field are discussed. *Key words* : Cattle - Sheep - Reservoir - Bluetongue - Virus - Sensibility - Sudan.

FAYZA (A.O.), ABU ELZEIN (E.M.E.), TAG ELDIN (M.H.), HAJER (I.E.). Sensibilidad de los carneros sudaneses al virus de la lengua azul aislado en ganado bovino al parecer sano en Sudán. *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (3) : 313-316

Este estudio intenta aclarar el papel de los bovinos al parecer sanos como reservorio del virus de la lengua azul con respecto a los carneros en Sudán. Confirma los trabajos anteriores y muestra que los bovinos pueden albergar el virus al cual el ganado ovino de dicho país es sensible. Las condiciones de transmisión experimental del virus entre las dos especies sugieren que el mejor para determinar la viremia en bovinos al parecer sanos consiste en inocular carneros sensibles con el virus bovino sospechado. Se discuten las condiciones de la viremia y la supervivencia del virus en la natura. *Palabras claves* : Bovino - Ovino - Huesped - Lengua azul - Virus - Sensibilidad a las enfermedades - Sudán.

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