Recovery of bluetongue virus serogroup from sera collected for a serological survey from apparently healthy cattle, from the Sudan

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SUMMARY

Virus of the bluetongue (BT) serogroup was recovered from 11 % of cattle sera collected from apparently healthy animals in Khartoum Province for the sole purpose of screening for BT antibodies. Since these sera did not contain BT antibodies, the donor cattle could have been scored as BT free in the serological survey.

Virus was initially isolated in chicken embryos inoculated intravascularly, and was further adapted to Vero cell cultures. Isolates were identified as belonging to the BT serogroup using the agar gel immunodiffusion (AGID) and complement fixation (CF) tests.

The results indicated that cattle in the Sudan could harbour BT virus without showing symptoms of the disease. Such an observation necessitates further work to clarify the role of cattle in the epidemiology of BT in the Sudan.

INTRODUCTION

In BT endemic regions, cattle usually experience an inapparent form of infection which can be demonstrated as a viraemia and by seroconversion. However, clinical disease and abortions without showing symptoms have also been reported (Metcalf *et al.* 1981).

In the Sudan, in spite of the existence of overt BT infection in sheep since the early 1950's (Anon. 1953), no reports of the disease in cattle are available. However, Mohamed *et al.* (1980) reported a case of BT in a calf in Khartoum Province, but they failed to support their observation with virus isolation.

The present study was initiated by the observation that during a serological survey for BT antibodies in cattle sera from the Sudan, some sera gave a specific reaction against the reference BT antiserum. That observation has been extended in this paper.

MATERIALS AND METHODS

A total of 261 cattle sera were examined. They were collected from apparently healthy adult animals from Khartoum province during February 1983. Reference BT soluble antigen and group specific antiserum, obtained from sheep infected with BT virus type 4, were kindly supplied by Dr W. Taylor, Animal Virus Research Institute, Pirbright, UK.

The AGID test for the detection of BT group specific antibodies

To detect antibodies against BT in the cattle sera, the slide method of the AGID test was used. Each glass slide was covered with 3 ml of 1 % Litex Agarose (Litex Accurate Chemical & Scientific Corp, Westbury, NY, USA) in Borate buffer pH 7·6. The test pattern consisted of a six-well rosette of 3 mm diameter wells around a central well, with 5 mm between the centre of each well. The reference virus antigen was added to the central well. Test sera were added to alternate wells. To the wells between these the reference serum was added. The slides were then placed in a humid chamber and were examined daily for the appearance of precipitin lines.

Virus isolation attempts from the cattle sera

Test sera which gave positive precipitin lines when reacted against the reference BT antiserum in the AGID tests, were selected for virus isolation attempts along with the sera which were completely negative for both BT virus antigen and antibodies. Each serum sample was diluted 1/10 in phosphate buffered saline (PBS) pH 7·2 containing 1000 IU/ml penicillin, 1 mg/ml streptomycin and 50 U/ml mycostatin. Samples were inoculated into 11-day old embryonated chicken eggs by the intravenous route (Goldsmit & Barzilai, 1968). Embryos dying after 48 h and onwards were removed aseptically and processed as described by Stott *et al.* (1982). Embryo suspensions were diluted 1/10 in Eagle's Minimium Essential Medium (MEM), inoculated onto Vero cell monolayers and maintained in MEM containing 5 % fetal calf serum and antibiotics. Cell cultures were incubated at 37 °C until any cytopathic effect involved 80 % of that cell sheet. The virus was then concentrated as described by Liendo & Castro (1981).

Sera which did not kill the chicken embryos or produce CPE on Vero cell cultures, were passaged blind three times before they were scored as negative.

Virus identification

To confirm that any isolated agent was BT virus, the AGID and CF tests (Della-Porta *et al.* 1981) were used. Uninfected tissue culture and chicken embryo suspensions were included as controls.

Comparison was made between the isolated virus, the original virus in the cattle serum and the reference BT soluble antigen using the AGID tests as follows: the reference BT antiserum was placed in the central well, the isolated virus was placed in the 12 and 6 o'clock wells of the rosette. To the two wells adjacent to the 12 o'clock well was placed the original cattle serum. To the remaining two wells the reference BT soluble antigen was placed.

RESULTS

Virus isolation and identification

The chicken embryos died within 4–6 days following inoculation. Infected embryos had a characteristic cherry red colour due to haemorrhages and associated oedema. On further passaging in chicken embryos death was recorded within 3 days post inoculation.

Table 1. *The AGID and virus isolation results on the sera from apparently healthy cattle*

No. of sera examined	No. positive for BT antibodies (%)	No. positive for BT virus antigen (%)	No. negative for BT antigen and antibody (%)
261	147 (56·3)	29 (11)	85 (32·7)

Table 2. *Results of complement fixation tests for the detection of BT virus antigen in suspensions of inoculated chicken embryos and Vero cells*

Virus material	Highest titre* ($\log_{10}$)	Lowest titre* ($\log_{10}$)	Mean* ($\log_{10}$)
Vero cell culture suspension passage 3	2·7†	2·1†	2·4
Vero cell culture concentrated virus	3·6	3·0	3·3
Chick embryo suspension passage 3	3·0	2·1	2·7
Chick embryo concentrated virus	3·6	3·0	3·3

* Of the 29 samples.
† Each titration was repeated three times, and this figure is the mean of the three experiments for each sample.

CPE characterized by the cells rounding-up was seen in the inoculated Vero cells in 4 days and progressed to 80 % within 8 days.

Table 1 shows that BT virus could be isolated from only 11 % of the sera examined. Those sera were also positive for BT antigen when they were initially tested in the AGID tests. No virus could be recovered from the sera which were negative for the BT virus antigen in the initial AGID tests.

Two discernable precipitin lines were produced when the reference BT antiserum was reacted against the isolated virus. Those lines completely merged with two lines produced as a result of the reaction between the original virus in the cattle serum, and the reference BT antiserum giving lines of complete identity.

The reference BT soluble antigen gave one line against the reference BT antiserum. This line completely merged with one of the two lines shared by the isolated virus and the virus contained in the original cattle serum.

Table 2 shows that BT-specific complement fixing antigen could be detected in suspensions made from inoculated chicken embryos and from infected Vero cells. Concentrated virus gave the highest titres, while suspensions from non-inoculated chicken embryos and Vero cell cultures gave negative results by complement fixation.

DISCUSSION

The present study was undertaken to see whether some sera from apparently healthy BT sero-negative cattle could harbour viable BT virus, and was not intended to advocate the use of sera as the material of choice for isolating BT virus from cattle.

The results presented here indicate that BT could only be isolated from the sera that contained BT precipitating antigens. This suggests that these sera were collected from animals in the early viraemic stage and before the appearance of the circulating BT antibodies.

The AGID results demonstrated that two antigens, which reacted with the BT reference antiserum, were contained in the original cattle sera. Those antigens gave a reaction of identity with two antigens which were present in the isolated virus preparations. One of these antigens also gave a reaction of identity with the BT reference soluble antigen. The other antigen which was only present in the original cattle serum and the isolated virus preparation could have been due to infectious BT virus particles.

Scrutiny of the literature reveals that surveillance of BT infection in animals has largely depended upon detecting BT antibodies in their sera (Jochim & Pearson, 1979; Metcalf *et al.* 1981; Gibbs *et al.* 1983). However, some animals with no detectable BT antibodies in their sera could have been viraemic at the time of bleeding and have been scored as BT negative in serological surveys although containing viable BT virus in their blood.

Attempts to isolate virus from the blood of animals found to be seronegative in serological surveys made to exclude their exposure to BT infection is rather impractical when several thousand sera have to be tested. Because this study has shown that some sera from apparently healthy animals contained BT virus antigens, detectable by gel diffusion, it would be reasonable to recommend the use of the simple micro-AGID or the more sensitive ELISA methods (Hubschle *et al.* 1981) to detect BT virus antigen in the sera or plasma of such animals.

Like other parts of the Sudan, Khartoum Province has been greatly hit by the drought of the last 4 years. In spite of this, repeated outbreaks of sheep BT were experienced in the province (Abu Elzein & Tag Eldin, 1985). On the other hand, the culicoides midges, which are known to transmit the disease in this area, were caught in abundance during some years of the drought (Boorman & Mellor, 1982); and BT and BT-related viruses were isolated from them (Mellor *et al.* 1984). Furthermore, the present study has also shown that virus of the BT serogroup could be isolated from the sera of cattle which were bled in February 1983 (during the drought). This indicates that BT virus activity has been going on in the province even during the long drought (1981–84). This could be explained by the continuous availability of favourable breeding conditions for culicoides all the year round along the banks of the Nile and the irrigation canals in Khartoum Province. However, in autumns with favourable weather conditions, the culicoides population increases greatly and consequently an increase in the BT virus activity would be expected.

At the time when the cattle used in the present study were bled, and for a few months before and after that, several outbreaks of BT in sheep were recorded in Khartoum province (Abu Elzein & Tag Eldin, 1985; Abu Elzein & Fayza, unpublished data). However, no clinical BT was ever reported in cattle, within the province or elsewhere in the country, before, during or after the time of sampling. Hence, whether cattle are a reservoir of the BT virus in the Sudan, remains to be elucidated.

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REFERENCES

ABU ELZEIN, E. M. E. & TAG ELDIN, M. H. (1985). The first outbreaks of sheep bluetongue in Khartoum Province, Sudan. Scientific & Technical Review, Office of International Epizootics **4**, 509–515.

ANONYMOUS (1953). Annual Reports, Department of Animal Resources, Sudan Government.

BORRMAN, J. & MELLOR, P. (1982). Notes on Culicoides (Diptera, Ceratopogonidae) from the Sudan in relation to the epidemiology of bluetongue virus disease. *Revue d'Elevage et de Medicine Veterinaire des Pays Tropicaux* **35**, 173–178.

DELLA-PORTA, A. J., McPHEE, D. A., WARK, M. C., ST. GEORGE, T. D. & CYBINSKI, D. H. (1981). Serological studies of two additional Australin bluetongues virus isolates CSIRO 154 & 156. *Veterinary Microbiology* **6**, 233–245.

GIBBS, E. P. J., GREINER, E. C., ALEXANDER, F. C. M., KING, T. H. & ROACH, C. R. (1983). Serological survey of ruminant livestock in some countries of the Caribbean region and South America for antibody to bluetongue virus. *Veterinary Record* **113**, 446–448.

GOLDSMIT, I. & BARZILAI, E. (1968). An improved method for the isolation and identifications of bluetongue virus by intravenous inoculation of embryonating chicken eggs. *Journal of Comparative Pathology* **78**, 477–487.

HUBSCHLE, O. J. B., LORENZ, R. J. & MATHEKA, H. D. (1981). Enzyme-linked immunosorbent assay for detection of bluetongue virus antibodies. *American Journal for Veterinary Research* **42**, 61–65.

JOCHIM, M. M. & PEARSON, J. E. (1979). Protocol for the immunodiffusion test for bluetongue. *Proceedings of the American Association for Veterinary Laboratory Diagnosticians*, pp. 463–471.

LIENDO, G. & CASTRO, A. E. (1981). Bluetongue in cattle: diagnosis and virus isolation. *Bovine Practitioner* **16**, 87–100.

MELLOR, P., OSBORNE, R. & JENNINGS, D. M. (1984). Isolation of bluetongue and related viruses from Culicoides spp. in the Sudan. *Journal of Hygiene* **93**, 621–628.

METCALF, H. E., PEARSON, J. E. & KLINGSBORN, A. L. (1981). Bluetongue in cattle: a serologic survey of slaughter cattle in the United States. *American Journal of Veterinary Research* **40**, 1057–1060.

MOHAMED, K. A., EISA, M., NAYIL, A. A., BALLOUGH, A. & ABD ELAZIZ, M.A. (1980). A fatal bluetongue infection in a calf: a case report. *Sudan Journal of Veterinary Research* **2**, 69–73.

STOTT, D. L., OSBURN, B. I. & BARBER, T. L. (1982). Recovery of dual serotypes of bluetongue virus from infected sheep and cattle. *Veterinary Microbiology* **7**, 197–207.