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Bluetongue virus and epizootic haemorrhagic disease of deer virus serotypes in northern Colombian cattle

By E. J. HOMAN

Department of Veterinary Science, University of Wisconsin, Madison

W. P. TAYLOR

Animal Virus Research Institute, Pirbright, England

H. LORBACHER de RUIZ

Faculty of Veterinary Medicine and Animal Science, University of Antioquia, Medellin, Colombia

and T. M. YUILL

School of Veterinary Medicine, University of Wisconsin, Madison

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SUMMARY

There is recent evidence of bluetongue (BT) and epizootic haemorrhagic disease (EHD) virus infection of cattle in the American tropics, including BT group reactive antibody in Colombian cattle. These observations prompted a study to determine serologically the specific BT and EHD virus types present, and time of infection and to collect *Culicoides* spp. as potential vectors. A prospective study of BT and EHD virus infection was done on two farms in the Colombian department of Antioquia. Sequential sampling of young cattle indicated acquisition of neutralizing antibody to BT virus serotypes 12, 14 and 17, and EHD virus serotypes 1 and 2. Insect captures showed a high association of *Culicoides insignis* with infected cattle.

INTRODUCTION

Bluetongue (BT) virus is thought to be distributed in the Americas between the latitudes of approximately 49° N and 40° S. However, reports of bluetongue disease in cattle are derived from the extremes of this range (Sellers, 1981; Tamayo, Alonso & Schoebitz, 1983). Recent studies in the Caribbean, Central and South America indicate high prevalence of BT group-specific antibody in the American tropics, but there is no report of clinical manifestations of the infection (Gibbs *et al.* 1983*a*; Gumm *et al.* 1984; Homan *et al.* 1984). EHD virus has been isolated from cattle in the United States and Canada (Sellers, 1981). In the American tropics antibody to EHD has been detected in cattle in the Caribbean islands, Guyana and Suriname (Gumm *et al.*, 1984) and Costa Rica (Homan & Taylor, unpublished observations). EHD and BT viruses are antigenically differentiable by neutralization tests, but in tests detecting group-specific antibody, such as agar gel precipitation, varying

degrees of cross-reaction may occur (Jochim, 1976; Della-Porta, McPhee & Parsonson, 1983). In the absence of acute cases of clinical BT and EHD for virus isolation attempts and of adequate facilities for orbivirus isolation and typing, a serological approach may yield valuable indicators of the virus types circulating in an area (Gumm *et al.* 1984). Mature animals that have been exposed to reinfection with several BT types may develop broadly cross-reactive neutralizing antibodies, making difficult an interpretation of the virus types present. However, young animals experiencing their first BT infection usually develop a clear monospecific response to the infecting virus type (Jeggo, Gumm & Taylor, 1983; Gumm *et al.* 1984). Sequential sampling can identify the time period in which infection occurs. This approach was adopted in attempting to determine the BT and EHD virus serotypes circulating in northern Colombia, in order to relate these findings to information available on serotypes prevalent in the Caribbean.

Despite the high prevalence of BT in tropical America, the vectors have not been determined. The *Culicoides variipennis* complex, which is the principal vector of BT and EHD in the United States, has not been reported south of Mexico (Wirth & Jones, 1957; Blanton & Wirth, 1979). Our earlier survey of BT antibody prevalence in northern Colombia had indicated a close inverse association with altitude (Homan *et al.* 1984) and it is to be expected that transmission patterns would differ with altitude.

A prospective study was therefore established at two altitudinally different locations in the department of Antioquia. Young cattle were sampled sequentially to determine (1) the time of appearance of BT and EHD antibodies, (2) the virus types infecting the cattle serologically, and (3) the *Culicoides* spp. populations associated with these cattle.

MATERIALS AND METHODS

Animal sampling

Sera were collected between June 1981 and October 1983 from dairy cattle in a herd located in the Valle de Aburra, at 1500 m above sea level. This herd is predominantly Holstein and Ayrshire, and all study animals were of these breeds. Both natural service and artificial insemination were in use. Calving occurred all through the year. The cows and heifers over seven months of age were tied up in covered stalls during the day and pastured at night on riverside fields. Young calves were housed in individual concrete pens which were roofed, but open sided.

A group of mature cows 3–13 years old was sampled over a 15-month period. Twenty heifer calves born from 1981 onwards were bled within a month of birth and thereafter at intervals of one to six months. Samples were not obtained before the ingestion of colostrum. Nine additional heifers aged 2–18 months at initiation of the study were also sampled.

Blood samples were collected in June 1982 and November 1983 from zebu cattle aged 9 months–2 years in a herd in the Caucasia region of Antioquia, at 500 m above sea level. These animals were maintained on range. Blood was collected by jugular venepuncture into evacuated tubes and serum separated within 24 h for storage at −10 °C until tested.

Serological tests

Undiluted serum samples were tested initially by the micro agar gel precipitation test (Jochim, 1976) to determine the presence of group-specific antibodies using BT antigen (produced in Vero cells) and control antisera provided by National Veterinary Services Laboratory, Ames, and subsequently by the more sensitive standard agar gel precipitin test (Pearson & Jochim, 1979) to detect the time of seroconversion, using BT antigen prepared in BHK cells by the Animal Virus Research Institute, Pirbright. Because many of the animals were vaccinated with foot and mouth disease vaccine propagated in BHK cells, controls were included to detect antibodies to those cells.

Type-specific antibodies to BT and EHD were determined by the microneutralization test (Herniman, Boorman & Taylor, 1983). This test was used only for sera of animals two years of age or less.

Insect collections

At the Valle de Aburra site two methods of insect capture were used. Initially, captures were made by aspiration of *Culicoides* species as they alighted to feed on cows held in the stalls between 18.00 h and 21.00 h. These captures were made over a total of 54 h between January and October 1982. Flies were preserved by discharging the aspirator into 70% alcohol. From July 1982 to October 1983 an adapted CDC light trap was used at this site for 73 trap nights. In place of the usual netting bag, a funnel was constructed leading into a detachable bottle of 70% alcohol. The trap was suspended at approximately 2 m height at the side of the cow stalls, and sources of competing light were eliminated. On 14 of the nights, light trapping was carried out in riverside pastures. Collections were made by one or both methods on an average of one night a week.

At the Caucasia site captures by aspiration were made for a total of 6 h between 17.30 h and 20.30 h using zebu calves as bait animals within corrals. Light trapping from 17.00 h to 06.00 h on four nights was done adjacent to corrals.

The species of *Culicoides* were identified (Wirth & Blanton, 1959, 1974) and representative specimens forwarded to Dr Willis Wirth (U.S. Department of Agriculture Systematic Entomology Laboratory at the National Museum) for confirmation of identification.

RESULTS

Antibody studies

The overall prevalence of BT group-specific antibodies in the Valle de Aburra herd from which the sample groups were derived was 56% (48 of 86 adult dairy cattle). Nine of 13 cows gave consistently positive AGPT reactions throughout a 15-month period. One was negative at all samplings. Three cows for which initial samplings were positive were occasionally negative on subsequent tests.

Of the 20 calves sampled from birth onwards, 14 showed maternal antibody in the initial sample. In 11 of these animals, sampling series permitted calculation of mean duration of maternal antibody as 2·2 months (range 0·5–5·5 months). Of 29

Table 1. *Period during which neutralizing antibody titre was acquired*

Calf	Month of birth	Bluetongue types				EHD types	
		12	14	17	Untyped	1	2
1	Jan. 1980	—	June–Dec. 1981 (320)	June-Dec. 1981 (120)	—	—	—
2	June 1980	—	—	—	June–Nov. 1981	—	—
3	Aug. 1980	—	by June 1981 (> 640)	—	—	—	June–Dec. 1981 (240)
4	Aug. 1980	—	June–Nov. 1981 (240)	—	—	—	—
5	Sep. 1980	—	June–Nov. 1981 (320)	—	—	—	—
6	Oct. 1980	—	June–Dec. 1981 (160)	—	—	—	—
7	Apr. 1981	Jan.–Feb. 1982 (480)	—	Jan.–Feb. 1982 (60)	—	—	Jan.–Mar. 1982 (120)
8	June 1981	—	—	Feb.-Mar. 1982 (60)	—	July-Aug. 1982 (160)	—
9	July 1981	—	—	—	Oct.–Nov. 1982	Nov. 1982–Oct. 1983 (120)	—
10	Nov. 1981	—	—	June–Aug. 1982 (160)	—	June–Aug. 1982 (160)	—
11	Jan. 1982	—	—	Oct.–Nov. 1982 (> 640)	—	—	—
12	Jan. 1982	Nov.–Dec. 1982 (640)	—	—	—	—	—
13	Feb. 1982	—	—	Dec. 1982–June 1983 (320)	—	—	—
14	Mar. 1982	—	—	June–Oct. 1983 (30)	—	—	—
15	Mar. 1982	—	—	—	Dec. 1982–June 1983	—	—
16	Mar. 1982	—	—	—	Dec. 1982–June 1983	—	—
17	Apr. 1982	—	—	Dec. 1982–June 1983 (> 640)	—	—	—
18	Apr. 1982	—	—	—	Nov. 1982–June 1983	—	—
19	Apr. 1982	—	—	—	Nov. 1982–June 1983	—	—
20	May 1982	—	—	—	Dec. 1982–June 1983	—	—
21	June 1982	—	—	—	June–Oct. 1983	—	—
22	July 1982	—	—	Dec. 1982–June 1983 (30)	—	June–Oct. 1983 (160)	—
23	Aug. 1982	June–Oct. 1983 (20)	—	—	—	June–Oct. 1983 (60)	—
25	Aug. 1982	—	Dec. 1982–Oct. 1983 (20)	Dec. 1982–Oct. 1983 (20)	—	Dec. 1982–Oct. 1983 (80)	—
26	June 1982	Dec. 1982–Oct. 1983 (120)	—	—	—	—	—
27	Nov. 1982	—	—	—	Dec. 1982–Oct. 1983	Dec. 1982–Oct. 1983 (240)	—

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Table 1 (*cont.*)

Calf	Month of birth	Bluetonge types				EHD types	
		12	14	17	Untyped	1	2
28	Nov. 1982	—	—	—	—	Dec. 1982–Oct. 1983	—
29	Oct. 1982	—	Dec. 1982–Oct. 1983 (240)	—	—	—	—
32	Dec. 1982	—	—	—	—	Dec. 1982–Oct. 1983 (480)	—
35	Dec. 1982	—	—	—	—	Dec. 1982–Oct. 1983 (120)	—

Figures in parentheses indicate reciprocal of neutralization titre.

calves and heifers, 28 developed active AGPT antibody to BT virus which was first detected at a mean age of 13·8 months (range 8–23 months).

Serum neutralization tests identified types of BT and EHD virus to which seroconversion had occurred. In the Valle de Aburra BT types 14 and 17 were present in 1981, types 12 and 17 in 1982 and types 12, 14 and 17 in 1983. At this location two seroconversions to EHD type 2 occurred in the period June 1981–March 1982 and nine to EHD type 1 between June 1982 and October 1983. Seroconversions to both BT and EHD occurred in nine calves. In addition, antibodies in several animals could not be related to one of the recognized BT types. Distribution of seroconversions over time is shown in Table 1.

In Caucasia, the prevalence of precipitin antibodies to BT in 49 cattle two or more years of age was 78 %. Two groups of animals 9–20 months old were bled once each. BT group-specific antibody was found in 7 of 8 and 27 of 28 individuals.

In this herd, neutralizing antibody to BT types 12, 14 and 17 was present in 1981–2. In 1983 no clearcut BT typing was obtained. Antibodies to EHD type 1 were detected in three calves and to EHD type 2 in one calf, all of which were exposed to infection in the period June 1982–November 1983.

Insect collections

At the Valle de Aburra site 55 light-trap collections included *Culicoides* spp. The 837 *Culicoides* from light-trap collections were identified. With the exception of 12 individuals, all of these were *C. insignis* (Lutz). Of 111 aspirated individuals, 109 were *C. insignis*. Remaining individuals from the two types of capture included *C. limai* (Barreto), *C. debilipalpis* (Lutz), *C. lutzi* (Costa Lima), *C. pusillus* (Lutz), *C. filariferus* (Hoffman) and *C. santanderi* (Brown). No difference was observed in the composition of collections obtained in pastures as opposed to cow stalls.

At the Caucasia site, 648 *Culicoides* were obtained on a total of four trap nights. With the exception of 34 individuals, all were *C. insignis*. Remaining species included *C. filariferus*, *C. pseudodiabolicus* (Fox) and *C. castillae* (Fox). Of 21 aspirated individuals, all except three were *C. insignis*, and the remaining specimens included one each of *C. pseudodiabolicus*, *C. jamaicensis* (Edwards) and *C. filariferus*.

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DISCUSSION

The presence of antibodies to BT types 12, 14 and 17 and EHD types 1 and 2 in northern Colombia is consistent with previous findings of antibody to the same types of BT, and EHD type 2 in the Caribbean islands, and types 14 and 17 and EHD types 1 and 2 in Guyana and Suriname (Gumm *et al.* 1984). These authors also found antibodies to BT types 1 and 6. The presence of antibodies to BT types 6, 12, 14 and 17 in Costa Rica has also been reported (Homan *et al.* 1984). It is of interest that of these serotypes only type 17 is found in the United States, where types 2, 10, 11 and 13 also occur (Barber, 1979; Gibbs *et al.* 1983*b*). This suggests that the countries bordering the Caribbean may form part of a separate and self-contained ecosystem when BT serotypes are considered. Further study is needed in these countries to determine how stable this pattern is over time.

Ten animals in the upland Valle de Aburra herd acquired precipitating antibody (eight of them in 1983) that could not be typed in the neutralization test using the recognized 22 BT serotypes. It is possible that samples were taken too early or too late in the development of a virus neutralizing response to permit typing; or alternatively that an additional virus type may be circulating. The sequence of antibody types found, although with limited numbers of animals, suggests that different serotypes of BT and EHD may alternate in a herd as new groups of susceptible animals become available. In determining serotype, we are dependent on the recognition of protein P 2 (Huismans & Erasmus, 1981). With the possibility that reassortment of the segmented genome takes place (Sugiyama, Bishop & Roy, 1981, 1982), the serotype is not necessarily an indication of the content of the remainder of the genome; other phenotypic properties of the virus, such as virulence, could vary although the serotype remains the same. Dual infections are probably a common occurrence in an endemic area where vectors are abundant. Individual animals differ in their antibody response to BT infection (Taylor, unpublished observations). However, in several of the study animals neutralization titres exceeded 640.

On the two farms there was a strong association of *C. insignis* (98% of all *Culicoides* captured) with infected cattle. However, the status of this species as a BT or EHD vector here remains to be established by virus isolation and transmission. Had aspiration been carried out at other times of the day, it is possible that other species would have been encountered in significant numbers (G. A. Mullen, personal communication). Nevertheless, *C. insigis*, which is distributed from Florida and Mexico through Central America to Brazil and Argentina (Wirth & Blanton, 1974), has been identified as a likely BT vector in Florida and in the Caribbean (Gibbs & Greiner, 1983; Greiner *et al.* 1984). While *Culicoides* were captured by light trap in high numbers at the lowland Caucasia site, light-trap captures at the Valle de Aburra site did not indicate a very high density of *Culicoides* and thus would suggest a very high vector efficiency. Results of the insect captures and seroconversions were compared but failed to yield any clear seasonal associations; *Culicoides* were captured at all times of the year and seroconversions occurred irrespective of season.

More complete information on distribution of BT and EHD serotypes may

have utility in regulating international trade in livestock. Countries having identical serotypes may wish to reconsider trade restrictions to accomplish control of BT or EHD.

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REFERENCES

BARBER, T. L. (1979). Temporal appearance, geographic distribution and species of origin of bluetongue virus serotypes in the United States. *American Journal of Veterinary Research* **40**, 1654–1656.

BLANTON, F. S. & WIRTH, W. W. (1979). The sandflies (*Culicoides*) of Florida. Arthropods of Florida, Vol 10. Florida Department of Agriculture and Consumer Service, p. 204.

DELLA-PORTA, A. J. MCPHEE, D. A. & PARSONSON, I. M. (1983). *Biochemical and serological comparisons of Australian orbiviruses: do we need sub-genera?* In *Double stranded RNA viruses* (R. W. Compans, D. H. L. Bishop), pp. 343–345. New York: Elsevier Science Publishing Co., Inc.

GIBBS, E. P. J. & GREINER, E. C. (1983). Bluetongue infections and *Culicoides* species associated with livestock in Florida and the Carribbean region. In *Double stranded RNA viruses* (ed. R. W. Compans, D. H. L. Bishop), pp. 375–382, Elsevier Science Publishing Co., Inc.

GIBBS, E. P. J., GREINER, E. C., ALEXANDER, F. C. M., KING, T. H. & ROACH, C. J. (1983a). Serological survey of ruminant livestock in some countries of the Caribbean region and South America for antibody to bluetongue virus. *Veterinary Record* **113**, 446–449.

GIBBS, E. P. J., GREINER, E. C., TAYLOR, W. P., BARBER, T. L., HOUSE, J. A. & PEARSON, J. E. (1983b). Isolation of bluetongue virus serotype type 2 from cattle in Florida: Serotype of bluetongue virus hitherto unrecognized in the Western Hemisphere. *American Journal of Veterinary Research* **44**, 2226–2228.

GREINER, E. C., BARBER, T. L., PEARSON, J. E., KRAMER, W. L. & GIBBS, E. P. J. (1984). Orbiviruses isolated from *Culicoides* in Florida. In *Bluetongue and related Orbiviruses* (ed. T. L. Barber, M. M. Jochim). New York: Allan Liss Press. (In the press).

GUMM, I. D., TAYLOR, W. P., ROACH, C. J., ALEXANDER, F. C. M., GREINER, E. C. & GIBBS, E. P. J. (1984). A serological survey of ruminant livestock in some countries of the Caribbean and South America for type specific antibodies to bluetongue and epizootic hamorragic disease of deer virus. *Veterinary Record* **114**, 635–638.

HERNIMAN, K. A. J., BOORMAN, J. P. T. & TAYLOR, W. P. (1983). Bluetongue virus in a Nigerian dairy cattle herd 1: Serological studies and correlation of virus activity to vector population. *Journal of Hygiene* **90**, 177–193.

HOMAN, E. J., LORBACHER, H., DONATO, A. P. & YUILL, T. M. (1984). A preliminary survey of the epidemiology of bluetongue in Costa Rica and Northern Colombia. *Journal of Hygiene* (In the press).

HUISMANS, H. & ERASMUS, B. J. (1981). Identification of the serotype specific and group specific antigens of bluetongue virus. *Onderstepoort Journal of Veterinary Research* **48**:51–58.

JEGGO, M. H., GUMM, I. D. & TAYLOR, W. P. (1983). Clinical and serological response of sheep to serial challenge with different bluetongue virus types. *Research in Veterinary Science* **34**, 205–211.

JOCHIM, M. M. (1976). Improvement of the agar gel precipitation test for bluetongue. *American Association of Veterinary Laboratory Diagnosticians, 19th Annual Proceedings*, 361–376.

PEARSON, J. E. & JOCHIM, M. M. (1979). Protocol for the immunodiffusion test for bluetongue. *American Association of Veterinary Laboratory Diagnosticians 22nd Annual Proceedings*, 463–471.

SELLERS, R. F. (1981). Bluetongue. In *Virus Diseases of Food Animals* Vol. 2 (ed. E. P. J. Gibbs), pp. 567–584. London: Academic Press.

SUGIYAMA, K., BISHOP, D. H. L. & ROY, P. (1981). Analysis of the genomes of bluetongue viruses recovered in the United States 1: Oligonucleotide fingerprint studies that indicate existence of naturally occurring reassortment BTV isolates. *Virology* **114**, 210–217.

SUGIYAMA, K., BISHOP, D. J. & ROY, P. (1982). Analysis of the genomes of bluetongue viruses recovered from different states of the United States and at different times. *American Journal of Epidemiology* **115**, 332–347.

TAMAYO, R., ALONSO, T. M. & SCHOEBITZ, R. (1983). Anticuerpos de lengua azul (bluetongue) en bovinos: primer informe en Chile. *Archivos de Medicina Veterinaria* (Valdivia, Chile) **15**, 49.

WIRTH, W. W. & BLANTON, F. S. (1959). Biting midges of the genus *Culicoides* (Diptera, Helidae) from Panama. Proceedings of the United States National Museum.

WIRTH, W. W. & BLANTON, F. S. (1974). The West Indian sandflies of the genus *Culicoides* (Diptera, Ceratopogonidae) United States Department of Agriculture, Agricultural Research Service Technical Bulletin **1474**.

WIRTH, W. W. & JONES, R. H. (1957). The North American Subspecies of *Culicoides variipennis* (Diptera: Helidae), United States Department of Agriculture Technical Bulletin **1170**, 1–35.