J. Hyg., Camb. (1985), **94**, 357–363
Printed in Great Britain

A preliminary survey of the epidemiology of bluetongue in Costa Rica and Northern Colombia

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(*Received* 3 *September* 1984; *accepted* 30 *November* 1984)

SUMMARY

Recent evidence of bluetongue (BT) virus infection of livestock in scattered localities in the neotropics prompted a serologic survey of cattle in Colombia and Costa Rica. In Costa Rica 48·1 % of 1435 bovine animals had BT virus antibody in the agar gel precipitation test (AGPT). In Colombia 51·8 % of 635 cattle were AGPT-positive for BT virus. Antibody prevalence ranged from over 50 % in the lowlands to 0 % in Costa Rica and 19 % in Colombian cattle above 2000 m altitude. Neutralization tests indicated that Costa Rican cattle had been exposed to BT virus types 6, 12, 14 and 17.

INTRODUCTION

Viruses of the bluetongue (BT) complex infect sheep, cattle and other ruminants. In sheep, some strains of BT virus produce acute disease (Erasmus, 1975). BT infection of cattle is usually inapparent. However, it may produce fever with ulceration and erosion of the oral mucosa and muzzle, accompanied by laminitis and lameness (Hourrigan & Klingspoorn, 1975). Infection of pregnant cows can result in abortion and resorption of the foetus, or mild teratogenic effects (Luedke, Jochim & Jones, 1977). Virus has been isolated from bovine semen, which may be an alternative mode of transmission (Breckon, Luedke & Walton, 1980).

The principal mode of BT virus transmission is by bite of *Culicoides* midges (Luedke, Jones & Jochim, 1967). Cows may be viraemic for extended periods after exposure (Luedke, Jochim & Jones, 1969). The virus remains intimately associated with red blood cells.

BT virus-associated disease in cattle of temperate latitudes has been reported in the United States (Hourrigan & Klingspoorn, 1975), South Africa (Bekker, deKock & Quinlan, 1934) and the Mediterranean countries (Lopez & Botija, 1958; Sellers, 1975). The distribution of the disease in sheep or the occurrence of antibodies to the virus in cattle includes tropical areas of Africa (Davies, 1978), India and Pakistan (Sellers, 1981) and Northern Australia (Coakley, Smith & Maker, 1980). Until recently, of 22 recognized BT virus serotypes, only 4 (types

10, 11 13 and 17) had been found in the United States (Barber, 1979). A fifth serotype, type 2, was isolated in Florida in 1982 (Gibbs *et al.* 1983*b*). The distribution of this serotype outside this state is not yet known. An additional serotype was isolated from a bull from Brazil while in quarantine (Groocock & Campbell, 1982). Further information concerning the distribution of BT in the tropical Americas is derived from the distribution of antibodies to BT. Gibbs *et al.* (1983*a*) report high prevalences of group-specific antibody in cattle, sheep and goats in some islands in the Caribbean as well as Guyana and Suriname. Neutralization tests on the serum of young cattle in these countries have indicated the presence of antibodies to serotypes 6, 12, 14 and 17 (Gumm *et al.* 1984). Group-specific antibodies were also reported in Mexico (Suzan *et al.* 1983), in Peru (Rosadio, Evermann & Di Martini, 1984) and in Chile (Tamayo, Alonso & Schoebitz, 1982). Nicaragua has reported the occurrence of suspected cases in sheep (FAO, 1982). A high prevalence of group-specific antibodies was reported in slaughter cattle in Puerto Rico (Metcalf, Pearson & Klingspoorn, 1981). The present study was undertaken to determine if cattle in Costa Rica and Northern Colombia are being infected by BT viruses, and if so, with what relative frequency.

MATERIALS AND METHODS

Geographical distribution

The two areas studied lie between the latitudes of 5° 50′ and 11° 15′ north, and comprised the Republic of Costa Rica and the Colombian departments of Antioquia and Cordoba. The study areas are separated by the Darien gap in Panamá and the Atrato River, major ecological barriers between Central and South America. Each area was subdivided into eight regions defined by ecological considerations (Table 1).

Retrospective antibody prevalence study

Sera were collected and tested in Colombia over the period March 1981–December 1982, and in Costa Rica between September 1981 and December 1982. In Colombia, blood collected in the field by jugular venepuncture into evacuated tubes was transferred to our laboratories there for separation within 2–3 days. In addition, 107 blood samples were collected at slaughter in the Medellin municipal abattoir. In Costa Rica approximately half of the blood samples were collected into evacuated tubes and shipped directly to our laboratory in Heredia for separation. The remaining sera were separated in the regional laboratories of the Ministry of Agriculture and forwarded frozen to our laboratory. Sera were stored at −10 °C until tested. In both countries several animals, usually 10–20, were sampled from each herd. Questionnaires were completed detailing the topography of each farm and the composition, management and clinical history of each herd.

Serological tests

Group-specific antibody. A micro modification of the agar gel precipitation technique (AGPT) (Jochim, 1976) was used. A rosette of seven wells 1·5 mm diameter and 2·5 mm apart were drilled in plexiglass templates. These were supported by tape 0·35 mm thick on microscope slides. The intervening space was

Table 1. *Climatic characteristics and prevalence of antibodies to bluetongue virus in subregions of study areas in Costa Rica and the Colombian departments of Antioquia and Cordoba*

	Climatic parameters (annual means)			Prevalence		
	Temperature (°C)	Rainfall (mm)	Relative humidity (%)	No. of farms sampled	Animals seropositive/ animals tested	Prevalence (%)
Costa Rica*						
Cordillera de Talamanca	19·0	2112	80	11	10/68	15
Cordillera Central	19·0	2750	84	24	43/196	22
Puntarenas, Panama border	27·5	4823	86	36	180/391	46
Meseta Central	19·7	1978	83	21	116/252	46
Puntarenas coastal strip	27·0	2445	78	10	79/143	55
Guanacaste	26·8	2225	76	16	151/228	66
Limon	24·6	4421	88	10	76/110	69
San Carlos	23·0	4529	86	5	35/47	75
Colombia†						
Yarumal	17·2	2779	—	5	4/85	5
Rio Negro	16·1	1901	67	6	33/80	42
Frontino	—	2810	—	7	50/101	50
Uraba	27·2	2401	85	4	8/14	57
Aburra	22·2	1560	67	3	66/108	62
Caucasia	27·2	3425	82	6	112/175	64
Monteria	27·1	1152	83	2	32/48	67
Fredonia	—	2415	—	1	24/24	100

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Table 2. *Relation of bluetongue antibody prevalence to altitude*

Altitude (m)	No. of farms	Total animals		Antibody prevalence (%)	95% confidence interval
		Tested	Positive		
		Costa Rica			
0–5000	46	571	349	61	57–65
501–1000	28	257	139	54	47–60
1001–1500	26	306	133	44	50–62
1501–2000	27	233	69	30	24–36
2001 and over	6	68	0	0	0– 6
		Colombia			
0–1000	13	261	176	67	61–73
1001–2000	12	246	129	53	47–59
2001 and over	9	128	24	19	13–27

filled with 0·9% agarose with 0·85% sodium chloride and 0·01% sodium azide. Antigen was placed in the centre well and the peripheral wells contained alternately test sera and control positive sera. Bluetongue group-specific soluble antigen and reference antisera were provided by the National Animal Disease Laboratory, Ames, Iowa. Slides were incubated at 8 °C for 72 h then washed in 0·85% sodium chloride. They were then stained with 0·1% thiazine red in 1·0% acetic acid, decolorized in 1·0% acetic acid and allowed to dry.

Type-specific antibody. Serum neutralization tests were performed on sera of Costa Rica cattle aged 6 months to 2 years. Tests were performed at the Animal Virus Research Institute, Pirbright, England, using a microneutralization test previously described (Herniman, Boorman & Taylor, 1983).

Statistics

Differences in prevalence between different zones were analysed by the Chi-square test.

RESULTS

In Costa Rica 1435 bovine sera were tested of which 690 (48·1%) gave positive AGPT reactions with BT virus antigen. In Colombia 635 bovine sera were tested, of which 329 (51·8%) gave positive reactions. The distribution of samples and antibody prevalence by region are shown in Table 1. Serological evidence of exposure to infection was widely distributed in both countries. Areas of highest antibody prevalence were those with hot humid climates. A highly significant ($P < 0·001$) inverse association of antibody prevalence with altitude was seen (Table 2). Below 500 m prevalence was greater than 50%. Prevalence was low (0% Costa Rica, 19% Colombia) above 2000 m. All cows tested from over 2500 m were negative. Apparent differences of prevalence between different breeds of cattle (Zebu–Brahma *vs* Holstein) could not be dissociated from differences in prevalence attributable to altitude. No significant differences were demonstrated between herds using natural service, artificial insemination or both.

Neutralization tests of 87 sera from seven Costa Rican farms showed two farms with clusters of antibody to type 14. Thirteen of 17 animals on one farm located on the Puntarenas coastal plain had antibodies to type 14 at a titre of 20, and

seven, a titre of $\geqslant 40$. Four of 10 animals from a second farm in the San Carlos region had antibody titres of $\geqslant 40$. In addition, one animal on the second farm was found to have a monospecific titre of 240 to type 6. Two animals at a farm in the Puntarenas–Panama border area had monospecific titres, one to type 17(640) and the other to type 12(120).

DISCUSSION

In Costa Rica and northern Colombia there is widespread distribution of antibodies indicative of exposure of cattle to BT group virus infection. The AGPT does not permit the differentiation of BT virus serotypes, and it cannot be used to determine whether the seropositive animals in these countries are carriers of recognized Western Hemisphere or other serotypes of BT or related orbiviruses. As latently infected animals may have fluctuating titres, and in view of the relative insensitivity of the AGPT, it is likely that the true BT prevalence has been underestimated.

The close association of BT antibody prevalence with altitude is very likely attributable to the densities of vector species. High densities of midges are probably needed to maintain the virus (Sellers, 1981). The tendency of different breeds of cattle to be distributed according to climatic zones, with *Bos indicus* breeds being found in large numbers only below 1000 m, results in a high BT prevalence in these breeds. The few Holstein cattle in Costa Rica that were sampled at low altitudes also were found to have a high prevalence of BT antibodies.

In neither Costa Rica nor Colombia are there reports of clinical BT disease in cattle, nor have virus isolates been made. Interestingly, the other reports of BT infection in the neotropics do not mention clinical disease in cattle, either. There are a number of factors which could contribute to the lack of clinical reports in Costa Rica and Colombia. Both countries are endemic for vesicular stomatitis virus, and Antioquia and Cordoba are within the areas of Colombia where foot and mouth disease occurs. Clinical BT in adult cattle must be differentiated from both these vesicular diseases (Hourrigan & Klingspoorn, 1975). It is likely that the reproductive and teratogenic effects of the virus would be under-reported, especially under the extensive ranching conditions of the hotter regions of the study areas. No detailed study has been carried out to determine the pathogenesis of bluetongue in *Bos indicus* breeds to determine if they are equally prone to clinically apparent manifestations, as are *Bos taurus* breeds. A lack of awareness by local veterinarians of the syndromes produced by BT virus infection is another factor. Costa Rica and northern Colombia both have very few sheep. The absence of this sentinel species for BT virus disease may contribute to the lack of clinical evidence of the infection. The apparent endemicity of BT in these countries should be considered a potential threat to sheep imported from non-endemic areas, similar to that encountered in endemic areas of Africa (Davies & Walker, 1974). Both countries have populations of deer. The contribution of these wild ruminants to the epidemiology of the virus is not known, but five of six whitetail deer (*Odiocoileus virginiana*) sampled in Costa Rica were AGPT positive.

Evidence was found for the presence of serotypes 14 and 6 during 1982–3 in Costa Rica. These serotypes were also found by Gumm *et al.* (1984) in the Caribbean

Islands. These workers also described the use of clusters of antibodies in a herd as an indicator of BT serotype activity.

Two major ecological barriers separate Costa Rica and Colombia – the Darien rain forest and the Atrato River swamps. Given the importance of the well-developed livestock industry on both sides of these barriers, the apparent endemicity of BT infection in cattle populations in Colombia and Costa Rica, and the rapid ecological changes associated with deforestation, agricultural development, and construction of the Pan American Highway across the Darien, studies are needed in this geographic area to address BT virus serotype distribution, transmission and pathogenesis.

This study was carried out as part of co-operative research and training agreements between the University of Wisconsin and the University of Antioquia and the National University of Costa Rica. The authors acknowledge the assistance of veterinarians of the Colanta Milk Cooperative and field veterinarians of the Costa Rican Ministry of Agriculture, as well as Drs Ignacio Correa, Oscar Bonilla, Fabio Zuluaga, Bolivar Suarez, Carlos Jiminez, Rolando Quesada and Oscar Johanning in the collection of serum samples. Maria Cristina Gallon gave expert technical assistance. Serum neutralization tests were performed at the Animal Virus Research Institute, Pirbright, England by Ian Gumm. Partial support was provided by each of the institutions involved and in Costa Rica by a USAID Title XII Strengthening Grant to the University of Wisconsin, College of Agriculture and Life Sciences.

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.

BEKKER, J. G., DEKOCK, G. W. & QUINLAN, J. B. (1934). The occurrence and identification of bluetongue in cattle: the so-called pseudo foot and mouth disease in South Africa. *Ondestepoort Journal of Veterinary Science and Animal Industry* **2**, 393–507.

BRECKON, R. D., LUEDKE, A. J. & WALTON, T. E. (1980). Bluetongue virus in bovine semen; virus isolation. *American Journal of Veterinary Research* **41**, 439–442.

COAKLEY, W., SMITH, V. W. & MAKER, D. (1980). A serologic study for bluetongue virus antibody in Western Australia. *Australian Veterinary Journal* **56**, 487–491.

DAVIES, F. G. (1978). Bluetongue studies with sentinel cattle in Kenya. *Journal of Hygiene* **80**, 197–204.

DAVIES, F. G. & WALKER, A. R. (1974). The distribution in Kenya of bluetongue virus and antibody and the *Culicoides* vector. *Journal of Hygiene* **72**, 265–272.

ERASMUS, B. J. (1975). Bluetongue in sheep and goats. *Australian Veterinary Journal* **51**, 165–170.

FOOD AND AGRICULTURE ORGANIZATION (1982). *FAO-WHO-OIE Animal Health Yearbook*. 58.

GIBBS, E. P. J., GREINER, E. C., ALEXANDER, F. C. M. & KING, J. H. (1983*a*). A 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. (1983*b*). Isolation of bluetongue virus serotype 2 from cattle in Florida: serotype of bluetongue virus hitherto unrecognized in the Western Hemisphere. *American Journal of Veterinary Research* **44**, 2226–2228.

GROOCOCK, C. M. & CAMPBELL, C. H. (1982). Isolation of an exotic serotype of bluetongue virus from imported cattle in quarantine. *Canadian Journal of Comparative Medicine* **46**, 160–164.

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 antibody to bluetongue and epizootic haemorrhagic disease of deer viruses. *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.

HOURRIGAN, J. L. & KLINGSPOORN, A. L. (1975). Bluetongue: the disease in cattle. *Australian Veterinary Journal* **51**, 170–174.

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

LOPEZ, A. C. & BOTIJA, C. S. (1958). Epizootie de fièvre catarrhale ovine en Espange. *Bulletin de l'Office International des Epizooties* **50**, 65–93.

LUEDKE, A. J. & JOCHIM, M. M. (1977). Bluetongue in cattle: effects of *Culicoides variipennis* transmitted bluetongue virus on pregnant heifers. *American Journal of Veterinary Research* **38**, 1687–1695.

LUEDKE, A. J., JOCHIM, M. M. & JONES, R. H. (1969). Bluetongue in cattle: viremia. *American Journal of Veterinary Research* **30**, 511–516.

LUEDKE, A. J., JOCHIM, M. M. & JONES, R. H. (1977). Bluetongue in cattle: effects of vector transmitted bluetongue virus on calves previously infected in utero. *American Journal of Veterinary Research* **38**, 1697–1700.

LUEDKE, A. J., JONES, R. H. & JOCHIM, M. M. (1967). Transmission of bluetongue between sheep and cattle by *Culicoides variipennis*. *American Journal of Veterinary Research* **28**, 457–469.

METCALF, H. E., PEARSON, J. E. & KLINGSPOORN, A. L. (1981). Bluetongue in cattle: a serologic survey of slaughter cattle in the United States. *American Journal of Veterinary Research* **42**, 1057–1061.

ROSADIO, R. F., EVERMANN, J. F. & DI MARTINI, J. C. (1984). A preliminary serological survey of viral antibodies in Peruvian sheep. *Veterinary Microbiology.* **10**, 91–96.

SELLERS, R. F. (1975). Bluetongue in Cyprus. *Australian Veterinary Journal* **51**, 198–203.

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

SUZAN, V. M., ONUMA, M., AGUILAR, R. E. & MURAKAMI, Y. (1983). Prevalence of bovine herpesvirus-1, parainfluenza-3, bovine rotavirus, bovine viral diarrhea, bovine adenovirus 7, bovine leukemia virus and bluetongue virus antibodies in Mexico. *Japanese Journal of Veterinary Research* **31**, 125–132.

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