

## BLUETONGUE IN CATTLE: TYPING OF VIRUSES ISOLATED FROM CATTLE EXPOSED TO NATURAL INFECTIONS

---

N. C. OWEN, R. M. DU TOIT and P. G. HOWELL, Veterinary Research Institute, Onderstepoort

---

In an attempt to elucidate the importance of bovines in the epizootiology of bluetongue, an investigation was undertaken whereby the incidence of this virus in cattle was determined. The general procedure and epizootiological findings of this investigation have been reported by Du Toit (1962). In his experiments, a number of bovines exposed to natural infection were bled at regular intervals into 10 per cent sodium citrate, and these samples injected into bluetongue susceptible sheep. The reacting sheep were bled into oxalated-carbol-glycerin solution (O.C.G.\*) during the febrile phase and the blood submitted for virus isolation and typing.

This paper describes the laboratory investigations with the virus types represented by the strains isolated.

To date 16 immunologically distinct bluetongue virus types have been established by serum-virus neutralization at this laboratory. Twelve of these types have already been described by Howell (1960), while the remaining four types (13, 14, 15 and 16) will be described shortly.

The object of this phase of the investigation was to determine whether bovines could be incriminated as bluetongue virus reservoirs, thereby accounting for the seasonal re-occurrence of the disease. For this purpose, five of the eight bovines used during the 1959/1960 bluetongue season (Du Toit) were selected at random and virus isolations made from the sheep which had received their blood.

### EXPERIMENTAL PROCEDURE

Virus isolations were made from the blood of the reacting sheep by initial propagation in eggs (Alexander, 1947) and adaptation to growth in primary lamb kidney cell cultures (Haig, McKercher & Alexander, 1956).

Primary lamb kidney cell cultures were prepared by standard techniques in roller tubes. The maintenance medium consisted of Hank's balanced salt solution containing 10 per cent bovine serum, 0.5 per cent lactalbumin hydrolysate and 0.01 per cent yeastolate.

The virus types were identified by the screen serum-virus neutralization technique described by Howell (1960) and, where possible, the typing confirmed by demonstrating a significant increase in homologous antibodies in the serum of the sheep from which the particular virus had been isolated.

---

\* O.C.G.: 500 ml. glycerine; 5 ml. carboric acid; 5 gm. potassium oxalate; and 500 ml. water.

Received for publication on 14 January, 1965.—Editor

## BLUETONGUE IN CATTLE

### EXPERIMENTAL RESULTS

Bluetongue virus was demonstrated in the blood of all five bovines as judged by the reactions in sheep. A total of 46 virus isolations was made from these reacting sheep over a period of 18 months.

The first isolation was made from a sheep that had received blood from bovine 9952 on 27 January, 1960. Thereafter circulating bluetongue viruses could be demonstrated in all the bovines at intervals until March, 1960. Three bovines showed circulating virus during April and one (254) gave a positive reaction in a sheep on 29 May, 1960. No further reactions occurred in sheep until 26 October, 1960 and thereafter all the bovines again showed circulating bluetongue virus with increasing regularity until February, 1961 when the investigation was terminated. The nature of the reactions in sheep has been given by Du Toit (1962). The types represented by the isolated strains are given in Table 1.

TABLE 1.—*Bluetongue virus types recovered from 5 bovines exposed to natural infection.*

	Bluetongue Season 1959/1960			Bluetongue Season 1960/1961		
	Recipient Sheep No.	Date injected	Virus type	Recipient Sheep No.	Date injected	Virus type
Bovine 254.	8326	9/3/60	15	7969	26/10/60	2
	7901	16/3/60	15	8312	2/11/60	2
	8199	7/4/60	15	8732	9/11/60	2
	8726	20/4/60	15	8191	25/ 1/61	5
	8193	29/5/60	15	8725	1/ 2/61	5
Bovine 248.	6298	3/2/60	7	7920	9/11/60	8
	8309	9/3/60	1	9914	4/ 1/61	12
	8378	16/3/60	1	8447	11/ 1/61	12
	8721	20/4/60	15	7934	8/ 2/61	5
	—	—	—	8736	15/ 2/61	5
Bovine 9952	8404	27/1/60	3	8395	28/12/60	2
	8429	3/2/60	3	9854	1/ 2/61	11
	7864	10/2/60	3	8726	8/ 2/61	8
	7959	17/2/60	4	7874	15/ 2/61	8
	8185	24/2/60	5	—	—	—
	7987	2/3/60	5	—	—	—
Bovine 9964	6804	27/1/60	1	8700	11/ 1/61	8
	8406	3/2/60	1	8733	25/ 1/61	8
	7994	17/2/60	1	8729	22/ 2/61	NN
	8188	24/2/60	1	8701	1/ 3/61	12
	8734	13/4/60	7	—	—	—
Bovine 9889	5996	27/1/60	5	9918	18/ 1/61	6
	7929	10/2/60	4	8238	25/ 1/61	6
	7944	12/2/60	4	9823	1/ 2/61	6
	7240	23/3/60	4	8696	8/ 2/61	NN

NOTE: NN denotes no neutralization

The results presented in Table 1 show the high incidence of bluetongue virus in the blood of cattle under natural conditions. Each animal was found to harbour a number of heterologous virus types during the latter part of the summer seasons investigated, while no virus could be demonstrated during the colder winter months.



Virus strains representing 11 of the established antigenic types were isolated during this period. In most instances a particular type was found to circulate for a period of less than 14 days before being superseded by the next. Type 1 could be demonstrated in the blood of bovine 9964 for 28 days and types 15 and 4 for 81 and 49 days in bovines 254 and 9889 respectively. During each season investigated six different bluetongue virus types were encountered, type 5 occurring in both seasons. Two isolations made during the second season failed to be neutralized by antiserum to the 16 established groups. At no stage during the investigation were any clinical signs of illness encountered in cattle from which the viruses were subsequently isolated.

It will also be noted that no single virus type was found to circulate in the blood of any one particular bovine during two consecutive seasons. Furthermore, except for type 5 which was present in both seasons, none of the types isolated during the 1959/60 season was found in these cattle during 1960/61.

#### DISCUSSION

Spreull, as far back as 1905, demonstrated that bluetongue virus could circulate for 21 days in a calf which had been artificially infected. Bekker, de Kock & Quinlan (1934) claimed to have produced clinical bluetongue in cattle in South Africa. Subsequently, De Kock, Du Toit & Neitz (1937), and Mason & Neitz (1940) demonstrated the presence of bluetongue virus in the blood of cattle exposed to natural infection, but could not confirm the symptomatology described by Bekker *et al.* In fact, they concluded that the condition described by the earlier workers was probably due to a form of ulcerative stomatitis and that bluetongue produced an inapparent infection in cattle.

It should, however, be noted that in several epizootics outside Africa, clinical bluetongue has been described in cattle (Komarov & Goldsmit, 1951; Silva, 1956; Lopez & Botija, 1958), and it is for this reason that the theory has been postulated (Haig, 1959; Howell, 1963) that the passive immunity transferred by the dam and early repeated natural inapparent infections suppress the symptomatology in enzootic areas.

The present investigations have shown that cattle in South Africa can harbour a number of antigenically different types of bluetongue virus without showing signs of illness. It, therefore, appears that when investigating suspected bluetongue in cattle, caution must be exercised against the co-incidental isolation of bluetongue virus, and its correlation with the symptomatology encountered.

These results support the contention that bluetongue causes an inapparent infection in cattle in this country.

On the grounds of this investigation cattle do not appear to be responsible for harbouring bluetongue virus during the interepizootic periods, as no particular virus type was found to persist from one active season to the next. However, the fact that large numbers of bovines become infected with a variety of different virus types during the summer months indicates that bovines can act as a source of virus for the insect vector, *Culicoides* spp. (Du Toit, 1944; Foster, Jones & McCrory, 1963), during epizootics of the disease.

The isolation of a number of heterologous virus types from each bovine during a relatively short period points to repeated reinfections, and also suggests that no basic immunity to the various types exists where cattle are concerned.

## BLUETONGUE IN CATTLE.

In most instances the presence of a single type could be demonstrated in the blood of a particular animal for less than 14 days. This corresponds to the period of 21 days recorded by Spreull (1905) in the case of calves and nine days recorded by Neitz (1933) in the blesbuck (*Damaliscus albifrons*). However, the reason for types 15 and 4 circulating for 81 and 49 days respectively, is not apparent and requires further investigation.

The isolation of a fairly large number of immunologically different types of bluetongue during each of the seasons investigated, demonstrates that several virus types can be simultaneously active within a limited locality.

Finally, the two isolations not neutralized by antiserum to the already established virus groups may represent further types of this virus.

### SUMMARY

Bluetongue viruses were isolated from five clinically healthy bovines exposed to natural infection over two consecutive bluetongue seasons. The isolated viruses were typed by serum-virus neutralization and found to represent 11 of the established antigenic groups. Two isolations failed to be neutralized and may represent further antigenic types of bluetongue virus.

No single virus type could be found to occur in the same bovine for two consecutive seasons and in most instances the particular virus type occurred in the blood of a bovine for a period of less than 14 days.

### ACKNOWLEDGEMENTS

The technical staff members of the virus and entomology sections are thanked for their very valuable assistance during this investigation.

### REFERENCES

- ALEXANDER, R. A., 1947. The propagation of bluetongue virus in the developing chick embryo with particular reference to the temperature of incubation. *Onderstepoort J. Vet. Sci.*, 22, 7-26.
- BEKKER, J. G., DE KOCK, G. & QUINLAN, J. B., 1934. The occurrence and isolation of bluetongue in cattle—the so-called Pseudo Foot and Mouth Disease in South Africa. *Onderstepoort J. Vet. Sci.*, 2, 393-507.
- DE KOCK, G., DU TOIT, R. M. & NEITZ, W. O., 1937. Observations on bluetongue in cattle and sheep. *Onderstepoort J. Vet. Sci.*, 8, 129-180.
- DU TOIT, R. M., 1962. Bluetongue—Recent Advances in Research. The role played by bovines in the transmission of bluetongue in sheep. *J.S. Afr. Vet. Med. Ass.*, 33, 483-490.
- DU TOIT, R. M., 1944. The transmission of bluetongue and horsesickness by Culicoides. *Onderstepoort J. Vet. Sci.*, 19, 7-16.
- FOSTER, N. M., JONES, R. H. & MCCRORY, B. R., 1963. Preliminary investigations on the insect transmission of bluetongue virus in sheep. *Amer. J. Vet. Res.*, 24, 1195-1200.
- HAIG, D. A., 1959. Bluetongue. *Proc. 16th Intern. Vet. Cong., Madrid 1*, 215-225.
- HAIG, D. A., MCKERCHER, D. G. & ALEXANDER, R. A., 1956. The cytopathic action of bluetongue virus in tissue culture and its application to the detection of antibodies in the serum of sheep. *Onderstepoort J. Vet. Res.* 27, 171-177.
- HOWELL, P. G., 1960. A preliminary antigenic classification of strains of bluetongue virus. *Onderstepoort J. Vet. Res.* 28, 357-363.
- HOWELL, P. G., 1963. Bluetongue. Emerging disease of animals. *F.A.O. Agricultural Studies* No. 61, 109-153.
- KOMAROV, A. & GOLDSMIT, L., 1951. A bluetongue-like disease of cattle and sheep in Israel. *Refuah Vet.* 8, 69-74.
- LOPEZ, A. C. & BOTIJA, C. S., 1958. Epizootie de fièvre catarrhale ovine en Espagne (blue tongue). *Bull. Off. Int. Epiz.*, 50, 65-93.
- MASON, J. H. & NEITZ, W. O., 1940. Erosive stomatitis of cattle. *Onderstepoort J. Vet. Sci.*, 15, 149-157.
- NEITZ, W. O., 1933. The blesbuck (*Damaliscus albifrons*) as a carrier of heartwater and bluetongue. *J. S. Afr. Vet. Med. Ass.* 4, 24-26.
- SILVA, F. S., 1956. Lingue azol ou febre cattal des ovines (bluetongue). *Rev. Cienc. Vet. (Lisboa)* 51, 191-231.
- SPREULL, J., 1905. Malarial catarrhal fever (bluetongue) of sheep in South Africa. *J. Comp. Path. and Therap.* 18, 321-337.