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The Susceptibility of Cattle to the Virus of Bluetongue.

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Introduction.

In another article we (Mason and Neitz, 1940) recorded the results of investigating a disease of cattle, erosive stomatitis. We showed that the cause was a filterable virus which was not that of bluetongue. In the course of this work we, and a number of our colleagues, were struck by the similarity of lesions produced by us to some produced by Bekker, de Kock and Quinlan (1934) with bluetongue virus isolated from cattle. According to these workers, the bluetongue virus produced, under experimental conditions, an elevation in temperature, obvious illness, and erosion and hyperaemia of the buccal mucous membrane in cattle. In bovines naturally infected in the field extensive mouth lesions, and skin and foot lesions were also seen.

In our article we showed that we could not isolate bluetongue virus from the local lesions or the blood of cattle artificially infected with erosive stomatitis. However, we considered that the question of the susceptibility of cattle to bluetongue, particularly the production of mouth lesions was so important that further investigation was called for. It will save repetition if it is now stated that the expression "no reaction", unqualified, means no reaction of any kind, thermal, constitutional, or local. All but three of the calves used were bred at Onderstepoort under tick-free conditions; the exceptions were calves reared at the farm Kaalplaas under veld conditions. The virus used was one of those isolated from cattle by Bekker *et al.*, and had been passaged serially 14 times through sheep.

Experiments.

Experiment 1.—This is summarized in Table 1. The calves used, 7697 and 7595, were bred at Onderstepoort.

TABLE 1.

Attempts to Infect Calves with Bluetongue Virus by Scarification, Intranasal Injection, and Subcutaneous Injection.

Animal.	Treatment.	Result.	Remarks.
C. 7697....	30/8/39. Scarified tongue, dental pad, and lower lip. Virus (blood, sh. 55116) applied	No reaction up to 18/9/39	Tiny white pimple on pad on 6th day; disappeared on 10th day.
C. 7697....	7/10/39, 10 c.c. virus (blood sh. 55875) into each nostril	No reaction up to 18/10/39	—
Sh. 55913..	18/10/39, 5 c.c., s.c. blood of calf 7697	No reaction.....	6/11/39, tested for immunity. Reacted (B.T.), and recovered.
C. 7697....	15/11/39, 5 c.c. virus i.v. (blood, sh. 55913)	No reaction up to 30/11/39	—
Sh. 56544..	30/11/39, 5 c.c., s.c. blood, calf 7697	Reacted (B.T.) and recovered	—
C. 7595....	30/8/39, scarified tongue, dental pad and lower lip. Saline applied (control to C. 7697)	No reaction up to 18/9/39	—
C. 7595....	7/10/39, scarified tongue, dental pad and lower lip. Virus (blood sh. 55875) applied	No reaction up to 15/11/39	—
C. 7595....	15/11/39, 5 c.c. virus s.c. (blood sh. 55913)	No reaction up to 7/12/39	—
Sh. 56545..	30/11/39, 5 c.c., s.c. blood, calf 7595	Reacted (B.T.) and recovered	—

(C. = calf; sh. = sheep; s.c. = subcutaneously; i.v. = intravenously).

The results show that known infective blood produced no symptoms or lesions in calves when injected by scarification or by the intranasal, subcutaneous, or intravenous routes. Calf 7697 did not become inapparently infected by the intranasal injection of virus (see negative transmission to sheep 55913) but both calves did become inapparently infected when the virus was given subcutaneously or intravenously (see positive transmission to sheep 56544 and 56545). The tiny white elevation that appeared on the lip of calf 7697 was almost certainly a healing portion of the scarification wound.

Experiment 2.—Calf 7585 (Kaalplaas) was inoculated intralingually in 2 places with virus (blood, sheep 52759) which was also rubbed into the scarified dental pad and lower lip. No reaction attributable to bluetongue virus occurred during the observation period of 24 days. On the 4th day a few, very small, very superficial erosions appeared on the scarification wounds; there is no doubt that these were of traumatic origin.

Experiment 3.—Two Onderstepoort calves, 7468 and 7700, and one Kaalplaas calf, 7494, each received 20 cc. of virus (blood, sheep 52697) intravenously and in addition received the same virus on

scarified portions of the tongue, dental pad, and lower lip. No reaction of any kind occurred during the observation period of 30 days. Sub-inoculations into sheep were carried out with the blood of calf 7468. Sheep 53696 received 5 cc. of blood 15 days after the attempted infection of calf 7468 and sheep 52667, 10 cc. intravenously 29 days after; neither reacted and both were later shown to be susceptible to bluetongue.

Not one of the 3 calves became visibly infected, and, in addition, it would appear that calf 7468 did not become inapparently infected. This failure cannot be attributed to the lack of infectivity of the blood of the donor, sheep 52697. Calf 7543 (Experiment 4) received some of the same blood on the same day as calves 7468, 7700 and 7494 and became inapparently infected.

Experiment 4.—An Onderstepoort calf 7543 received 10 cc. of the blood of sheep 52697 intravenously and the same blood was applied to scarifications on the tongue, dental pad, and lower lip.

No reaction of any kind occurred until the 15th day. At this time, roughly circular, very superficial erosions about 0·75 cm. in diameter were observed on each side of the lower lip. The erosions looked as if they had been punched out. The base was slightly redder than the normal mucous membrane. Salivation although increased was not excessive. Scrapings were removed from the erosions and held in phosphate buffer of pH 7·4, and formed the inoculum for another experiment to be noted later. The erosions healed in 5 days. No other reaction occurred during the next 15 days. The subinoculations carried out are recorded in Table 2.

TABLE 2.

Subinoculoations carried out from Calf 7543 *of Experiment* 4 (*inoculated* 9.2.39).

Animal.	Date.	Inoculum.	Result.	Immunity Test.
Sh. 53713	20/2/39	Blood: 5 c.c., s.c.....	Reaction.....	31/3/39. No reaction.
Sh. 53686	20/2/39	Blood: 5 c.c., s.c.....	Reaction† B.T.	—
Sh. 53614	24/2/39	Erosion scrapings, i.v....	Reaction.....	23/3/39. No reaction.
Sh. 53623	24/2/39	Erosion scrappings rubbed on to scarifications in mouth	No reaction..	—
Sh. 53619	10/3/39	Blood: 5 c.c., s.c.....	Reaction.....	31/3/39. No reaction.
Sh. 53720	13/3/39	Blood: 0·01 c.c., s.c....	No reaction..	11/4/39. † B.T.
Sh. 53727	13/3/39	Blood: 0·001 c.c., s.c...	No reaction..	11/4/39. Reaction. Recovered.
Sh. 53630	30/3/39	Blood: 10 c.c., i.v.....	No reaction..	24/4/39. Reaction Recovered.
Sh. 53623	30/3/39	Erosion scrapings rubbed on to scarifications in mouth	No reaction..	3/5/39. Reaction. Recovered.

(† = died; other contractions as for Table 1).

Blood was removed from calf 7543 on 24.2.39 (15 days after infection) and stored in the refrigerator. On 4.3.39, 10 c.c. was inoculated intravenously into calf 7325 (Onderstepoort). No reaction

of any kind occurred during the 14 days observation period. Blood of this calf taken on the 10th day produced bluetongue in a sheep (53710).

The one definite result of this experiment is that calf 7543 became inapparently infected with bluetongue (see positive transmission to calf 7325, 15 days, and to sheep 53619, 29 days). Small but definite local lesions, in the form of superficial erosions, appeared on the lower lip, and scrapings from these erosions contained bluetongue virus. (The scraping produced no lesions when applied to scarifications in the mouth of sheep 53623.) However, one cannot be certain that the scrapings, *per se*, were infective; it is possible that the blood removed with the erosions contained sufficient virus to produce the disease. In an attempt to check this point 2 sheep (53720 and 53727) were inoculated subcutaneously with 0·01 cc. and 0·001 cc. respectively of blood of calf 7543. No reaction occurred. The reason for this may have been the time at which the blood was taken—32 days after the original infection of the calf. At this stage the blood would possibly have been non-infective even in a large dose; it will be noticed that 10 cc. taken on the 49th day failed to set up bluetongue.

Experiment 5.—An attempt was made to ascertain whether the erosion scrapings of calf 7543 (Experiment 4) would produce lesions if inoculated into the buccal mucous membrane of bovines. With this end in view, the tongue, dental pad, and lower lips of a normal calf 7682 (Kaalplaas) and of a bluetongue-immune calf 7711 (Onderstepoort) were scarified and inoculated with an emulsion of scrapings, and at the same time the emulsion was given intravenously to sheep 53614 (noted in Table 2). Calf 7711 was considered to be immune for the following reason. On 15.10.38, it received virus (blood, sheep 52903) on the scarified buccal mucous membrane. No reaction (local or general) occurred. On 1.2.39 it received 20 cc. of virus (blood, sheep 52759) intravenously and did not react during the observation period of 23 days; its blood (5 cc. and 10 cc. amounts), taken on the 9th and on the 57th day after the intravenous injection of virus did not produce bluetongue in sheep (52749 and 52904) and these sheep were later shown to be susceptible to bluetongue virus. It may be added that at the time (30.3.39) of taking the second sample of blood, calf 7711 received 10cc. of virus (blood, sheep 53696) intravenously. No reaction occurred, and its blood, taken 11 days later, failed to produce bluetongue in sheep 53648. It would thus appear that the original inoculation by scarification on 15.10.38 produced an inapparent infection; unfortunately we took no steps at the time to prove this by the sub-inoculation of blood into sheep.

The application of the erosion scrapings of calf 7543 to the scarified buccal mucous membranes of calves 7682 and 7711 on 23.2.39 produced no reaction whatever, although the same material produced bluetongue when given intravenously to sheep 53614. As mentioned under experiment 4, there is no proof that the scrapings contained the virus; sufficient blood may have been removed with the scrapings to account for the result. Nevertheless, one is in the position to state an inoculum, containing sufficient virus

to set up bluetongue when given intravenously to a sheep, failed to produce any recognizable reaction when applied to the scarified buccal mucous membrane of a calf.

Experiment 6.—To the scarified buccal mucous membrane of an Onderstepoort calf (7405), virus (blood, calf 7543, see Experiments 4 and 5) was applied. Calf 7543 was infected by intravenous inoculation on 9.2.39, blood was removed on 24.2.39, and held in the refrigerator until 4.3.39, and on that day was inoculated into calf 7405. On the 7th day, 3 small (0·25 to 0·75 cm. in diameter) roughly circular, very superficial erosions appeared on the lower lip of calf 7405. The lesions had a "punched-out" appearance, were non-inflammatory and had a yellowish-grey base. Healing was complete on the 13th day. No thermal or constitutional reaction occurred. Scrapings of these lesions, removed on the 6th day after their first appearance, did not set up bluetongue when given intravenously to a sheep and blood, taken 14 days after the original scarification, also failed to produce bluetongue in a sheep.

Experiment 6*a*.—In 2 calves, 7543 (Experiment 4) and 7405 (Experiment 6), erosions had appeared on the lower lip after the administration of bluetongue virus, in the case of 7543 by intravenous injection and application to scarifications on the buccal mucous membrane, and in the case of 7405 by application to scarifications only. The lesions were of the mildest nature, caused no inconvenience, would have been missed but for careful search and were unaccompanied by a systemic reaction. We could not convince ourselves that they were the result of infection by bluetongue virus but considered their most probable cause was the trauma produced by the scarification. If the reaction in calf 7405 was due to bluetongue, there was every likelihood that it would become immune. The only way of proving this was to inoculate it with virus and after a suitable interval to subinoculate its blood into a susceptible sheep. The absence of a reaction in the sheep would indicate that the calf was immune. The results are collected in Table 3.

The results were quite clear-cut. Calf 7405 was not immune as shown by the positive transmission to sheep 53641; calves 7586 and 7682 were not immune and calf 7711 was immune as proved by the failure of transmission to sheep 53648. We can conclude that what caused the erosions on the lip of calf 7405 did not, at the same time, produce immunity to bluetongue.

Discussion.

In our opinion, we failed to produce a recognizable disease in cattle with bluetongue virus (blood of infected sheep or cattle). In four animals, insignificant lesions appeared in the mouth. Those appearing in 2 animals (7697, Experiment 1 and 7585, Experiment 2) were so small that only a careful search revealed them and there is no doubt that they were the result of the scarification wounds. Calf 7543 (Experiment 4) was infected by inoculating blood intravenously and applying it to scarifications on the buccal mucous membrane. Small, very superficial, non-inflammatory erosions appeared on the lower lip on the 15th day after infection. Both the

blood and the erosion scrapings contained bluetongue virus, but the scrapings, when applied to scarifications on the buccal mucous membrane of a susceptible calf (7682, Experiment 5), failed to produce a lesion. In the last experiment of this kind virus was rubbed into scarifications on the tongue, lower lip, and dental pad of calf 7405 (Experiment 6). On the 7th day erosions, similar to those found in calf 7543, appeared on the lower lip. In spite of this, calf 7405 did not develop immunity to bluetongue (Experiment 6a). The point that we are about to make will be better appreciated if the calves, the route of injection of virus, and the presence or absence of any local reaction are tabulated.

TABLE 3.

Experiment to ascertain whether Calf 7405 had developed Immunity to Bluetongue (Exp. 6a).

Animal.	History.	Present Inoculum.	Subinoculations.	Result of Subinoculation.
Calf 7405.....	4/3/39. Scar. buccal m.m. B.T. virus. Erosions produced (Exp. 6)	30/3/39. 10 c.c. i.v. virus (blood, sheep 53696)	11/4/39. 10 c.c. i.v. blood calf 7405 into sheep 53641	Reacted (B.T.) and recovered I.T. No reaction.
			21/4/39. 5 c.c. i.v. blood, calf 7405 into sheep 53650	No reaction. I.T. Reacted and recovered.
Calf 7586.....	Normal O.P. calf	30/3/39. As calf 7405	11/4/39. 10 c.c. i.v. blood, calf 7586 into sheep 53705	Died (B.T.).
Calf 7682.....	23/2/39. Scar. buccal m.m. with erosion scrapings of calf 7543. No. reaction (Exp. 5)	30/3/39. As calf 7405	11/4/39. 10 c.c. i.v. blood, calf 7682 into sheep 53734	Died (B.T.).
Calf 7711.....	Immune to B.T. (Exp. 5)	30/3/39. As calf 7405	11/4/39. 10 c.c. i.v. blood, calf 7711 into sheep 53648	No reaction. I.T. Reacted (B.T.) and recovered.

(B.T. = bluetongue; I.T. = immunity test; O.P. = Onderstepoort; i.v. — intravenously; scar. = scarified; m.m. — mucous membrane).

It will be observed that a local lesion was produced only when the buccal mucous membrane had been scarified. When the inoculation was by the intranasal, subcutaneous, or intravenous routes, mouth lesions were not produced. In three instances inoculation by the combined scarification and intravenous routes led to no local lesion although the blood used contained virus. In another three cases, scarification alone (in one instance, saline applied and in 2 others, erosion scrapings applied) did not lead to the formation of an erosion.

TABLE 4.

The Route of Injection and the Presence or Absence of a Local Lesion.

Calf.	Route of Injection.	Local Lesion.
7697	Scarification (blood).....	Positive (tiny pimple).
7697	I.N. (blood).....	Negative.
7697	S.C. (blood).....	Negative.
7595	Scarification (saline).....	Negative.
7595	I.V. (blood).....	Negative.
7585	Scarification (blood).....	Positive (tiny erosions).
7468	Scarification and I.V. (blood).....	Negative.
7700	Scarification and I.V. (blood).....	Negative.
7494	Scarification and I.V. (blood).....	Negative.
7543	Scarification and I.V. (blood).....	Positive (small erosions).
7325	I.V. (blood).....	Negative.
7682	Scarification (erosion scrapings)....	Negative.
7711	Scarification (erosion scrapings)....	Negative.
7405	Scarification (blood).....	Positive (small erosions).
7586	I.V. (blood).....	Negative.
7682	I.V. (blood).....	Negative.
7711	I.V. (blood).....	Negative.

(I.N. intranasal; other abbreviations as in Table 1).

We consider that the results point to the scarification, *per se*, playing the big, if not the only, part in the production of the tiny erosions. And even if we grant that the bluetongue virus may have had a share in the process, we consider that these minute, difficult-to-find lesions are not to be compared as to size and gravity with the lesions found by Bekker *et al.* in naturally-infected bovines. The only definite conclusion we can draw from our work is that bluetongue virus produces an inapparent disease in cattle. Although the animals appeared healthy and exhibited no thermal, constitutional, foot, or skin lesions, yet virus could be demonstrated in their blood 29 days but not 49 days after the infective inoculation.

In many respects our results differ from those of Bekker *et al.* They claim to have produced mouth lesions, thermal reactions, and in a few cases, constitutional (obvious illness) reactions. We are not impressed by the temperature charts of their calves 5201 and 5257 (pp. 506 and 507) infected by intralingual inoculation of virus. The temperature of calf 5257 rose to 103·1° F. on the 4th day and thereafter remained at 102° F. or below except for one rise to 102·6° F. Such temperatures are definitely within normal limits. That of calf 5201 reached limits (103·8° to 104° F) that could be termed abnormal but a rather low starting temperature (101·1° F.) makes the chart look more impressive than it really is. One is struck by the early-appearing thermal rise in their calves, very often on the 3rd day and once on the 2nd day. At the present moment, the much more susceptible sheep commences to react to their virus ("Bekker") on the 5th day at the earliest and usually not until the 6th or 7th day. Another point that requires explanation is why mouth lesions not infrequently appeared before the first rise in temperature or why they appeared without a rise in temperature at all. For example, calf 9 (p. 457) had an elevation in temperature

on the 10th day, but on the 5th day superficial ulcers appeared on the upper lip. Again, it is a little difficult to reconcile the appearance of the ulcers on the lip of calf 5407 (p. 463, photograph on p. 418) with the inoculation of bluetongue virus. The temperature rose to 104° F. on the 3rd day, stayed at this level for 24 hours and thereafter remained within normal limits. Yet, although the reaction was almost negligible, definite ulcers were present on the lower lip on the 3rd day. We have a very clear recollection of these particular lesions and in size, "severity," and degree of inflammation they were greatly in excess of our tiny, very superficial erosions. They resembled much more closely the erosions we produced with erosive stomatitis virus. Whilst early-appearing hyperaemia, excoriation, and erosion-formation could be symptoms of bluetongue in cattle, we would point out that hyperaemia of the buccal mucous membrane in bluetongue-infected sheep seldom appears until the thermal reaction is well advanced and that coronitis appears at a still later date.

Although de Kock, du Toit and Neitz (1937) and de Kock, van Heerden, du Toit and Neitz (1937) isolated bluetongue virus from the blood of cattle living under veld conditions, they did not record the presence of mouth lesions in such animals or in bovines experimentally infected, and this despite full knowledge of, and participation in, the work of Bekker *et al.*

As stated in the introduction to this article, we commenced this piece of research after working on erosive stomatitis of cattle. We had been able to transmit this disease by the application of erosion scrapings to the scarified buccal mucous membrane, but had not succeeded in demonstrating bluetongue virus either in local lesions or blood. To our knowledge, Bekker *et al.* did not attempt the reproduction of lesions by applying erosion or ulcer scrapings locally. We can only speculate on the result, but there is no doubt that the failure to carry out the experiment was a serious omission. A counter-argument could be that lesions were produced by the intravenous inoculation of virulent blood alone. But, if the local lesions had been produced by a virus of the erosive stomatitis type, the indiscriminate "mouthing" of healthy and infected cattle, and the failure to adopt rigid isolation and disinfection measures would have been sufficient to ensure infection without the intervention of bluetongue virus.

A possible criticism of our work is that our virus had been subjected to many passages (14) through sheep and had in the process lost pathogenicity for cattle. However, we would recall that Bekker *et al.* claim to have produced the lesion in calf 5407 with virus that had been passed through 2 sheep in series. We would also recall that the "Bekker" strain of virus produces, at the present time, as severe a disease in sheep as it did immediately after isolation from cattle. We intend to repeat part of the work with a virus from cattle whenever we are fortunate enough to isolate such a strain.

Thus, because of our own results and because of the reasons given in our cricitism of the work of Bekker *et al.*, we cannot unreservedly accept their view that bluetongue of cattle is

characterized by a rise in temperature, constitutional symptoms, and mouth or foot lesions. However, we do appreciate that a different set of circumstances such as a virus direct from cattle, cattle highly susceptible to bluetongue, and altered nutritional, environmental, and climatic conditions, might give different results.

Conclusions.

(1) Bluetongue virus inoculated intravenously, subcutaneously, intranasally, or through scarifications on the buccal mucous membrane did not cause any apparent illness in cattle.

(2) Inoculated cattle developed a *maladie inapparente*.

(3) The ability of bluetongue virus to cause lesions in the buccal cavity of cattle is doubted.

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