

The Isolation of *Cl. Welchii*, Type B, from Foals affected with Dysentery.

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To our knowledge the only reference to the occurrence of "lamb dysentery" (bloedpens) in foals is that of Montgomerie and Rowlands (1937). They record that the only abnormality seen in the foal they examined was "an acute congestion of the small intestine throughout its length with numerous discrete ulcers of the mucous membrane". From these ulcers they succeeded in isolating *Cl. welchii*, Type B. We have had one experience of the same disease in foals and the following communication summarizes our findings.

HISTORY OF THE DISEASE.

The two outbreaks of which we have knowledge occurred in the foals of a thoroughbred stud farm in the Cape Province, in an area in which lamb dysentery is present, but although about 30 ewes were running on this farm none of their progeny died of the disease nor could a previous history of the illness be elicited.

Apparently the disease made its appearance on this particular farm in 1936, and at the time of the field investigation it was stated that a like condition had not been seen on other stud farms of the district; however, inquiries made subsequently elicited the information that it had occurred occasionally.

The 1936 Outbreak.

Forty-three foals were born in the season and seven died of dysentery. In five instances the mares were from 9 to 23 days overdue and in two cases they foaled at full term; thus late foaling was not necessarily a factor in the causation of the disease. The foal of the nineteenth dam was the first to be infected, after which cases occurred right up to the end of the season. Foaling took place in loose boxes, the floors of which were covered with dry powdery horse dung, over which, in some instances, straw was spread.

The 1937 Outbreak.

The outbreak commenced at the beginning of the foaling season and at the time of the visit of one of us (E.M.R.), 7 of 17 foals had died. One would have been justified in predicting a 50 per cent. morbidity rate in the foals of the 26 mares which remained to foal if no preventive measures were taken. As in the 1936 outbreak, some mares carried their foals over time, but this appeared to have no etiological significance; all were in good condition and had adequate milk. The in-foal dams were kept in a large, bare camp and daily received hay thrown on the ground. When due to foal they were transferred to a small enclosure near loose boxes and allowed to foal there; on the ground of this enclosure a considerable amount of dung had been allowed to collect.

Symptoms.—In all the foals involved in the 1936 and 1937 outbreaks symptoms were noticed within 48 hours after birth and, as a rule death supervened within a further 24 hours; however, one affected foal lived for 5 days. Between the 24th and the 48th hour after birth the foal stopped sucking and appeared to be in pain; diarrhoea was marked, the faeces being very fluid in consistency and reddish-brown in colour. The animal gradually became weaker and died in a comatose state about 12 to 24 hours later. This description, given by the manager, was confirmed in a foal seen by one of us during the field investigation; it may be added that the temperature was normal and that, just before death, the breathing was very rapid.

Pathological Changes.—Two foals which had died of dysentery were autopsied, and, in both, the only macroscopic lesions noticed were in the alimentary tract. The small intestine was markedly hyperaemic, the wall showing a number of dark red spots (? commencing ulcers) up to a centimetre in diameter. These were more frequent in the ileum than in any other portion of the small gut, but at no level was actual ulceration present. The caecum and colon were only slightly hyperaemic and the intestinal contents, from the small intestine to the rectum, were fluid in consistency and dark red in colour.

THE ISOLATION OF *Cl. welchii*, TYPE B.

The blood and internal organs yielded, in artificial culture, no germs of a pathogenic nature and, although a special search was made for paratyphoid bacilli, none were found. Another probable cause was a member of the *Cl. welchii* group of anaerobic organisms, and from the small gut contents of two foals we succeeded in isolating such a germ.

Foal 1.

The supernatant fluid of the centrifuged intestinal contents was lethal for mice (intravenous injection) in a dose of 0.01 cc. Type A antitoxin, type B, 1930 variety antitoxin (i.e. containing the alpha and beta but not the epsilon fraction) and type D antitoxin had no

apparent neutralizing effect on this material; type B antitoxin 1922 variety (i.e. containing the epsilon fraction in addition to the alpha and beta) and a mixture of types B, 1930 variety and D antitoxins neutralized the toxin contained in the supernatant.

By appropriate methods, a pure culture of a welch-like germ was isolated, and toxins were prepared by growing it in donkey-flesh meat-broth for one and for five days respectively. Antiserum was prepared by hyperimmunizing a goat with the one-day toxin.

Morphologically and culturally the culture isolated could not be distinguished from known strains of *Cl. welchii*, type B. Its fermentative reactions were as follows:—Acid and gas production in saccharose, galactose, lactose, laevulose, and sorbite; acid without gas in glucose, maltose, and glycerine (slight); and no reaction in mannite, dulcite, and inulin; gelatin and Loeffler's serum medium were liquefied, inspissated horse serum was pitted but not liquefied and litmus milk reacted with a "stormy fermentation". The post-mortem picture in a guinea-pig killed by the intramuscular inoculation of 1.0 cc. of an 18 hours' broth culture was, in all respects, similar to that produced by *Cl. welchii*, type B.

Table 1 records the results obtained by titrating the one-day toxin (precipitated with ammonium sulphate and dried) against various antitoxins.

TABLE 1.

Amount of Various Antitoxins Required to Neutralize the One-day Toxin.

(M.L.D. of toxin=0.002—0.003 cc.)

Neutralizing dose of antitoxin (cc.).

| Toxin (cc.). | Type A GG 2748. | Type B 1930 variety. 1789. | Type B 1922 variety. C2540. | Type B OP. | Type D Ex 15. | Foal. |
|--------------|-----------------|----------------------------|-----------------------------|------------|---------------|-------|
| 0.005..... | >0.1 | — | — | — | — | — |
| 0.1..... | — | >0.1 | 0.02 | 0.04 | — | 0.04 |
| 0.025..... | — | 0.0015 | — | — | — | — |
| 0.003..... | — | — | — | — | >0.1 | — |

(G.G.2748, C.2540 and Ex 15 were obtained from the Wellcome Laboratories; C.2540 was a current issue of lamb dysentery antitoxin; type B, OP was a mixture of type B 1930 variety and type D antitoxins made at Onderstepoort; "foal" antitoxin was made by hyperimmunizing a goat with the one-day foal culture toxin.)

These results show that the toxin was of the lamb dysentery or bloodpens bacillus type (see Mason, 1935). Types A and D antitoxins (0.1 cc. amounts) did not neutralize 1 M.L.D.; type B antitoxin (C.2540 0.02 cc.), containing the beta and the epsilon fractions, neutralized about 50 M.L.D.; 0.1 cc. of type B antitoxin (1789), devoid of the epsilon fraction, did not neutralize 50 M.L.D. but 0.0015 cc. was effective against about 10 M.L.D. Mice which received 0.1 cc. of a mixture of 0.5 cc. of toxin and 0.5 cc. of 1789 antitoxin died, whereas those receiving 0.05 cc. lived. A series of mixtures were made, each containing 0.1 cc. of toxin and 0.1 cc. of D (Ex 15) antitoxin and to each of these mixtures different amounts of 1789 antitoxin were added; under these conditions 0.003 cc. of the latter antitoxin produced neutrality. These experiments show that 0.1 cc. of toxin contained about 2 M.L.D. of the epsilon toxic fraction; if this portion was neutralized with a type D antitoxin, a relatively small amount of a non-anti-epsilon-containing type B serum was effective against a test dose of about 50 M.L.D. of the "foal" toxin.

In Table 2, the results obtained in titrating the five-day toxin (precipitated and dried) are given.

TABLE 2.

Amount of Various Antitoxins Required to Neutralize the Five-day Toxin.

(M.L.D. of toxin=0.003—0.004 cc.)

Neutralizing dose of antitoxin.

| Toxin (cc.). | Type A GG2748. | Type B 1930 variety. 1789. | Type B 1922 variety. C2540. | Type B OP. | Type D Ex 15. | Foal. |
|--------------|----------------|----------------------------|-----------------------------|------------|---------------|-------|
| 0.003..... | >0.1 | >0.1 | — | — | — | — |
| 0.1..... | — | — | 0.04 | 0.08 | 0.06 | 0.015 |

The results given above show the five-day toxin consisted, probably entirely, of the epsilon fraction. No titration to detect small amounts of the beta portion was conducted and the alpha fraction was not investigated.

The reciprocal toxin-antitoxin titration was carried out by using the one- and the five-day toxins of the bloodpens strain of *Cl. welchii*, type B, and an antitoxin made in a goat with the foal strain. One of us (Mason, 1935) has already shown that the bloodpens strain is serologically identical with the original lamb dysentery organism. The results were as follows:—0.1 cc.—0.15 cc. of serum neutralized 100 M.L.D. of the one-day toxin and 0.004 cc. neutralized 60 M.L.D. of the five-day toxin.

Foal 2.

The supernatant fluid of the spun contents of the small intestine was not lethal for mice, on intravenous injection, in a dose of 0.1 cc. A welch-like germ isolated from this material was identical morphologically and culturally, with that obtained from foal 1. A detailed serological examination of the toxin was not carried out but it was shown that a test dose of a one-day toxin, containing at least 10 M.L.D., was neutralized by type B antitoxin, 1922 variety (C.2540) and by a mixture of type B, 1930 variety (1789) and type D (Ex 15) antitoxins, but not by either separately.

PREVENTION.

Between visiting the farm and establishing a diagnosis 11 foals (Nos. 15-26) had been born and none had developed dysentery; foals 12 and 14 had died during the visit. Although anti-serum was sent off immediately the causation was known, only one foal received an injection and this, weak at birth, developed dysentery and died. One other foal (No. 37), not passively protected, succumbed to the disease. It is fortunate that the farm manager omitted to use the antitoxin because, if he had used it, we might have attributed the lowered disease incidence to the effect of the serum and not to other causes. It is possible that the recommendation to permit foaling only in the open resulted in a reduction in the losses from 7 out of the first 17 foals to 2 out of the following 26. However, it is probable that the infection was present (even if in less degree) in the foaling camp as well as in the boxes because the disease occurred in two foals which had not been near boxes.

SUMMARY.

An outbreak of dysentery in foals is recorded. The symptoms and post-mortem lesions have much in common with those of lamb dysentery. From the intestinal contents of two foals, *Cl. welchii*, type B (the lamb dysentery or bloedpens bacillus) was isolated.

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