Experiment II.—Calf 343, Three and a half Months' Old.

The design of this experiment was to feed bracken, but at the start to give a generous allowance of swedes, in order to see whether the addition of this food, believed to be rich in anti-scorbutic vitamine, would arrest the appearance of symptoms of bracken poisoning, or exert any influence on them.

On 5th September 1920 Calf 343 received 4 lbs. of green fresh-cut bracken, together with a generous ration of chopped swedes. This ration was fed daily until 3rd October—twenty-eight days—when it had consumed approximately 112 lbs. of green bracken. On 3rd October the animal looked ill and refused food. The temperature was 105.2° F. On 4th October the temperature was 107° F., the pulse was feeble and soft, and the faeces were very dark in colour (admixture with blood). The animal was very weak and refused food of any description. It was found dead on 5th October—thirty days from the start of the experiment. The *post-mortem* lesions were typical of bracken poisoning.

These experiments supply further definite evidence that green bracken is poisonous—in the autumn at least—and that it is accountable for the trouble characterised by symptoms and lesions of acute scurvy, and which is sometimes regarded as a specific disease affecting a number of animals on the same pasture at about the same time. They also furnish evidence that the trouble does not arise from avitaminosis, but that it is probably due to a specific poison which has not yet been isolated.

The author hopes to publish in a future paper the results of experimentation on species other than cattle, and on the effects following administration of extracts obtained from green bracken.

THE SPECIFIC IDENTITY OF BACILLUS PARABOTULINUS.

By H. R. SEDDON, D.V.Sc., Veterinary Institute, University of Melbourne.

INTRODUCTION.

IN a previous paper the author¹ has given an account of an anærobic, toxin-forming bacillus, which, whilst resembling *Bacillus botulinus* very closely in certain respects, yet presented certain characters which were deemed sufficient to warrant the organism being regarded as a distinct species, and the name *Bacillus parabolulinus* was therefore proposed for it.

This organism, *Bacillus parabolulinus*, was recovered from a bone of an animal that had died of what in Tasmania is locally termed Midland cattle disease, but the condition commonly occurs also in at least three States of the mainland of Australia. The condition, further, seems to be identical with lamziekte of South Africa.

¹ "Journal of Comparative Pathology and Therapeutics," Vol. XXXV., 1922, Part 3, p. 147.

A study of *Bacillus paratuberculosis* showed that the administration of bacteria-free filtrates of cultures was followed by the same symptoms as those seen in natural cases of the disease, and the organism was therefore considered to be concerned in the etiology of the condition.

On account of the fact that the symptoms induced are those of progressive bulbar paralysis the condition was described under the title of toxic bulbar paralysis, as it was felt that the geographical and other names used in Australia do not convey a meaning expressive of either the cause or the nature of the condition.

It was shown also that in the horse the administration of toxic filtrates of *Bacillus paratuberculosis* lead to symptoms identical with those due to botulinus toxin, whether from human or equine sources; and, as forage poisoning (cerebro-spinal meningitis, so-called) in horses has been shown by several workers in America to be due to botulism, it would seem possible that there are at least three organisms capable of causing forage poisoning in horses, viz., *Bacillus botulinus* (Types A and B) and *Bacillus paratuberculosis*.

Cases of bulbar paralysis in both horses and cattle are of not infrequent occurrence in Australia, but so far as the horse is concerned no determination has yet been made as to which organism or organisms are responsible for the condition in this country. In the case of cattle it would appear that *Bacillus paratuberculosis* is the common cause, and not *Bacillus botulinus*; for, though the toxin of this latter organism is capable of inducing symptoms of bulbar paralysis, it has been shown by Hart and Hayes¹ that the ox is relatively insusceptible, the administration of massive doses of toxin being necessary to cause symptoms.

In order therefore to determine definitely the relationship of *Bacillus paratuberculosis* to *Bacillus botulinus* investigations have been continued, and the results of toxin-antitoxin tests are given below, together with a short summary of the other points of difference between these two organisms.

COMPARISON OF *BACILLUS BOTULINUS* (TYPES A AND B) AND *BACILLUS PARATUBERCULOSUS*.

(a) *Morphological and Cultural Characters*.—In the earlier paper it was pointed out that while the two types of *Bacillus botulinus* were identical morphologically and culturally (as is well known), *Bacillus paratuberculosis* showed certain differences in that (1) it was distinctly larger, (2) it formed a wholly branched colony in solid media, and (3) it failed to show gas formation in glucose media. It was upon these grounds that the organism isolated here was differentiated from *Bacillus botulinus*.

(b) *Toxin Formation*.—As is well known, Types A and B of *Bacillus botulinus* both produce powerful toxins, and these toxins are identical in that they both give rise to the same symptoms; but, as has been pointed out by Graham,² whereas Type A is highly fatal for chickens (in which it induces Limberneck), Type B

¹ "Journal of the American Veterinary Medical Association," Vol. LVII. (10), 1920, p. 638.

² "Journal of Infectious Diseases," Vol. XXVIII., 1921, p. 317.

toxin is not. Further, it was pointed out many years ago by Leuchs, and has been confirmed by others, that the antitoxin to one type does not protect against the toxin of the other type, and *vice versa*.

The bacteria-free filtrates of cultures of *Bacillus paratuberculosis* likewise induce symptoms identical with those due to botulinus toxin; and, though exact determinations of relative susceptibility have not been made, it has further been shown that cattle and horses possess much the same susceptibility to these filtrates of *Bacillus paratuberculosis* (whereas Hart and Hayes¹ have shown that such is not the case with botulinus toxin). The administration of paratuberculosis filtrates to chickens, in such experiments as have been as yet performed, indicates their behaviour to be similar to that of the toxin of Type B *Bacillus botulinus*.

Further work has led to the production of much more powerful filtrates of *Bacillus paratuberculosis* than those previously recorded, *e.g.*, of one product subcutaneous injection into a guinea-pig at the rate of '0005 cc. per kilogramme body weight led to death in sixty-three hours, and other products have shown almost as high a titre. Even so, it is not believed that the limits of toxicity have been reached, and further enquiry is being made into the factors associated with maximal toxin production.

The evidence presented in our earlier paper that *Bacillus paratuberculosis* produced a true toxin was as follows:—

1. The administration of germ-free filtrates led to symptoms identical with those induced by whole cultures. (These filtrates were obtained after passage of culture through Chamberland F. or Doulton candles, and were tested culturally for sterility.)
2. Such symptoms ensued only after a well-marked period of incubation, varying according to dosage and route of administration. This period of incubation was commonly from fifteen hours to several days; even after intravenous inoculation of large doses into guinea-pigs no symptoms were manifested for at least six hours.
3. The administration of small doses of such filtrates sufficed to set up the characteristic symptoms and death.
4. The toxicity of a filtrate was diminished on heating to 60° C. for fifteen minutes and entirely destroyed by heating to 80° C. for a like period.
5. The fact that filtrates possessed a toxic property analogous to that of botulinus toxin, *i.e.*, were toxic when administered by the mouth.

It was admitted that as no antitoxin had up till then been prepared against these toxic filtrates the claim that a true specific exotoxin was present in these cultures could not be wholly substantiated.

PRODUCTION OF ANTITOXIN.

Repeated attempts have been made to immunise small animals against the toxic filtrates of *Bacillus paratuberculosis*, in addition to which two horses have also been employed. The experiment with the first of these horses had to be discontinued for another cause,

¹ Loc. cit.

and the second horse has received as yet only comparatively few injections; a test of its serum reveals the presence of antibodies, but its serum is as yet of too low a potency for critical experiment.

At first graduated doses of unaltered toxin, starting with a small fraction of a lethal dose, were used, but without success, and the animals (guinea-pigs and rabbits) invariably succumbed sooner or later as the doses were increased.

For the next attempt toxin heated to 60° C. for an hour was employed for the earlier injections, and by this means two guinea-pigs out of six have been successfully immunised. The remainder of these guinea-pigs died during the immunisation process. One of those immunised at one time exhibited marked symptoms of paralysis, from which, however, it gradually recovered; the other at one time showed slight symptoms, and it is noteworthy that with it the initial injections were very much smaller.

In determining the potency of the toxins employed the minimal lethal dose (m.l.d.) has been interpreted as the smallest quantity which, administered subcutaneously to a 350 gramme guinea-pig, has led to death in less than forty-eight hours.

The following toxin-antitoxin experiments were performed with the serum from one of these guinea-pigs (No. 72). The initial dose for this guinea-pig was $\frac{1}{80}$ th m.l.d. of heated toxin (potency determined after heating); nine injections of heated toxin were given, after which the animal received six injections of non-heated toxin, the last injection, nine days before bleeding, being 40 m.l.d. This guinea-pig showed symptoms of paralysis after the third injection ($\frac{1}{10}$ th m.l.d.), in consequence of which treatment was suspended for nearly four months. No symptoms have been shown following the subsequent injections, and at time of killing the animal weighed 900 grammes and was in excellent condition.

In order that cross-immunity experiments might be carried out a polyvalent botulinus antitoxin has been prepared in a cow (Cow 6) by repeated injection of cultures of both types of *Bacillus botulinus*. The strains used were *Bacillus botulinus* A1 obtained from the Lister Institute through the courtesy of the Director, Dr C. J. Martin, and *Bacillus botulinus* (Type B), No. 40, from Dr K. F. Meyer's collection, kindly supplied by Dr Hilda Hempl Heller.

TOXIN-ANTITOXIN EXPERIMENTS.

In these experiments guinea-pigs of 225 to 270 grammes weight were used, and the test was carried out as follows: The toxin-antitoxin mixtures, made up to a constant volume (3 cc.) with saline, were placed in conical glasses, the contents thoroughly mixed, incubated for one and a quarter hours at 37° C., and then injected subcutaneously.

[TABLE.

Experiments.

<i>Guinea-pig.</i>	<i>Antitoxin.</i>	<i>Toxin.</i>	<i>Result.</i>
336	Parabotulinus, '2 cc.	Parabotulinus, 50 m.l.d.	Lived.
338	do.	Botulinus A, 1 m.l.d.	Died 2nd day.
340	do.	Botulinus B, 1 m.l.d.	Died 3rd day.
337	No antitoxin	Parabotulinus, 1 m.l.d.	Died 2nd day.
339	do.	Botulinus A, 1 m.l.d.	do.
341	do.	Botulinus B, 1 m.l.d.	Died 3rd day.
342	Botulinus, 1 cc.	Parabotulinus, 1 m.l.d.	Died in 20½ hrs.
344	do.	Botulinus A, 2 m.l.d.	Lived.
346	do.	Botulinus B, 2 m.l.d.	do.
345	do.	Botulinus A, 5 m.l.d.	do.
347	do.	Botulinus B, 50 m.l.d.	do.
343	Serum normal guinea-pig, '5 cc.	Parabotulinus, 1 m.l.d.	Died in 22 hrs.

- Antitoxins . Parabotulinus, Serum of Guinea-pig 72.
 Botulinus (polyvalent), Serum of Cow 6, 13th September 1922.
- Toxins. . Bacillus parabotulinus of 11th September 1922. M.l.d. '004 cc.
 Bacillus botulinus, Type A, A1 of 30th September 1922. M.l.d. 1 cc.
 Bacillus botulinus, Type B, B. 40 of 21st July 1922. M.l.d. '01 cc.

In other experiments it has been shown that 1 cc. of this parabotulinus antitoxin is capable of protecting against at least 50 m.l.d. of toxin. Even the administration of 100 m.l.d. of toxin, with a similar quantity of antitoxin, was followed by but slight symptoms and ultimate recovery.

It will be noted that the m.l.d. of Type A botulinus toxin is very large; this was a very weak toxin, and though this strain has a low degree of toxicity much more powerful products have at times been obtained. The dose selected as the m.l.d. for Type B botulinus toxin was really less than 1 m.l.d., and this is to be ascribed to the toxicity of this filtrate having diminished somewhat since its potency was determined. These factors, however, only serve to make the test more crucial.

The following conclusions are drawn from the above:—

(a) That the antitoxins employed, parabotulinus and polyvalent botulinus, protect against their homologous toxins.

(b) That parabotulinus antitoxin fails to protect against either type of botulinus toxin, and, conversely, a polyvalent botulinus antitoxin fails to protect against parabotulinus toxin.

(c) That normal guinea-pig serum possesses no antitoxic value against parabotulinus toxin. (In another experiment it has been shown that normal ox serum affords no protection against botulinus toxin.)

(d) That, from immunity tests alone, there is sufficient evidence to regard *Bacillus paratubulinus* as being distinct from *Bacillus botulinus*.

Other tests, though not so comprehensive as that given above, have given the same results.

CONCLUSIONS.

1. That *Bacillus paratubulinus* produces a true toxin, *i.e.*, a soluble exotoxin obtainable by filtration, causing symptoms only after a definite period of incubation, and capable of inducing the formation of an antitoxin.

2. That, though the toxins of *Bacillus paratubulinus* and of *Bacillus botulinus* are identical in their action, the antitoxin to the one does not protect against the other, and *vice versa*.

3. As it has been shown previously that *Bacillus paratubulinus* differs from *Bacillus botulinus* (Types A and B) both morphologically and culturally, and it is now demonstrated by toxin-antitoxin tests to be distinct, the specific identity of *Bacillus paratubulinus* is claimed.

CRITICISM OF DR. SCHARRER'S PAPER ON BOVINE STAGGERS.

By W. H. ANDREWS, D.Sc., M.R.C.V.S., Senior Research Officer
Onderstepoort.

THE isolation of the toxic principle of *Matricaria nigellæfolia*, and the study of its actions in the bovine body, would have great theoretical interest, in view of the nature of the intoxication and the fact that all domestic animals other than bovines appear to be relatively very insensitive. The discovery of a successful method of treatment would have considerable practical importance for many farmers in the Natal Midlands. The claims advanced in this connection by Dr. Scharrer in the article which appeared in the June number of this Journal, however, appear to invite criticism, and some of his statements can hardly be allowed to pass unchallenged.

In the first place, it is regrettable that the paper contains no description of any of the methods employed by the author (with the exception of a few remarks on chemical tests and procedures, to which further reference is made later) and no details whatever are given with respect to any experimental case of the intoxication. In no case is one able to consider the methods employed, the precautions against possible error which were adopted, and the course and nature of the resulting intoxication. This complete absence of definite experimental data deprives the reader of any opportunity of forming his own estimate of the grounds on which conclusions are based, and therefore of the justification for, or