TESTING CATTLE WITH JOHNIN

BEING A REPORT ON THE VALUE OF THE INTRADERMAL TEST ON CATTLE

BY THE AGRICULTURAL RESEARCH COUNCIL'S COMMITTEE ON JOHNE'S DISEASE

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1. INTRODUCTION

IN the last century a wasting disease of cattle in which diarrhoea was the prominent symptom was described by some veterinarians, but was at first often confused with tuberculosis although the distinctive lesions found in the latter disease were not present at post-mortem examination. However, it is only during the last forty years that Johne's disease[1] has been clearly differentiated from bovine tuberculosis.

The credit for the discovery of the causal organism rests with Johne and Frothingham who, in 1895, described inflammatory changes in the intestine

[1] We suggest the avoidance of the term 'paratuberculosis' for the disease, since this term is not infrequently used to describe all infections with acid-fast bacilli which are not clearly within the group *Mycobacterium tuberculosis hominis, bovis* or *avium* respectively. Johne's bacillus obviously falls in the category of 'paratuberculosis', but there are other acid-fast organisms in the group.

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associated with the presence of a very large number of acid-fast bacilli resembling the tubercle bacillus (*Mycobacterium tuberculosis*). These organisms were situated in the altered mucous membrane and in the lymphatic glands of the mesenteric tract. The authors suggested that the infection was a form of avian tuberculosis.

It was ten years before the occurrence of a similar disease was reported from other sources. In 1906 Bang made a careful study of bacterial enteritis in cattle and recognized that the chronic form associated with the bacillus of Johne and Frothingham was distinct from tuberculosis. In 1909 he discovered that a preparation made from the avian tubercle bacillus, if injected subcutaneously, produced a characteristic febrile reaction in cattle infected with Johne's bacillus.

The first cases recognized in this country were described by M'Fadyean (1907) who gave a detailed account of several cases. He suggested the term Johne's disease and noted that the condition was widely spread in Great Britain.

Failure to secure cultures of the causal organism delayed further progress, but Twort (1910) obtained growths on a medium composed of inspissated egg (Dorset's egg medium) to which dead tubercle bacilli had been added. Subsequently Twort & Ingram (1913) substituted *M. phlei* for *M. tuberculosis* and obtained good growths of Johne's bacillus both on solid and liquid media. M'Fadyean, Sheather & Edwards (1916) found that equally good results could be obtained on media enriched with glycerolated extracts of various acid-fast organisms.

Twort & Ingram (1913) prepared a diagnostic 'johnin' from cultures by a method similar to that used for making tuberculin from the tubercle bacillus. Their results were confirmed by M'Fadyean *et al.* (1916).

Dunkin (1928) prepared johnin from cultures of three strains grown on broth enriched with potato and liver extracts and containing *M. phlei*. The product was concentrated by heat and used for the diagnosis of Johne's disease by the double intradermal (D.I.D.) method. This type of johnin sometimes gave rise to a large bean-like swelling in a negative animal which was liable to cause confusion. Dunkin (1933) therefore turned his attention to simpler media and succeeded in obtaining an active product by growing a strain on Henley's synthetic medium. At first he used medium containing *M. phlei*, but he later succeeded in inducing his strain to grow in the absence of any acid-fast organism.

About the same time Glover (1932–3*b*) reported the preparation of a synthetic-medium johnin on a slightly modified Long's medium containing a glycerolated extract of *M. phlei*. With considerable difficulty he also trained the organism to grow on culture fluid containing no extract of acid-fast bacilli and subsequently produced johnins of good potency. The synthetic medium johnins of Dunkin were concentrated by heat, whereas those of Glover were prepared by separating the active principle by saturation of the dilute johnin with ammonium sulphate.

These improved johnins, when injected intradermally (I.D.) into infected cattle, produced a soft diffuse swelling like that of a tuberculin reaction, but usually less pronounced.

In 1928 Dunkin described the results of testing 204 cattle in certain herds with johnin by the D.I.D. method, and recorded that sixty-two gave positive, 139 negative and three doubtful reactions. He also tested over forty clinical cases of Johne's disease and found that all reacted except a very few which were in a very advanced stage of the disease; all the reactors were examined post-mortem and the clinical diagnosis was confirmed.

Houthuis (1932*a*) published results of testing cattle with johnin by the double I.D. method.

According to Minett (1935) who quotes a private communication, Houthuis tested forty-two cattle, of which thirty-eight were shown to have Johne's disease by very thorough post-mortem examination including histological methods when necessary.

Of twenty-eight tested with Dunkin's johnin twenty-five reacted, and of these twenty-four were shown to be infected with Johne's bacillus and one with tuberculosis only. Forty-one were tested with Sheather's johnin[1] and twenty-seven reacted; twelve did not react, of which eight were suffering from very advanced Johne's disease and the other two were also shown to be infected.

Glover (1932–3*b*) also carried out tests in infected herds with both heat-concentrated and precipitated johnins applied simultaneously by the D.I.D. method. In negative animals the heat-concentrated johnins gave slightly larger swellings, while in reacting animals the two products were about equal in potency.

Minett (1933) conducted tests by the D.I.D. method with johnin prepared from cultures on Sauton's synthetic medium containing extract of *M. phlei*. He tested 499 adult cattle belonging to seven herds situated in six counties in England. When both positive and doubtful reactors were included, the average incidence of presumed infection in these herds was 28% with extremes of 7 and 56%. In 1935 he recorded the results of testing fifty-nine animals, and fifty-two of these which reacted on one or more occasions were examined post-mortem. Thirty-nine of them were found definitely to be infected and eleven more in all probability. Six did not react to johnin though shown to be infected post-mortem; two of them were in very poor condition and were advanced cases of the disease, which probably accounted for their lack of response to the test. The remaining four were clinically in fair condition though in two advanced disease was present.

He found that when animals were repeatedly tested the reaction varied in intensity and at the re-test might be slight or even absent. The reaction was usually slight as compared with that given with tuberculin in tuberculous

[1] A heat-concentrated product obtained by growing a well-acclimatized strain of Johne's bacillus on Sauton's synthetic medium and containing *M. phlei*.

animals. He considered that repeated tests of a herd periodically were needed to ensure the detection of all infected cattle. He found no evidence that the presence of tuberculosis had any effect on the I.D. reaction to johnin.

In 1932 when this Committee was appointed by the Agricultural Research Council, it was recognized that Johne's disease was widespread in England and very prevalent in some areas. At that time a good deal of doubt was felt by members of the veterinary profession as to the reliability of the johnin diagnostic test. However, a number of experienced veterinarians were convinced from clinical observation that Johne's disease could be greatly decreased or entirely eliminated from a herd by methodical testing followed by removal or segregation of reactors. A large number of cattle had been tested by a few workers, but it had only been possible to compare the result of the test with a thorough post-mortem examination in a small proportion of the animals tested. It had been reported that some of the infected cattle did not react to the test, though this failure was chiefly in advanced cases of the disease, and that the reactors might show no symptoms either at the time of testing or for some months afterwards, nor in some cases could evidence of infection be detected post-mortem. This was not really remarkable since Johne's disease is a chronic infection, its progress may be very slow and the early pathological changes may be difficult to detect. On the other hand, it had been stated that sometimes cases of tuberculosis reacted to johnin though they were apparently not infected with the bacillus of Johne's disease.

For many years, avian tuberculin was used by various methods as a diagnostic agent for the detection of Johne's disease and there can be no doubt that a proportion of infected animals will react to this reagent. With the introduction of johnin and the recognition of its ability to elicit a specific reaction, it became logical to substitute johnin for avian tuberculin. The D.I.D. method of testing for tuberculosis has been used for some years in this country. The Committee decided, therefore, to confine its attention to this method.

Since different methods of preparing johnin were in use and no generally recognized standard existed, it was obviously of importance to examine and compare different kinds of johnin prepared in different laboratories.

2. General account of experimental work by the committee

The chief aim of the Committee was to find out which form of johnin was the most reliable and gave the most easily read results. It was also of importance to ascertain whether the observers agreed in reading the test reactions and especially how far the post-mortem examinations confirmed the diagnosis made with the different reagents.

The Committee has made three experiments, or series of tests of cattle, with johnin of different kinds. The cattle were slaughtered immediately after the tests had been read, and were examined post-mortem by macroscopic and microscopic methods. Cultural tests of the tissues were made for

M. tuberculosis and for Johne's bacillus and in addition guinea-pigs were inoculated for *M. tuberculosis*. The pathological work was shared by three laboratories and a representative of each laboratory took part in reading the reactions to the D.I.D. tests at the 72nd hour. In the first two experiments the D.I.D. tests were made and the post-mortem examinations were carried out at one of the laboratories. The third series of tests was made at the Islington abattoir.

The first experiment was made on ten animals which were known to react to johnin. The cattle were tested with three kinds of johnin and one avian tuberculin. Almost all the animals proved to be tuberculous and the results of the tests were inconclusive as regards the merits of the different johnins, but the impression was gained that avian tuberculin was less effective than the johnins.

The second experiment was made on twelve cattle of which four were believed to be normal, four infected with Johne's disease and not tuberculous, four infected with tuberculosis and not Johne's disease. The cattle were tested with two types of synthetic medium johnin. Both were concentrated by heat, but one was prepared in a medium containing *M. phlei* and the other in *phlei*-free fluid. No definite conclusion could be arrived at as to which johnin was the better since the reactions were often slight and the opinions on the diagnosis were not uniform.

For the third experiment the Committee made arrangements to test 300 cattle at the Islington abattoir. The animals to be tested were unselected, but when the tests had been read an attempt was made to choose for post-mortem a representative number in each of four groups, viz. (*a*) reacting to neither johnin nor tuberculin; (*b*) reacting to both johnin and tuberculin; (*c*) reacting to tuberculin but not to johnin; (*d*) reacting to johnin but not to tuberculin. The reagents used were (1) tuberculin prepared with synthetic medium and concentrated by precipitation with ammonium sulphate; (2) johnin prepared in synthetic medium without *M. phlei* and concentrated by heat; (3) johnin prepared in synthetic medium with *M. phlei* and concentrated with ammonium sulphate.

These experiments were very laborious and slow since the primary culture of Johne's bacillus may only become visible after 4–6 months and testing a culture by the inoculation of guinea-pigs to exclude tubercle bacilli requires 6 months observation at least.

3. DESCRIPTION OF THE MAIN (THIRD) EXPERIMENT

(*a*) *Sources of animals and places of examination*

During the period February 1936 to November 1938, 104 cattle were slaughtered and examined post-mortem. The examination of thirty-six was undertaken at the Research Institute of Animal Pathology, Camden Town, London, by Dr F. C. Minett; thirty-two at the Institute of Animal Pathology

Cambridge (twenty by Mr R. E. Glover, and twelve by Mr M. O. J. McCarthy), and thirty-six at the Farm Laboratories of the National Institute for Medical Research at Mill Hill (twenty by Mr G. W. Dunkin and sixteen by Mr R. E. Glover). The majority (ninety-three) which were tested and slaughtered at the Islington abattoir were commercial cattle brought to the market for slaughter and their history was quite unknown. Ten other animals (138, 139, 140, 141, 142, 146 and 127 from one source, and 125, 126 and 169 from elsewhere) were also included; they were from herds in which Johne's disease had been previously diagnosed.

(*b*) *Reagents used*

The cattle were tested with both johnin and tuberculin.

Synthetic-medium tuberculin was obtained from the Institute of Animal Pathology, Cambridge, and had been prepared and concentrated by precipitation by the method given in detail elsewhere (Glover, 1932–3*a*; Buxton & Glover, 1939).

Two forms of johnin were used:

(i) *Heat-concentrated* (H.C.). This was prepared at the Farm Laboratories of the National Institute for Medical Research at Mill Hill, using Henley's synthetic medium (Dunkin, 1933). The medium contained in flasks was steamed for 1 hr. daily for 3 days, incubated for a week as a sterility test and then a piece of actively growing film of the Johne's bacillus was floated on the surface. Under good conditions, macroscopic evidence of growth was apparent after 10 days and by 3 months growth was abundant, covering the surface of the medium to a depth of a quarter of an inch; by this time the medium had assumed a light yellow colour. The cultures were shaken up, filtered through two layers of paper and the filtrate reduced to one-tenth of the original volume of the medium by evaporation over a water-bath. The concentrated product while still hot was again filtered under pressure through sand and paper, and was then ready for use.

(ii) *Precipitated* (Pt.). The precipitated johnin was prepared at the Institute of Animal Pathology, Cambridge. The selected strain of Johne's bacillus, which differed from that used for the heat-concentrated johnin, was grown on a synthetic medium of the type used for the preparation of the synthetic-medium tuberculin, but enriched by the addition of a glycerolated extract of *M. phlei* (Glover, 1932–3*b*). After 3½–4½ months in the incubator, the cultures were steamed, filtered and precipitated with ammonium sulphate in exactly the same manner as the synthetic-medium tuberculin. The final product was made up in a concentration of one-twentieth of the original culture fluid and was tested on known reactors and non-reactors to johnin.

(*c*) *The methods of conducting the post-mortem examinations*

The three laboratories taking part in the work followed approximately the same methods, which had been agreed on beforehand, viz.

(i) After slaughter the following organs were removed for examination to

the laboratories: the intestine and mesenteric glands (unless tuberculous lesions were visible), the submaxillary, pharyngeal, bronchial, mediastinal, hepatic and iliac lymph glands.

(ii) Tuberculosis: If characteristic naked eye lesions were present, smears were examined for tubercle bacilli. If no characteristic lesions were seen, two guinea-pigs were inoculated subcutaneously from glands of each group including two separate groups of mesenteric glands. The glands were ground up and treated with 15% antiformin at the laboratory temperature for 30 min. The resulting emulsion was washed and (1) given subcutaneously to the guinea-pigs and (2) inoculated into culture media for Johne's bacillus which were incubated at 37° C. The guinea-pigs and culture tubes were kept at least 6 months.

(iii) Johne's disease: Smears of the intestinal mucous membrane were stained and examined, and if these were not positive, portions of the intestinal wall, especially the ileo-caecal valve and ileum, were fixed and examined histologically after staining by the Ziehl-Nielsen method. Cultures were made from scrapings of the large and small intestine, ileo-caecal valve and lymph glands after treatment with antiformin and washing. The culture medium used was the liver-extract-*M. phlei*-egg medium recommended by Dunkin (1928) with or without gentian violet. The culture tubes were incubated 6 months at 37° C. unless growth appeared earlier. Growth was examined by animal inoculation and subcultured.

(*d*) *Reactions to tuberculin and johnin and results of post-mortem examination*

The reactions of 103 of the cattle (one of the 104 was slaughtered and examined at Camden Town untested) to tuberculin and johnin are shown in Table 1. The animals examined by each individual observer are grouped together in the order in which they were killed. The result of the post-mortem examination is added in the last columns.

(*e*) *The relation between the reactions to the intradermal tests and the results of the post-mortem examinations*

In summarizing the agreement or disagreement between the reactions to johnin and the post-mortem evidence of Johne's disease the following considerations must be borne in mind:

(1) Since in practice the test is used to exclude all infected animals from a herd, a 'doubtful' reaction must often be accepted as positive, because it is desirable to eliminate all possible cases of the disease.

(2) The presence of early or slight infection with Johne's bacillus is very difficult to detect post-mortem; the disease may be confined to a few lymphatic glands; the presence of the bacillus can often only be discovered by culture on special medium—a technique which may not always be successful.

Table 1

Observers: (1) Dr Minett; (2) Mr Dunkin; (3) Mr Glover.

A. Cattle dealt with at Research Institute in Animal Pathology, London, by F. C. MINETT

			Reactions			Tuberculosis			Johne's disease				
				Johnin					Smears (and/or			Diagnosis	
No. of animal	Date of slaughter	Observer	Tuberculin	H.C.	Pt.	P.M.	G.P.*	Culture	P.M.	histology)	Culture	T.B.	J.D.	Remarks
8	20. ii. 36	1	+	−	−	+	.	.	+	(H. +)	+ (G.P.)	+	+	Advanced J D.
		2	+	−	−									
		3	+	−	−									
11	20. ii. 36	1	+	? +	? +	+	.	.	−	? + (H. −)	−	+	? −	
		2	+	+	+									
		3	+	+	+									
18	5. iii. 36	1	−	+	+	−	−	−	+	+ (H. +)	+ (G.P.)	−	+	
		2	−	+	+									
		3	−	+	+									
23	5. iii. 36	1	−	?	?	−	−	−	−	− (H. −)	−	−	−	
		2	−	+	+									
		3	−	+	+									
24	25. iii. 36	1	−	+	−	+	.	.	−	(H. −)	−	+	−	
		2	−	+	+									
		3	+	+	+									
27	26. iii. 36	1	?	+	+	−	−	−	+	+ (H. +)	+ (G P.)	−	+	
		2	+	+	+									
		3	−	+	+									
28	26. iii. 36	1	+	−	−	+	.	.	−	(H. +)	+ (G.P.)	+	+	Moderately advanced J D.
43	11. vi. 36	1	−	+	?	−	−	−	−	+	+ (G.P.)	−	+	
		2	−	+	+									
		3	−	+	+									
46	11. vi. 36	1	+	−	−	+	.	.	? −	− (H. −)	−	+	−	
		2	+	+	−									
		3	+	+	−									
59	16. xi. 36	1	−	+	?	−	−	−	−	− (H. −)	−	−	−	
		2	−	+	+									
		3	−	+	+									

* G.P. = guinea-pig inoculation.

61	16. xi. 36	1	?	+	+	–	+*	–	–	– (H. –)	–	+	–	*Med. gld. only in 1 of 2 G.P.
		2	+	+	+									
		3	+	+	+									
64	30. xi. 36	1	+	+	? +	+	.	.	–	– (H. –)	–	+	–	
		2	+	+	–									
		3	+	+	–									
69	30. xi. 36	1	–	+	–	–	–	–	–	– (H. –)	–	–	–	
		2	–	+	–									
		3	–	+	–									
75	1. ii. 37	1	–	–	–	–	–	.	–	(H. –)	–	–	–	
		3	–	–	–									
82	1. ii. 37	1	–	+	+	–	–	–	–	(H. –)	–	–	–	
		3	–	–	–									
86	1. iii. 37	1	–	+	+	–	–	–	–	– (H. –)	–	–	–	
		2	–	+	+									
		3	–	+	+									
92	1. iii 37	1	–	+	+	–	–	–	–	? (H. –)	–	–	–	? Early J.D.
		2	–	+	+									
		3	? +	+	+									
106	3. v. 37	1	+	+	+	+	.	.	–	– (H. –)	–	+	–	
		2	+	+	+									
		3	+	+	+									
110	3 v 37	1	+	–	–	+	.	.	–	– (H. –)	–	+	–	
		2	+	–	–									
		3	+	–	–									
119	7. vi. 37	1	–	–	–	–	–	–	–	– (H. –)	–	–	–	
		2	–	–	–									
		3	–	–	–									
120	7. vi. 37	1	–	?	–	–	–	–	–	– (H. –)	–	–	–	
		2	–	–	–									
		3	–	+	–									
123	9. vi. 37	1	–	+	+	?	–	.	+	+		–*	+	* Probably
		3	–	+	+									
124	31. i. 38	1	–	–	–	–	.	–	+	+	+ (G.P.)	–	+	Advanced J.D.
136	21. iii 38	1	+	+	+	–	–	–	–	– (H. –)	–	–	–	
		2	+	+	+									
		3	+	+	+									
137	21. iii. 38	1	? +	+	+	–	–	–	–	– (H. –)	–	–	–	
		2	+	+	+									
		3	+	+	+									

Table 1 (*continued*)

No. of animal	Date of slaughter	Observer	Reactions: Tuberculin	Reactions: Johnin H.C.	Reactions: Johnin Pt.	Tuberculosis: P.M.	Tuberculosis: G.P.	Tuberculosis: Culture	Johne's disease: Smears (and/or histology) P.M.	Johne's disease: Smears (and/or histology)	Johne's disease: Culture	Diagnosis: T.B.	Diagnosis: J.D.	Remarks
142	11. IV. 38	1	+	+	+	–	–	–	–	–	–	–	–	
		2	+	+	+					(H. –)				
		3	+	+	+									
144	10. IV. 38	1	–	+	+	–	–	–	–	–	–	–	–	? Avian T.B. present
										(H. –)				
154	16. V. 38	1	+	?	?	+	.	.	–	–	–	+	–	
		3	+	+	+					(H. –)				
156	16. V. 38	1	+	+	+	+	.	.	–	–	–	+	–	
		3	+	+	+					(H. –)				
167	27. VI. 38	1	–	+	+	–	–	–	–	–	–	–	–	
		3	–	+	+									
168	27. VI. 38	1	–	–	–	–	–	–	–	–	–	–	–	
		3	–	–	–									
178	31. X. 38	1	–	+	+	–	–	–	–	–	–	–	–	
		3	–	+	+					(H. –)				
181	31. X. 38	1	–	+	+	–	–	–	–	–	–	–	–	
		3	–	+	+					(H. –)				
184	23. XI. 38	1	+	+	+	+	.	.	–	–	–	+	–	
		3	+	+	+					(H. –)				
192	23. XI. 38	1	? –	+	+	–	–	–	–	–	–	–	–	
		3	–	+	+					(H. –)				
			B. Cattle dealt with at Institute of Animal Pathology, Cambridge (Numbers 7–117 by R. E. Glover and numbers 128–193 by M. O. J. McCarthy)											
7	20. II. 36	1	–	–	–	+	+	.	–	.	–	+	–	? Advanced T.B.
		2	–	–	–									
		3	–	–	–									
14	20. II. 36	1	+	+	+	+	+	+	–	(H. ? +)	? T.B.	+	? –	Tuberculous enteritis present
		2	+	+	+									
		3	+	+	+									
17	5. III 36	1	+	? –	? –	Slt.	+	+*	–	–	–	+	–	* Avian T.B. from mes. gld. Bovine from thor. gld.
		2	+	?	?	+								
		3	+	+	+									

25	5. iii. 36	1	−	+	+	−	−	.	+	+	+	−	+	
		2	?	+	+									
		3	+	+	+									
31	26. iii. 36	1	+	?+	?+	+	+	.	+	+*	?+†	+	+	* From intestine † From iliac gland ? Johne
		2	+	+	+									
		3	?+	?+	+									
35	26. iii. 36	1	+	−	−	+	+	.	−	−	−	+	−	
		2	+	−	−									
		3	+	−	−									
41	11. vi. 36	1	+	+	+	+	+	.	?+	−	+	+	+	
		2	+	+	+									
		3	+	+	+									
44	11. vi. 36	1	−	−	−	−	−	.	−	.	−	−	−	
		2	−	−	−									
		3	−	−	−									
50	16. xi. 36	1	−	?−	−	−	−	.	−	−	−	−	−	Healed (? avian) lesions in mes. glds.
		2	−	+	−									
		3	−	+	−									
53	16. xi. 36	1	+	−	−	+	+	.	−	.	−	+	−	
		2	+	−	−									
		3	+	+	+									
66	30. xi. 36	1	+	+	+	Slt.	.	?*	−	.	−	?*	−	* ? Avian T.B.
		2	+	+	+	+								
		3	+	+	+									
68	30. xi. 36	1	−	−	−	−	−	.	−	.	−	−	−	
		2	−	+	+									
		3	−	−	−									
78	1. ii. 37	1	−	−	−	−	−	.	+	+	+	−	+	Fairly well advanced J.D.
		2	−	−	−									
79	1. ii. 37	1	?+	+	+	−	−	.	+	+	+	−	+	
		2	−	+	+									
90	1. iii. 37	1	−	?+	−	−	−	.	?−	− (H. −)	?−*	−	?−	* Mes. gld. Subcultures failed to grow
		2	−	−	−									
		3	−	+	+									
96	1. iii. 37	1	−	+	+	−	−	.	−	−	−*	−	−	* Contaminated
		2	−	+	+									
		3	−	+	+									
99	3 v. 37	1	+	+	+	+	+	+	−	.	−	+	−	Avian and bovine T.B. from mes. gld.
		2	+	+	+									
		3	+	+	+									

Table 1 (*continued*)

No. of animal	Date of slaughter	Observer	Reactions: Tuberculin	Reactions: Johnin H.C	Reactions: Johnin Pt.	Tuberculosis: P.M.	Tuberculosis: G.P.	Tuberculosis: Culture	Johne's disease: Smears (and/or histology) P.M.	Johne's disease: Smears (and/or histology)	Johne's disease: Culture	Diagnosis: T.B.	Diagnosis: J.D.	Remarks
105	3. v. 37	1	−	+	+	?+*	−	−	−	−	−	−	−	* Abscess in 1 mes. gld. with scanty acid-fasts (? healed avian T.B.)
		2	−	+	+									
		3	−	+	+									
111	7. vi 37	1	−	?	−	?+*	−	.	?+†	− (H. −)	−	−	−	* Calcif. les. in 2 mes. glds. with a few acid-fasts. Proved avian T.B. † Some thickening of intests.
		2	−	+	−									
		3	−	+	+									
117	7. vi. 37	1	−	−	−	−	−	.	−	.	−	−	−	Avian T.B. from glds.
		2	−	+	−									
		3	−	−	−									
128	21. iii. 38	1	−	−	−	−	−	−	−	.	−	−	−	
		2	−	−	−									
		3	−	−	−									
129	21. iii. 38	1	+	+	+	−	−	−	+	*	−	−	+	* Typical Johne, but only 1 bac. seen in 8 smears
		2	+	+	+									
		3	+	+	+									
140	11. iv. 38	1	?	+	?+	−	−	−	+	+	*	−	+	* Failed to grow
		2	−	+	−									
		3	−	+	+									
141	14. iv. 38	1	−	+	−	−	−	−	−*	−	−	−	−	* Slight rubor
		2	−	+	−									
		3	−	?+	?+									
148	16. v. 38	1	+	+	+	−	−	−	−*	−	−	−	−	* Slight rubor
		3	+	+	+									
149	16 v 38	1	?	+	+	−	−	−	?−*	−	−	−	?−	* Slight thickening of i.c.v. small gut. ? Early Johne
		3	+	+	+									
164	27. vi. 38	1	+	+	+	−	−	−	?−*	−	−	−	?−	* Slight thickening and rubor. ? Early Johne
		3	+	+	+									
165	27. vi. 38	1	−	−	−	−	−	−	−	−	−	−	−	
		3	−	−	−									
175	31. x. 38	1	−	+	+	−	−	−	?+*	−	−	−	−	* Rubor and slight thickening of i c v.
		3	?	+	+									

176	31. x. 38	1	−	?	+	−	−	−	?+*	−	−	−	?−	* Rubor and thickening in caecum. Slight rubor in small intestine but no thickening
		3	−	+	+									
188	23. xi. 38	1	+	+	+	−	−	−	?+*	−	−	−	−	* Slight rubor of valve
		3	+	+	+									
193	23. xi. 38	1	+	+	+	−	−	−	?+*	−	−	−	?−	* Typical enteritis in small gut. Strawberry valve. Some rubor in large gut
		3	+	+	+									

C. Cattle dealt with at National Institute for Medical Research, Mill Hill

(Numbers 6–114 by G. W. Dunkin and numbers 125–189 by R. E. Glover)

6	20. ii. 36	1	+	−	−	+	.	.	+	.	+	+	+	Fairly well advanced J.D.
		2	+	−	−									
		3	+	−	−									
13	20. ii. 36	1	−	+	+	−	−	.	+	.	+	−	+	
		2	−	+	+									
		3	−	+	+									
21	5. iii. 36	1	+	?	?	+	.	.	+	.	+	+	+	
		2	?+	?	?									
		3	+	+	+									
26	5. iii. 36	1	−	+	+	−	−	−	+	.	+	−	+	
		2	−	+	+									
		3	−	+	+									
33	25. iii. 36	1	+	+	+	+	.	.	+	+	*	+	+	* Failed: ? bad media
		2	+	+	+									
		3	+	+	+									
34	25. iii. 36	1	+	+	+	+	.	+	+	.	+	+	+	
		2	+	+	+									
		3	+	+	+									
37	11. vi. 36	1	+	+	−	+	.	−*	+	+	−*	+	+	* ? Bad media
		2	+	+	−									
		3	+	+	−									
45	11. vi. 36	1	+	+	−	+	.	.	+	+	−*	+	+	* ? Bad media
		2	+	+	−	•								
		3	+	+	−									
47	16. xi. 36	1	+	+	+	+	.	*	+	.	*	+	+	* Contaminated
		2	+	+	+									
		3	+	+	+									

Table 1 (*continued*)

No. of animal	Date of slaughter	Observer	Reactions: Tuberculin	Reactions: Johnin H.C.	Reactions: Johnin Pt.	Tuberculosis: P.M.	Tuberculosis: G.P.	Tuberculosis: Culture	Johne's disease: Smears (and/or histology) P.M.	Johne's disease: Culture	Diagnosis: T.B.	Diagnosis: J.D.	Remarks
56	16. xi. 36	1	−	?	−	?	+	+	−	−	+	−	
		2	+	+	−								
		3	+	+	−								
62	30. xi. 36	1	−	−	−	−	−	−	−	−	−	−	
		2	−	+	+								
		3	−	+	+								
63	30. xi. 36	1	−	+	+	−	−	−	−	−	−	−	
		2	−	+	+								
		3	−	+	+								
77	1. ii. 37	1	−	?	−	−	−	−	−	−	−	−	
		3	−	+	−								
83	1. iii. 37	1	−	+	+	−	−	−	+	*	−	+	* Negative at 2½ months, then lost
		3	−	+	+								
91	1. iii. 37	1	+	?	−	+	.	?+*	?−	?−*	+	?−	* Br. gld. +. Although no animal tests attempted, probably *M. tuberculosis*
		2	+	+	+								
		3	+	+	+								
94	1. iii. 37	1	−	+	+	+	.	−	?−	−	?−*	+	?−	* ? Mes. gld. Culture lost
		2	+	+	+								
		3	+	+	+								
100	3. v. 37	1	−	+	+	−	−	−	?+	.	?−	−	+	
		2	−	+	+								
		3	−	+	+								
103	3. v. 37	1	−	−	−	−	−	?+*	?	.	?+*	−	−	* ? +Pharyngeal gland suspected, proved avian T.B.
		2	−	−	−								
		3	−	−	−								
113	7. vi. 37	1	?	+	+	+	.	.	?	+*	−	+	?−	* From mes. and sub. max. glds.
		3	+	+	+								
114	7. vi. 37	1	+	+	?+	?	−	−	+*	−	−	−	?−	* Linear markings on m.m. suggestive of Johne
		3	+	+	+								
125	2. iii. 38	3	−	+	.	−	−	.	+	+	+	−	+	

126	5. iii. 38	3	–	–	.	–	–	.	+	+	+	–	+	? Moderately advanced J.D.
127	14. iii. 38	3	–	+	.	–	–	.	+	+	+	–	+	
132	21. iii. 38	1	–	?	–	–	–	.	–	–	–	–	–	
		2	–	–	–									
		3	–	?	–									
134	21. iii. 38	1	?+	–	–	–	–	.	–	–	–	–	–	
		2	+	+	+			.						
		3	?+	+	+									
138	11. iv. 38	1	?+	+	+	–	–	.	+	+	–*	–	+	* ? Bad media
		2	+	+	+									
		3	+	+	+									
139	11. iv. 38	1	–	+	+	–	–	.	+	+ (H.+)	–	–	+	
		2	–	+	+									
		3	–	+	+									
146	27. vi. 38	1	–	+	+	–	–	.	+	+	+	–	+	
		3	–	+	+									
147	16. v. 38	1	–	+	+	–	–	?*	–	–	–	–	–	* ? Avian T.B. almost completely attenuated
		3	+	+	+									
155	16. v. 38	1	?+	?	–	–	–	.	–	?	–	–	–	
		3	+	+	–									
159	27. vi. 38	1	–	–	–	–	–	?*	–	.	?*	–	–	* Avian T.B.
		3	–	–	–									
169	12. vii. 38	3	–	+	.	–	–	.	?+	? (H.–)	–	–	–	
170	31. x. 38	1	+	+	+	?–	–	.	?–	–	–*	?†	–	* Contaminated † ? Healed avian T.B.
		3	+	+	+									
174	31. x. 38	1	–	+	+	–	–	.	–	–	–*	–	–	* Contaminated
		3	–	+	+									
183	23. xi. 38	1	+	+	+	–	–	.	–	.	–	–	–	
		3	+	+	+									
189	23. xi. 38	1	–	–	?+	–	–	.	–	–	–	–	–	
		3	–	–	–									

(3) In order to avoid contaminating bacteria in the cultures it was necessary to use antiformin to prepare the animal tissues before inoculating the culture medium with them. The strength of antiformin used was not always the same and the higher strengths may sometimes have prevented growth of the Johne's bacillus.

(4) An unknown proportion of positive reactors without post-mortem evidence of Johne's disease is probably due to infection with the avian tubercle bacillus. This latter usually requires a special technique for its detection which was not used regularly in this experiment.

Table 2. *Summary of results. Reactions to tuberculin and johnin*

Category	Tuberculin	Johnin	Nos. of animals	Total cases where P.M. findings agreed with reactions as regards Johne's disease	Total animals
A	–	–	(7), (44), (50), (68), (75), (77), 78, (90), (103), (117), (119), (120), 124, 126, (128), (132), (159), (165), (168), (189)	17 (85%)	20
B	+	+	11, 14, (21), (31), (33), (34), (41), (47), 61, 64, 66, 91, 94, 99, 106, 113, 114, (129), 136, 137, (138), 142, 134, 148, 149, 154, 156, 164, 183, 184, 188, 193, 170	8 (24·2%)	33
C	+	–	6, 8, 28, (35), (46), (53), (56), (110), (155)	6 (66·6%)	9
D	–	+	(13), (18), 23, 24, (25), (26), (27), (43), 59, 62, 63, (79), (83), 86, 92, 96, (100), 105, 111, (123), (125), (127), 147, (139), (140), 141, 144, (146), 167, 169, 174, 175, 176, 178, 181, 192	15 (41·7%)	36

Brackets indicate that post-mortem examination confirmed the johnin I.D. test.

Note. In five further animals the opinions on the reactions to the two johnins were equally divided; of these, two (37 and 45) were considered post-mortem to be positive and three (17, 69 and 82) negative.

The detailed record of the 103 animals in Table 1 shows the results as regards the I.D. tests with the two johnins, the reading of the reactions by different observers and the evidence of disease detected post-mortem. It is seen that a definite conclusion was not reached on these points in a number of cases.

In Table 2 the results are summarized and, where there has not been unanimity in reading the I.D. reaction, the result tabulated has been based on the opinion of the majority of the observers on the reaction to both johnins. The final opinion of the pathologist who made the post-mortem examination

has been adopted as to the presence or absence of the disease. The animals are grouped in four categories so as to show the reactions to tuberculin as well as to johnin, since in some cases the presence of tuberculosis may have influenced the johnin reaction.

In Table 3 the cases of agreement and disagreement between the johnin test during life and the post-mortem examination are collected into separate columns.

Table 3. *Comparison of johnin test and post-mortem evidence of Johne's disease*

	Agreement		Disagreement		
Johnin test	−	+	−	+	
Post mortem	−	+	+	−	
	7	13	6	11*	136
	35	18	8	14*	137
	44	21	28	23	141
	46	25	78	24*	142
	50†	26	124	59	144†
	53	27	126	61*	147†
	56	31*		62	148
	68	33*		63	149*
	75	34		64*	154*
	77	41		66†	156*
	90	43		86	164*
	103‡	47		91*	167
	110	79		92	169
	117‡	83		94*	170†
	119	100		96	174
	120	123		99‡	175
	128	125		105†	176*
	132	127		106*	178
	155	129		111‡	181
	159‡	138		113*	183
	165	139		114	184*
	168	140		134	188
	189	146			192
					193*
Totals	23	23	6		46

* Complicated by bovine tuberculosis.

† Possible avian infection.

‡ Avian strain of *M. tuberculosis* recovered.

Note. In five further animals the opinions on the reactions to the two johnins were equally divided; of these, two (37 and 45) were considered post-mortem to be positive and three (17, 69 and 82) negative.

In Table 2 the numbers of animals in which the post-mortem and the johnin test agreed are placed in brackets. The animals in categories A and C which all gave negative reactions to johnin numbered twenty-nine (20 + 9), but three in category A (78, 124, 126) and three in category C (6, 8, 28), six in all, were found to have Johne's disease post-mortem, and in twenty-three (17 + 6) no evidence of this disease was found. Therefore in twenty-three (79·3%) was there agreement between the test during life and examination after death.

Of the sixty-nine animals in categories B and D which all gave positive reactions to johnin, twenty-three (33·3%) were recorded as cases of Johne's disease post-mortem and in forty-six (66·6%) this disease could not be

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detected. The agreement between the two methods of diagnosis was therefore only in about 33% of the positively reacting animals.

Of the forty-six which gave a positive johnin reaction unsupported by post-mortem evidence, twenty-four which were in group B also reacted to tuberculin and the presence of tuberculosis may in some cases have been the cause of the positive reaction to johnin.

If the evidence were taken at its face value, the experiment would suggest that in a herd examined by the D.I.D. johnin test alone, two-thirds of those condemned might be incorrectly diagnosed and about one-fifth of the cases of undoubted disease missed. However, the recognized difficulty in detecting infection with Johne's bacillus post-mortem makes it impossible to draw final conclusions from this experiment.

(*f*) *Examination of the separate factors in the experiment*

Since the previous experience of johnin testing by several observers has led to the belief that I.D. testing with johnin gives fairly accurate results, especially as regards the reliance to be placed on positive reactions, the different steps in the experiment have been further scrutinized.

(i) *Choice of the two johnins and the similarity of the results obtained with them.*

Both johnins were prepared with synthetic culture media like modern tuberculin, to avoid the excessive reactions in normal animals which sometimes occur when broth is used; they were concentrated in different ways, by heat (H.C. johnin) and precipitation with ammonium sulphate (Pt. johnin) respectively; the latter contained an extract of the acid-fast *M. phlei*, but the former did not; both had been previously tested on healthy and infected cattle. The reactions obtained with the two johnins agreed as a rule, but among 103 cattle twelve instances occurred in which there were decided differences between the reactions, and in some other animals slighter degrees of dissimilarity were found.

The H.C. johnin produced more and sometimes larger reactions. In no instance was there a positive reading with Pt. johnin when the reaction to H.C. was negative. In two cases (C, 37 and 45) the reaction was positive to H.C. and negative to Pt. when Johne's disease was present; in twelve cases (A, 46, 64, 69, 120; B, 50, 90, 111, 117, 141; C, 56, 77, 155) a definitely positive reaction was recorded with H.C. but not with Pt. when signs of Johne's disease were absent post-mortem; six cases (A, 6, 8, 28, 124; B, 78; C, 126) did not react at all to johnin, but were found to be diseased post-mortem.

It may be noted here that three cases (B, 50, 111, 117) which reacted to H.C. but not to Pt. were found to be infected with avian tubercle bacillus but not with Johne's bacillus.

(ii) *Different readings of double intradermal reactions.*

The observers did not always agree in their estimate of the reactions to johnin, and on thirty-three occasions only two and on seven only one observer read the result at the 72nd hour.

Since the plan of accepting the opinion of the majority of the observers in the case of varied readings was not very satisfactory, separate lists have been made of those cases in which there was no difference of opinion. There were twenty-seven positive reactions on which all three observers were agreed, twenty-one read by two on which there was no disagreement and four were read by only one; that is, fifty-two positive reactions without any difference of opinion. There was unanimity also in seventeen cases with negative reactions (nine by three observers; five by two observers; three by one). There were thirty-four cases in which the readings varied or were doubtful. If only the undisputed reactions are compared with the post-mortem results, it is found that out of fifty-two positive reactors nineteen (36·5%) were definite cases of disease, twenty-seven negative and six doubtful; of the seventeen negative reactors, eleven were found to be negative and six positive post-mortem. The agreement in these cases selected on account of the unanimous reading of the reactions is about the same as when the majority opinion on all the animals is taken.

It does not appear that the difference between the observers or any fault in the reading can account for the discrepancy between D.I.D. reaction and post-mortem examinations.

(iii) *The diagnosis of Johne's disease post-mortem.*

The post-mortem criteria of the presence of Johne's disease are difficult to lay down exactly, but there are three important facts which should be established:

(*a*) macroscopic thickening and congestion of the mucous membrane in parts of the large and small intestine, especially of the ileo-caecal valve;

(*b*) the presence of numerous acid-fast bacilli, typically in large clumps, in smears of scrapings of the mucous membrane and mesenteric lymph glands; and

(*c*) culture of Johne's bacillus on special media from scrapings which have been treated sufficiently, but not too severely, with antiformin to kill contaminating bacteria. 15% antiformin for 30 min. was normally but not always satisfactory. The cultures grow very slowly and growth may not be visible for 4–6 months.

Either the typical presence of the bacillus in scrapings or sections under the microscope, or its successful culture and subsequent examination, may be accepted as proof of the presence of Johne's disease, and typical naked eye appearance of the gut alone is probably sufficient evidence.

Unfortunately the naked eye appearances may be only very slightly abnormal or not noticeable, the bacilli may not be numerous in the parts examined and the cultures are very liable to fail chiefly owing to contaminating bacteria.

The difficulty in establishing the existence of Johne's disease is much greater than in the case of tuberculosis, especially as no small laboratory animal has yet been found to be susceptible to the disease.

(*g*) *Isolation of Johne's bacillus*

Among the 103 animals examined post-mortem there were twenty cases from which Johne's bacillus was isolated in culture. These were A, 8, 18, 27, 28, 43, 124; B, 25, 31, 41, 78, 79; C, 6, 13, 21, 26, 34, 125, 126, 127, 146. Of these ten (A, 18, 27, 43; B, 25, 41, 79; C, 13, 26, 34, 146) gave positive reactions to I.D. testing with both johnins, except for one doubtful reading of the reaction to Pt. of 43; and two more (C, 125 and 127) reacted positively to H.C., but Pt. was not used.

Six of the twenty (A, 8, 28, 124; B, 78; C, 6, 126) were negative reactors, the first five with both johnins, but 126 was tested with H.C. only; two (C, 21 and B, 31) gave doubtful I.D. reactions.

The failure of a few cases of definite Johne's disease to react to johnin is in accordance with previous observations. The absence of reactions to the I.D. test has usually been attributed to the advanced stage of the disease. Of the above mentioned six cases three (78, 124, 126) reacted to neither tuberculin nor johnin, and Johne's disease but no tuberculosis was found post-mortem; the other three (6, 8, 28) reacted to tuberculin, but not to johnin, and tuberculosis and Johne's disease were both present. The stage of Johne's disease in these animals was noted as 'fairly well advanced' (78, 6), 'advanced' (124, 8), and 'moderately advanced' (126, 28).

(*h*) *Isolation of avian tubercle bacillus*

In some cases (by R.E.G.), a special effort was made to isolate avian tubercle bacilli by inoculating an extra guinea-pig from each emulsion of gland material, killing it after one month and culturing from the local gland and spleen.

In five animals (B, 17, 99, 111, 117; C, 159) fully virulent cultures of the avian type of tubercle bacillus were isolated, and, in two of these (B, 17 and 99), bovine *M. tuberculosis* was also present. In one other (C, 103) an avian strain was isolated. In six further cases the presence of this type was suspected, but could not be confirmed (A, 144; B, 50, 66, 105; C, 147, 170). Of the six confirmed cases, only the two in which bovine tuberculosis was present (B, 17 and 99) reacted positively to tuberculin and only these two reacted positively to both johnins; but two only (C, 103 and 159) were completely negative in all readings to both johnins.

(*i*) *Presence of* (*bovine*) *tuberculosis*

There is a suggestion that the presence of tuberculosis (bovine) may influence the reactions to johnin in the fact that fewer reactors to johnin (8 = 24·2%) were found to have Johne's disease post-mortem in category B, where the tuberculin reaction was also positive, than in category D (15 = 41·7%) in which the animals only reacted to johnin. Also thirteen of the thirty-three cattle in category B which reacted to johnin and tuberculin were found to be tuberculous post-mortem without evidence of Johne's disease. It seems

probable that in some cases tuberculosis accounted for the reactions to johnin.

There were, in this series of 103 animals, fifteen which gave positive reactions to both tuberculin and johnin though no tuberculosis was found post-mortem, and in only two of them was Johne's disease found to be present. Besides these cases there does not appear to be any evidence that Johne's disease may sensitize an animal to tuberculin. There is also the experience in the tubercle-free herd at Compton in which there are many reactors to johnin but none to tuberculin.

4. DISCUSSION

The value of the double intradermal Johnin test

In estimating the value of the johnin test for diagnosis the quality of the preparation of johnin used is obviously very important. Testing the specificity and potency of johnin is difficult since clinical diagnosis is often uncertain except in advanced cases which are apt not to react satisfactorily. Diagnosis by examination of the faeces is slow and uncertain; Houthuis was of opinion that 10% of cases could be diagnosed in this way. As a consequence it is not at all easy to obtain a series of certainly infected and uninfected cattle on which to test the reagent.

It has often been urged that the use of a highly potent johnin is very important and in the earlier series of tests, when post-mortem examinations have been made, most of the failures in correct diagnosis have been the negative reactors with disease found post-mortem, and the positive reactors have usually turned out to be infected. In this present (main) experiment, on the other hand, it is the cattle giving a positive reaction but appearing to be negative post-mortem, which are in excess. This experiment therefore suggests a possible defect in the specificity of the johnin rather than in its potency. Perhaps the difference in the direction of results between this and earlier work is in part due to the majority of recorded post-mortem examinations having then been made on animals in known infected herds and under suspicion on account of loss of condition.

How far the large proportion of reactions without evidence of disease post-mortem may be due to imperfections in the methods of pathological examination is uncertain, but awaits further work on the clinical following up of reactors and also on methods of culture.

Minett (1935) could not prove the presence of disease post-mortem in seven out of forty-six reactors, he also records five or six cases in which there were no macroscopic signs of disease and no Johne's bacilli to be seen in microscopic preparations yet in which cultures were obtained.

M'Fadyean remarked on the frequency of trivial naked-eye changes though many bacilli could be found, and also stated that in some cases the bacilli were very few and scattered in the diseased tissues.

The reasons therefore which may be suggested to account for positive

reactions to johnin in the absence of evidence of disease post-mortem are (1) early cases of disease not detected post-mortem; (2) the presence of tuberculosis, bovine or avian; (3) lack in specificity of the johnin.

5. Conclusions

1. The comparison between reactions to the double intradermal (D.I.D.) test with johnin and the post-mortem showed:

(*a*) An agreement of twenty-three in sixty-nine positive reactions, or nineteen in fifty-two if only those reactions are accepted concerning which there was no difference of opinion.

(*b*) That out of twenty-nine negative reactors six were found to have Johne's disease post-mortem, i.e. in twenty-three was there agreement; or considering only the seventeen which were unanimously called negative reactors, six were positive and eleven negative post-mortem.

(*c*) That if out of the 103 cattle tested only the twenty cases from which Johne's bacillus was isolated are considered, ten reacted positively to both johnins, and two more to H.C.—the only Johnin used; two gave doubtful reactions.

2. The reasons for the large disagreement between clinical reaction and post-mortem results are to a great extent speculative:

(*a*) Failure to detect the infecting bacillus present in early or slight cases of the disease probably accounted for a considerable number of disagreements.

(*b*) The existence of bovine or avian tuberculosis may have accounted for some unexplained positive reactions.

(*c*) There was sufficient agreement between the reactions to the two johnins to make it unlikely that possible defects in these preparations would be responsible for very many errors, but the difficulty in testing johnin for specificity and potency must leave room for a certain margin of error.

3. Further research work is much needed:

(*a*) Especially by regular testing and following up segregated reactors.

(*b*) To improve the specificity and estimate the potency of johnin.

(*c*) To improve cultural methods for isolating Johne's bacillus.

(*d*) To find a susceptible small laboratory animal by which diagnosis, especially post-mortem diagnosis, can be improved.

(*e*) To investigate methods for detection of avian tuberculosis in cattle, and to inquire into the occurrence of positive reactions to johnin of animals infected with bovine and avian tuberculosis.

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