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CULTURE OF MYCOBACTERIUM JOHNEI

By

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The State Veterinary Serum Laboratory uses complement fixation on blood samples and microscopical examination of faeces and rectal mucosa samples in its routine diagnosis of Johne's disease. The complement fixation method has been described in detail previously by the writer (1) and therefore will not be discussed further in the present work. Microscopy of faeces samples for content of *M. johnei* is a simple and rapid method but only satisfactory in the event of a positive result. *Doyle* (1959) reported that 25 to 30 per cent of diseased animals will excrete bacteria in their faeces in sufficient number to be demonstrated by microscopy. Diagnosis can be achieved in a greater number of cases by microscopic examination of rectal mucosa scrapings. In the writer's experiments, such examination has given positive results in about 25 per cent more cases than microscopy of faeces (1).

A more accurate examination of faeces and rectal mucosa can be made by using both the microscopic technique and the culture method. However, the culture method is timeconsuming, since often about six months will elapse before a final reply can be given. Thus this method is of limited value in practice.

WRITER'S EXAMINATIONS

Comparison has been made between the value of two culture media for isolation of *M. johnei*, and the growth propensity of the bacteria has been examined in microscopically positive cases. In addition, the number of positive cultures was evaluated in relation to the microscopical findings.

Methods.

The inoculation material was homogenized in mortar with 10 per cent sulphuric acid, in the case of samples examined before June 1959 for 15 minutes, and for samples treated after that date for 30 minutes. After the addition of 10 ml. physiological saline and centrifugation for 10 minutes at 3,000 to 4,000 r.p.m., the supernatant was poured off and the sediment suspended in 2 to 3 ml. Besredka medium. Using a Pasteur pipette with bent point, two drops of the suspension were spread on the surface of two tubes of Löwenstein medium to which 5 per cent killed bovine tubercle bacteria (t. b. membranes) had been added, and on two tubes of Löwenstein medium to which both t. b. membranes and I.N.H. ("Tibinid") had been added.

All cultures were observed for three months. The criteria for positive culture were: characteristic colony morphology and characteristic microscopical appearance of the individual bacteria in the colonies. In the event of doubt concerning the nature of the resulting colonies, and in many cases as a control measure, inoculation was also carried out on to the media generally used in the diagnosis of tubercle bacteria, *viz.* Löwenstein medium with and without I.N.H., and Besredka medium.

Materials.

Since January 1958 cultures have been made from faeces, pieces of small intestine, mesenteric lymph nodes, etc. both for the purpose of diagnosis and for the sake of experiment. The experiments have been of the greatest importance in those instances where bacteria were demonstrated by culture though none had been found by direct microscopy. Apart from the diagnostic significance in individual cases, such results were valuable in the assessment of the reliability of the complement fixation method. Negative culture results have also been useful in that respect (1). The culture method is also valuable from the point of view of identification of acid-fast rods of not quite characteristic morphology. Furthermore, the method has been used in a number of cases in which microscopy had definitely revealed the presence of Johne organisms. These latter examinations were of more experimental nature and served to investigate, for example, the growth ability of the bacteria and their possible preference for certain media.

Results.

Inoculation was made of 2,107 specimens on to two tubes of Löwenstein medium with t. b. membranes (Table 1). Microscopy was not carried out in 36 cases. In 292 of the remaining 2,071 cases (14.1 per cent) direct microscopy had revealed acid-fast rods which, on the basis of their appearance, were judged to be Johne organisms. Such bacteria could not be demonstrated by microscopy in 1,779 specimens (85.9 per cent).

In 187 cases culture gave growth of Johne organisms on both of the inoculated tubes ("A"). Two of the specimens had not been examined by microscopy; direct microscopy had revealed Johne organisms in 139 of the remaining 185 (75.1 per cent); 46 (24.9 per cent) had been negative by microscopy.

In 46 cases there was growth on one of the inoculated tubes only ("B"). Microscopy had been positive in 22 (47.8 per cent) of these, while the remaining 24 (52.2 per cent) were negative by microscopy.

Table 1. Löwenstein medium with t. b. membranes.

Reading Two tubes	Code	No micro-scopy	Micr. positive		Micr. negative		Micr. pos. & micr. neg.	
			No.	%	No.	%	No.	%
2 +	A	2	139	75.1	46	24.9	185	8.9
1 +, 1 —	B	0	22	47.8	24	52.2	46	2.2
1 +, 1 cont.	C	0	15	50.0	15	50.0	30	1.4
Culture pos. Total no.		2	176	67.4	85	32.6	261	12.6
2 —	D	11	67	5.2	1225	94.8	1292	62.4
1 —, 1 cont.	E	7	15	6.1	231	93.9	246	11.9
2 cont.	F	16	34	12.5	238	87.5	272	13.1
Culture neg. Total no.		34	116	6.4	1694	93.6	1810	87.4
Total		36	292	14.1	1779	85.9	2071	100

Explanation of symbols:

Micr.: Microscopical examination of smear.
+: Growth of typical colonies of *Mycobacterium johnei.*
cont.: Contaminated (non-specific growth).
—: No growth.

In 30 cases ("C") one of the culture tubes was so contaminated that growth of Johne organisms would not have been recognized by ordinary observation, while the other tube showed colonies of Johne organisms. Microscopy had revealed acid-fast rods in 15 (50 per cent) of these specimens.

In 1,303 cases there was no growth on either of the culture tubes ("D"). Eleven of these had not been examined microscopically and 67 (5.2 per cent) of the remainder were positive.

In 253 cases ("E") one of the culture tubes was contaminated while the other showed no growth whatsoever. Seven of these had not been examined microscopically and 15 (6.1 per cent) of the remaining 246 were positive by that method.

In 288 cases ("F") both culture tubes were contaminated. Sixteen had not been examined microscopically and 34 (12.5 per cent) of the remainder were positive.

In 1,953 cases inoculation was also made on to two tubes of Löwenstein medium to which both I. N. H. and t. b. membranes had been added (Table 2).

Culture gave growth of Johne organisms on both tubes in 188 cases ("A"). Two of these had not been examined micro-

Table 2. Löwenstein medium with I. N. H. and t. b. membranes.

Reading Two tubes	Code	No microscopy	Micr. positive		Micr. negative		Micr. pos. & micr. neg.	
			No.	%	No.	%	No.	%
2 +	A	2	136	73.1	50	26.9	186	9.6
1 +, 1 —	B	0	22	45.8	26	54.2	48	2.5
1 +, 1 cont.	C	1	16	50.0	16	50.0	32	1.6
Culture pos. Total no.		3	174	65.4	92	34.6	266	13.7
2 —	D	2	72	5.9	1143	94.1	1215	62.4
1 —, 1 cont.	E	1	14	6.0	221	94.0	235	12.1
2 cont.	F	1	28	12.2	202	87.8	230	11.8
Culture neg. Total no.		4	114	6.8	1566	93.2	1680	86.3
	Total	7	288	14.8	1658	85.2	1946	100

Explanation of symbols: As in Table 1.

scopically, 136 (73.1 per cent) of the remainder were positive, and 50 (26.9 per cent) negative by direct microscopy.

In 48 cases there was growth on one of the inoculated tubes only ("B"). Twenty-two (45.8 per cent) of these had been positive by direct microscopy, while in the other 26 cases (54.2 per cent) it had not been possible to demonstrate Johne organisms.

In 33 cases one of the two tubes was contaminated while colonies of Johne organisms grew on the other ("C"). One specimen had not been examined microscopically; in 16 of the other specimens (50 per cent) direct microscopy had already revealed the presence of Johne organisms.

In 1,217 cases there was no growth in either of the culture tubes ("D"). Two of the inoculated specimens had not been examined microscopically; 72 (5.9 per cent) of the remainder were positive by microscopy.

In 236 cases ("E") one of the two culture tubes was contaminated, while the other showed no growth. One of the specimens had not been examined microscopically; 14 (6 per cent) of the remainder showed content of acid-fast rods by microscopy.

Cultures from 231 specimens were completely contaminated ("F"). One of these had not been examined microscopically, and 28 (12.2 per cent) of the remainder were positive.

DISCUSSION

It would appear that culture on the two media (Tables 1 and 2) has given results which are similar within the individual groups (A, B, C, D, E, F). Thus I. N. H. seems to have absolutely no effect on the growth of *Mycobacterium johnei.*

Johne organisms were demonstrated by culture in three groups: "A" (growth on both tubes), "B" and "C" (growth on one tube while the other was without growth or contaminated). If each inoculation on two tubes is regarded as one independent test irrespective of the medium used (as mentioned, the principle was that the specimens were inoculated on two tubes of each medium), it will be seen that of 527 samples which were positive by culture, 350 (66.4 per cent) were also positive by microscopy. In 33.6 per cent of the cases, therefore, a positive bacteriological diagnosis was obtained only by culture.

In group "D" (both tubes without growth), 2,507 samples were examined. In 139 cases (5.5 per cent) the microscopical

examination was positive. Thus, in these cases the culture method failed.

Of a total of 4,017 samples examined by microscopy, 580 (14.4 per cent) had been positive by that method, and of these 350 (60.3 per cent) were also positive by culture. Contamination of one or both tubes (groups "E" and "F") in 91 out of the 580 cases (15.7 per cent) must be assumed to have prevented recognition of a number of positive cultures. As mentioned above, out of the same 4,017 specimens, 527 (13.1 per cent) had given positive culture and 66.4 per cent of these also positive microscopy.

As will be seen from Tables 1 and 2, the frequency of specific growth on both tubes ("A") was about 50 per cent higher than the frequency of specific growth on one tube only ("B" and "C") among the samples positive by microscopy. Among the specimens negative by microscopy, the frequency of specific growth on only one tube was about twice as high as the frequency of specific growth on both tubes. Thus, the employment of more tubes enhances the chance of obtaining positive diagnosis by culture.

It has not been possible by culture to achieve a greater number of positive results than by microscopy, but by means of culture a larger cumulative number of positive bacteriological diagnoses has been obtained among the specimens examined.

REFERENCES

1. *Ringdal, G.:* Diagnosis of Johne's Disease in Cattle. Nord. Vet.-Med. 1960, *12*, 513.
2. *Doyle, T. M.:* Diseases Due to Bacteria 1959, *1*, 319.

SUMMARY

The addition of I. N. H. to Löwenstein medium has no effect on the growth of *Mycobacterium johnei.*

In 33.6 per cent of 527 specimens where culture gave positive result, direct microscopy had not revealed the presence of Johne organisms.

In 39.7 per cent of 580 cases where microscopy was positive, no Johne organisms could be demonstrated by culture.

The culture method will enable the establishment of positive bacteriological diagnosis in a number of cases where microscopy has failed to reveal the presence of bacteria. However, the prolonged time necessary for the examination diminishes its value in practice.

ZUSAMMENFASSUNG

Züchtung von Paratuberkel-Bakterien.

Zutat von I. N. H. zu Løwenstein's Nährboden hat keine Wirkung auf das Wachstum der Paratuberkel-Bakterien.

In 33.6 % von 527 Proben, wo die Kultivierung positives Resultat gegeben hatte, hatte eine direkte mikroskopische Untersuchung keinen Gehalt von Paratuberkel-Bakterien gezeigt.

In 39.7 % von 580 Proben, wo eine mikroskopische Untersuchung positives Resultat gab, wurde beim Züchten kein Gehalt von Paratuberkel-Bakterien nachgewiesen.

Eine Kultivierung kann eine bakteriologisch positive Diagnose für eine Anzahl von Proben stellen, wo eine mikroskopische Untersuchung den Bakteriengehalt nicht enthüllen konnte. Die langwierige Untersuchungszeit wird jedoch die praktische Bedeutung des Züchtens beschränken.

RESUMÉ

Dyrkning af paratuberkelbakterier.

Tilsætning af I. N. H. til Løwenstein's substrat er fundet uden virkning på paratuberkelbakteriers vækst.

I 33.6 % af 527 prøver, hvor dyrkningsundersøgelse havde givet positivt resultat, var ved direkte mikroskopisk undersøgelse ikke påvist indhold af paratuberkelbakterier.

I 39.7 % af 580 prøver, hvor mikroskopisk undersøgelse havde givet positivt resultat, blev ved dyrkning ikke påvist indhold af paratuberkelbakterier.

Ved dyrkningsundersøgelse vil kunne stilles bakteriologisk positiv diagnose på en del prøver, hvor en mikroskopisk undersøgelse ikke har været i stand til at afsløre bakterieindhold. Den lange undersøgelsestid vil dog begrænse dyrkningens praktiske betydning.

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