

INFECTION OF ADULT CATTLE WITH *MYCOBACTERIUM TUBERCULOSIS AVIUM*

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A RECENT investigation, the results of which are published in the next paper, was concerned with the value of the double intradermal johnin test in detecting cases of Johne's disease in cattle. The Committee of the Agricultural Research Council, which was responsible for the initiation of these experiments, wisely confined its attention to the presence or absence of Johne's disease in the animals under test, and the instructions which were drawn up for the guidance of the participating laboratories were framed with the object of obtaining the maximum information on this point.

Several enquiries have shown that the existence of the avian type of the tubercle bacillus in cattle is more widespread than had been suspected. The subject has been investigated in this country by Minett (1932), Glover & Griffith (1934), Kelland, Frood & Doyle (1938), Harbour (1941) and others, and has been reviewed in a report issued by Buxton & Glover (1939) at the request of the Agricultural Research Council. The literature relevant to the problem has been surveyed in these articles and requires no additional comment. Attention may be directed, however, to the reaction induced by johnin in cattle affected with the avian bacillus. Thus Minett (1932), in the course of some observations on the control of Johne's disease which involved periodic testing with johnin, noted that some of the reactors failed to show recognizable changes of the disease at the post-mortem examination. Examination of material obtained from six animals resulted in the isolation of five strains of *Mycobacterium tuberculosis avium*: three were of full virulence, while two appeared to be attenuated. Glover & Griffith (1934) also obtained avian strains of full virulence from two adult animals which were positive to the johnin test.

In view of the importance of infection of cattle with *M. tuberculosis avium* as a factor of some significance in routine tuberculin testing, it was decided that advantage should be taken of the investigation mentioned above to examine the organs of the reacting cattle for the avian bacillus. The work was commenced at the Institute of Animal Pathology, Cambridge, and was completed at the Farm Laboratories, National Institute for Medical Research. It is not intended to forestall the main results of the Committee's investigation, which doubtless will be published in due course, but as the attempted isolation of avian strains was entirely separate, it is felt that there is justification for the publication of a separate note.

The animals, which were of various breeds and ages, were mainly unselected and were not specially suspected as carriers of the avian bacillus. They were tested in groups of about ten at a time, using an ammonium sulphate precipitated synthetic medium tuberculin, a heat concentrated synthetic medium johnin and an ammonium sulphate precipitated johnin.

In each instance the initial dose was 0.1 ml. injected intradermally, and readings were taken at the 48th hour: in those animals in which the reactions were not clearly positive, a second injection of 0.1 ml. was given, and the final inspection made at the 72nd hour. Immediately the test was completed, the animals were subjected to a post-mortem examination. Representative lymphatic glands together with portions of small and large intestine were removed together with any lesions which appeared to be suspicious. During the course of the investigation 35 animals were examined and clear-cut evidence of avian infection was found in five (14.3%), while in five (14.3%) the post-mortem examination, or cultural findings, suggested that this type may have been present.

The direct isolation of the avian type of bacillus by cultural means from bovine tissue presents certain difficulties, owing to (1) the trivial nature of the lesions in many cases, and (2) the existence of the organism in lymphatic glands without macroscopic changes (Buxton & Glover, 1939). Fortunately, the guinea-pig, although relatively insusceptible to *M. tuberculosis avium* in so far as the production of progressive lesions is concerned, is nevertheless capable of harbouring the organism for considerable periods: indeed, the available evidence suggests that up to a point the bacilli actually multiply in the guinea-pig tissues. Feldman (1936) has shown that the avian bacillus can be recovered from the spleen up to 70 days after subcutaneous inoculation and our own work has confirmed his observation.

In the present series it was decided that, in so far as the glands were concerned, reliance should be placed on the guinea-pig as a means of recovering the organism and no attempt was made at isolation by direct cultural methods, although mesenteric gland tissue was seeded on media suitable for the isolation of Johne bacilli. The selected tissues were triturated with sand and normal saline solution and the mixture, after light centrifugation, was injected subcutaneously into the guinea-pigs. In a few instances, a preliminary treatment with 2.5% KOH was necessary to reduce the contaminating organisms, but in most cases they were so few that they produced no untoward effect in the guinea-pigs.

Three guinea-pigs were used for each set of glands selected for the biological test. Two animals were kept for 6 months to control the incidence of bovine tuberculosis, while the third was killed about the 28th day, and suspensions of the gland adjacent to the site of inoculation and of the spleen were seeded on to Herrold's egg yolk medium and the same medium with 5% glycerine. Several tubes of each type of medium were used. In the case of the local gland, it was necessary to disintegrate the tissue very thoroughly in a sterile mortar before transferring the material to the culture medium, but splenic tissue, which is so much more diffuent, was spread directly on the egg medium by means of a broad spade. It was considered advisable to kill the guinea-pigs by stunning and bleeding from the throat, since the presence of excessive amounts of blood may inhibit the growth of the tubercle bacilli.

If the results of the cultures from the guinea-pig tissues were positive, they were subjected to further tests on guinea-pigs, rabbits, fowls and sometimes pigeons. In every instance an attempt was made by serial doses to determine the virulence of the organisms.

It may be noted that the intestines were not subjected to the biological test, but were treated with antiformin and then seeded on to media suitable

Table 1

No. of animal	Tuber- culin test	Johnin test	Post-mortem findings	Micro- exam.	Cultures from guinea-pigs	Remarks
17	+	+	Caseous lesions bronchial gland.* Mesenteric gland no visible lesions	0	Cultures of avian type isolated	* <i>M. tuberculosis bovis</i> , isolated. Avian culture of full virulence
50	-	sl. +	Completely calcified lesions in four mesenteric glands	-	0	Possibly healed avian infection
66	+	+	Caseo-calcareous lesions in left bronchial and a mediastinal gland	+	0	Nature of organism not confirmed by culture
99	+	+	Extensive caseous lesions thoracic glands.* Mesenteric glands no visible lesions	0	Cultures of avian type isolated	*Guinea-pigs died of generalized tuberculosis. Avian culture of full virulence
105	-	+	Lesions in one mesenteric gland containing thin caseo-pus	+	0	Possibly healed avian infection
111	-	+	Calcified lesions in two mesenteric glands	+	Cultures of avian type isolated directly from gland and from guinea-pig	Avian culture of full virulence
117	-	-	-	0	Culture of avian type from guinea- pig inoculated mesenteric glands	Avian culture of full virulence
147	+	+	-	0	Culture of avian type from guinea- pig inoculated iliac gland	Culture avirulent
159	-	-	-	0	Culture of avian type from guinea- pig inoculated mixed glands	Avian culture of full virulence
170	+	+	Numerous calcified lesions in mesen- teric glands	-	0	Possibly healed avian infection

for the growth of *M. paratuberculosis*. In two instances strains of an avian type were obtained in this manner. As guinea-pigs were not injected with this material, no evidence was obtained as to whether the biological test would have been as successful as the cultural method for the recovery of the organisms from this site.

The results of the tuberculin and johnin tests, the post-mortem findings and the inoculation of the guinea-pigs, are shown in Table 1.

Attention may be drawn to the lack of correlation between the results of the tuberculin and johnin tests, the presence or absence of tuberculous lesions at the post-mortem examination and the recovery of organisms of the avian type. In the five cases in which avian strains of full virulence were isolated, two were reactors to both tuberculin and johnin, one was negative to tuberculin and positive to johnin, while two were negative to both products. It must be pointed out, however, that the double reactors were animals which were shown to be affected with the bovine as well as the avian types. On the other hand, amongst the animals from whom atypical strains were recovered or from whom it proved impossible to isolate tubercle bacilli, three were reactors to both bovine and avian tuberculin, while two were negative to bovine tuberculin but positive to johnin.

Five animals (17, 99, 111, 117 and 159) yielded strains of full virulence.

Avian strains of full virulence for the fowl and the rabbit were isolated from each animal in this group. The extent of the lesions showed considerable variations ranging from moderate caseo-calcareous foci to a complete absence of macroscopic evidence of disease. It may be noted, however, that the two severest cases (17 and 99) were examples of double infection with the bovine and avian types, in which it was probable that the more extensive lesions were due to the bovine type. In view of the comparative rarity of reported cases of the dual infection the details of the post-mortem changes are of interest.

No. 17. The right bronchial gland was increased to about four times its normal size and beset with caseous centres up to 3 cm. in diameter. There were no visible lesions in the other glands which were examined. Guinea-pigs inoculated with a suspension from the thoracic glands died of generalized tuberculosis within eight weeks and a bovine strain of full virulence was isolated from each animal. A guinea-pig injected with a mesenteric gland suspension was killed 46 days later. There was no evidence of tuberculosis, but cultures from the spleen yielded a strain of the avian type which was fully virulent for rabbits and fowls.

No. 99. Both bronchial and several mediastinal glands were much enlarged and contained numerous caseo-calcareous lesions up to 1 cm. in diameter. There were no visible lesions elsewhere in the carcase. The thoracic glands produced generalized tuberculosis in guinea-pigs from which a bovine strain was recovered. In this instance two guinea-pigs inoculated with a suspension of mesenteric glands were killed at the 31st and 102nd days respectively; cultures from their spleens and local glands failed to yield tubercle bacilli. On the other hand, direct cultures from the same glands on the media used for the detection of Johne's bacillus yielded about half a dozen smooth, moist colonies. Good growths were obtained in subcultures on plain egg agar and glycerine egg agar. The strain was apathogenic for the guinea-pig in a dose of 10 mg., but killed fowls with widespread lesions. It also produced a typical Yersin reaction in the rabbit after intravenous inoculation.

It will be observed that in both these cases there were no macroscopic lesions in the glands from which the avian bacilli were recovered.

There were two animals (117 and 159) in which the organs and glands showed no evidence of tuberculosis. An avian culture was obtained from 117 through the inoculation of a guinea-pig with a mesenteric gland suspension. It proved to be of full virulence for the rabbit and the fowl. In the case of

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159 a virulent organism of the avian type was isolated from a guinea-pig inoculated with a mixed suspension of the glands of the head, thorax and liver.

In one instance (111) trivial foci were found in two mesenteric glands. They were completely calcified while smears showed only a few poorly stained bacilli. Cultures of the avian type were isolated (a) from the spleen of a guinea-pig injected with a suspension of an affected gland, and (b) by direct seeding of the original material on John's medium.

The absence of visible lesions, coupled with the presence of avian bacilli, confirms the results which had been reported in a previous paper (Buxton & Glover, 1939). The failure of the tissues to react macroscopically was probably due to an infection of the glands by small numbers of bacilli insufficient to cause visible foci.

Five animals (50, 66, 105, 147 and 170) yielded no growth or atypical strains

In the previous group the evidence of infection with the avian type of organism was complete. There were five animals, however, in which there were indications that avian bacilli may have been responsible, either for the reaction of Johnin, or for the production of tuberculous lesions. Three of these cattle (66, 147 and 170) were reactors to both bovine tuberculin and Johnin, while two (50 and 105) were negative to tuberculin and positive to Johnin.

No. 147 showed no macroscopic lesions at post-mortem examination. Cultures from guinea-pigs inoculated with various glands were negative, with the exception of one animal which received a suspension of an iliac gland. Both the local lesion and the spleen of the guinea-pig yielded a few colonies which grew equally well on egg agar and glycerine egg agar. The colonies were moist and smooth. Subcultures gave a very moist, diffuent growth with the appearance of the avian type. The strain proved to be of very low pathogenicity. Inoculated subcutaneously into guinea-pigs (10 and 1 mg.) it produced a local caseo-purulent lesion with no evidence of metastasis. Fowls and rabbits were completely refractory to doses of 0.1 and 0.01 mg. intravenously, and to 10 mg. by the mouth (fowl only).

It has been presumed that the avian bacillus may undergo modification in the bovine tissue, and Minett (1932) has described strains isolated from adult animals which were of substandard virulence. In this instance the process of attenuation would appear to have progressed still further, the sojourn in the tissues of the bovine animal having completely modified the virulence: alternatively the strain may have been a paratubercle bacillus which behaved culturally as an avian strain. Assuming that a latent infection elsewhere in the body was not overlooked, the reaction to the biological test may be attributed to this organism.

No. 66 presented widespread caseo-calcareous lesions in the left bronchial and mesenteric glands. Smears showing short acid-fast bacilli in small numbers were found in the former, and as the infection was presumed to be of the bovine type no biological test was undertaken. Guinea-pigs inoculated with the mesenteric glands failed to show any evidence of tuberculosis and the cultures from their organs were negative. A suspension of the mucous membrane of the small and large intestines, treated with antiformin, was negative for John's bacilli, but a few colonies were obtained on two tubes showing the typical appearance of the avian type.

A first generation on fresh media failed to grow; moreover, repeated attempts to obtain subcultures were unsuccessful.

It is unfortunate that in this instance the type of bacillus present in the thoracic gland was not determined. It is considered probable that it was of the bovine type since the reaction to bovine tuberculin was more intense than the response to johnin. The cultures obtained from the intestines appeared within the first month of incubation and grew well although the media contained glycerine. They were not, therefore, of the dysgonic bovine type; moreover, they developed so rapidly as to rule out any suspicion that they might be growths of Johne bacilli. It is highly probable that the strain was of the avian type, but satisfactory proof that this was the case is lacking. Finally, in three animals it proved impossible to isolate acid-fast bacilli.

No. 170. In this animal the mesenteric chain of glands showed numerous calcified lesions up to 0.5 cm. in diameter. They were composed of a yellowish, dry, gritty material which readily shelled out from a surrounding fibrous capsule. There were no visible lesions elsewhere. No acid-fast bacilli were seen in smears from the calcareous foci, but histologically the glands showed typical tuberculous changes. A number of guinea-pigs was inoculated with affected mesenteric glands and killed at intervals ranging from 39 to 137 days. No lesions were found in any of these guinea-pigs, while cultures from local glands and spleens were negative.

No. 50. The lesions were confined to four mesenteric glands which were completely calcified, each showing a focus of 0.5 cm. in diameter. No acid-fast bacilli were found in smears. A number of guinea-pigs inoculated with suspensions of mesenteric glands were killed at intervals ranging from 43 to 107 days after injection. They were entirely free from visible tuberculosis and cultures remained negative.

No. 105. There were no lesions in this animal with the exception of one mesenteric gland which contained an abscess with a thin viscous pus. A few short acid-fast bacilli were seen in smears. Cultures from this animal were negative and guinea-pigs inoculated failed to develop any lesions.

It would seem legitimate to conclude that in these instances the animals had completely overcome a moderately severe tuberculous infection which may have been either of a bovine or of an avian type. It is significant that the reactions to johnin were stronger than those to tuberculin, a fact which seems to point to a possible infection with the avian type of bacillus.

SUMMARY

A series of 35 unselected cattle tested with synthetic medium tuberculin and synthetic medium johnin was subjected to post-mortem examination. In five instances (14.3%) avian strains of full virulence for the usual experimental animals were recovered by the inoculation of guinea-pigs with glandular suspensions or by cultural methods used for the isolation of Johne's bacillus: in two of these cases an avian type was isolated from the glands of the alimentary tract and a bovine type from the thoracic glands. In addition there were five cases (14.3%) in which evidence of avian infection was not complete. From one a strain was obtained which was culturally identical

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with an avian type, but it was almost completely avirulent for laboratory animals. In two, acid-fast bacilli were seen in smears from the thoracic and mesenteric glands respectively, while in two, completely calcified glands were observed which were typically tuberculous, but were devoid of acid-fast bacilli (microscopical examination of smears and sections). Cultures from the organs of guinea-pigs inoculated with glandular suspensions from the four last-mentioned cases failed to grow tubercle bacilli. In these circumstances the evidence that the infection was of the avian type is largely presumptive, but the nature and extent of the lesions suggests that such may have been the case.

With regard to the tuberculin and johnin tests, clear cut reactions to johnin were obtained in seven instances, a doubtful reaction in one and negative reactions in two. One-half of the animals were also positive to tuberculin.

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