

## Avian tuberculosis in pigs: miliary lesions in bacon pigs

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### SUMMARY

An outbreak of tuberculosis caused by *Mycobacterium avium* type 2 is described which resulted in the total condemnation of 26 carcasses and partial condemnation of tissues and organs of a further 200 animals. Circumstantial evidence is presented that hens running in the farmyard were the source of the infection.

Examinations of the carcasses and organs of the diseased pigs suggested that the accepted pathogenesis of the disease is incorrect and a new hypothesis is presented. The problems for the meat inspector in differentiating tuberculosis from 'milk-spot liver' are discussed and recommendations made.

The findings of the study are discussed in the light of 'The Meat Inspection Regulations 1963' and it is recommended that where tuberculosis is suspected there is no longer any necessity to split the carcasses. The public health implications of this study are discussed.

### INTRODUCTION

'There is little evidence that infection spreads extensively from pig to pig and the incidence of tuberculosis in this species depends mainly on its exposure to tuberculous products from cattle, fowls or man' (Francis, 1958). In Great Britain between 1952 and 1956, of 45 isolates made from pigs, 20 were *Mycobacterium avium* and 25 were *M. bovis*. In contrast, because of the virtual eradication of bovine tuberculosis by the State Veterinary Service, of 139 strains of mycobacteria isolated from pigs between 1962 and 1966, 128 were *M. avium* and the remaining 11 were *M. bovis* (Lesslie *et al.* 1968). McDiarmid (1964) reported avian tuberculosis

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in many species of wild birds, and Lesslie and Birn (1967) suggested that transmission of tuberculosis from fowls to pigs could occur on farms where the species are kept in close proximity. Nevertheless, in 1975 the epidemiology of avian tuberculosis in swine was still thought to be in need of clarification (Thoen, Jarnagin & Richards, 1975).

Circumstantial evidence of transmission from fowls to pigs is presented in this paper and the occurrence of tubercular lesions in pig carcasses is discussed in the light of 'The Meat Inspection Regulations 1963'. These regulations were drawn up primarily for tuberculosis in cattle and pigs caused by *M. bovis*. Following the condemnation of many bacon pig carcasses our investigation into the pathogenesis of avian tuberculosis in pigs was undertaken to determine whether the regulations were really adequate for avian tuberculosis in the pig.

## METHODS

### *Farm investigation*

The farm was self-contained and comprised 100 sows with a throughput of 1800 pigs a year sold at 180 lb at about six months of age. During the summer the sows were kept out of doors and in winter they were run in covered yards. Some pigs were fattened on the farm of origin and the remainder on a fattening unit a few miles away. The first sign of a problem was an increase in the number of heads condemned at slaughter for tuberculosis. Over a period of seven months 26 carcasses and approximately 200 heads and sets of offals were condemned out of a total of 1272 pigs slaughtered.

Investigation on the farm showed that no cattle or sheep had been kept for the preceding 7–8 years. Wild birds had access to the feeding troughs in many of the pens. Free-range hens had been kept on the breeding farm until six months before the disease problem appeared, but they had not been performing well and so were disposed of, excepting six which continued to roam the pig yard.

At the fattening unit 70 hens were housed near but had no direct contact with the pigs. It was decided to tuberculin test the hens using avian tuberculin injected into the wattle, as described by Francis (1958), but the owner killed the six in the farmyard and burned the carcasses. The hens at the fattening unit were tested and 69 were negative to the test. The reactor was slaughtered, but there was no lesion of tuberculosis and *M. avium* was not isolated. All sows were tuberculin tested by the method described by Lesslie *et al.* (1968) using avian and mammalian tuberculins. All were negative to the test.

In an attempt to reduce the losses by condemnation, pigs were sent for slaughter at 140 lb live weight. However, six months after the last of the breeding farm hens were killed, the outbreak ceased. It was concluded that these hens were the source of infection for the pigs.

### *Laboratory investigations*

#### *Post-mortem examination*

Twelve bacon pigs which had been condemned at the abattoir because of tuberculosis were obtained and the organs examined for tubercular lesions; each carcass was dissected and the lymph nodes excised. Each lymph node was cut in

two, and half was fixed in buffered neutral formalin, the remainder being used for the cultural examinations. Portions of liver, spleen and lung were similarly processed.

In addition, various 'plucks' and 'guts' were received from 41 other animals (for details see Table 2). The material was processed as described below.

#### *Cultural examinations*

The liver, lung and spleen of each pig were examined separately. The lymph nodes were bulked in the following groups: those draining the alimentary tract (including liver); those draining the respiratory tract; those draining the abdominal cavity; those draining the limbs. Tissues from the condemned viscera were divided into similar groups—but there were no carcass lymph nodes, and there were few specimens from the respiratory tract.

It was not possible to examine the tissues as they became available and so they were stored at  $-20^{\circ}\text{C}$ . After removal from storage they were immersed in a boiling water bath for 8 s, and then ground with sterile sand in a pestle and mortar. Molar sodium hydroxide was added and grinding continued. When the gross sediment had settled the supernatant was poured off into a universal container and kept at  $37^{\circ}\text{C}$  for 20 min. Using phenol red as an indicator the contents were neutralized with 2 M hydrochloric acid.

The specimen was centrifuged at 1000 g for 1 min to remove sand and tissue particles. The supernatant was removed and centrifuged for 20 min at 3000 g and the deposit resuspended in a small quantity of sterile saline. This was used to inoculate three Lowenstein–Jensen and three Stonebrink slopes. The medium was incubated at  $37^{\circ}\text{C}$  for up to a month. Colonies were stained by the modified Ziehl–Neelsen method described by Laidlaw (1978). Acid-fast organisms were sent to the central Veterinary Laboratory, Weybridge, for identification.

#### *Histological examinations*

Fixed tissues were embedded in paraffin wax; sections were cut at  $5\ \mu\text{m}$  and stained with haematoxylin and eosin and by the modified Ziehl–Neelsen method.

## RESULTS

### *Post-mortem examinations*

The lesions in lymph nodes were typical tubercles, whereas those in the liver, lung and spleen were not. Affected lymph nodes were swollen and the lesions could often be seen before an incision was made (Fig. 1). Caseation and calcification were seen and often the necrotic mass would shell out. Lesions varied from less than 1 mm in diameter to more than 4 cm; in some nodes more than one focus was seen. Of the head lymph nodes, only the sub-maxillaries were seen to be diseased. There was no regular distribution of lesions in the mesenteric lymph nodes; the variation was endless. In one pig only the gastric lymph nodes were affected – in others only the caecal; in many, lesions were irregularly present along the chain and in some all mesenteric lymph nodes were seen to be diseased.

The gross appearance of the lesions in the liver varied from a few translucent seed-like structures on the surface and in the depths of the organ (Fig. 2) to extensively spreading confluent lesions resembling those of 'milk-spot liver'

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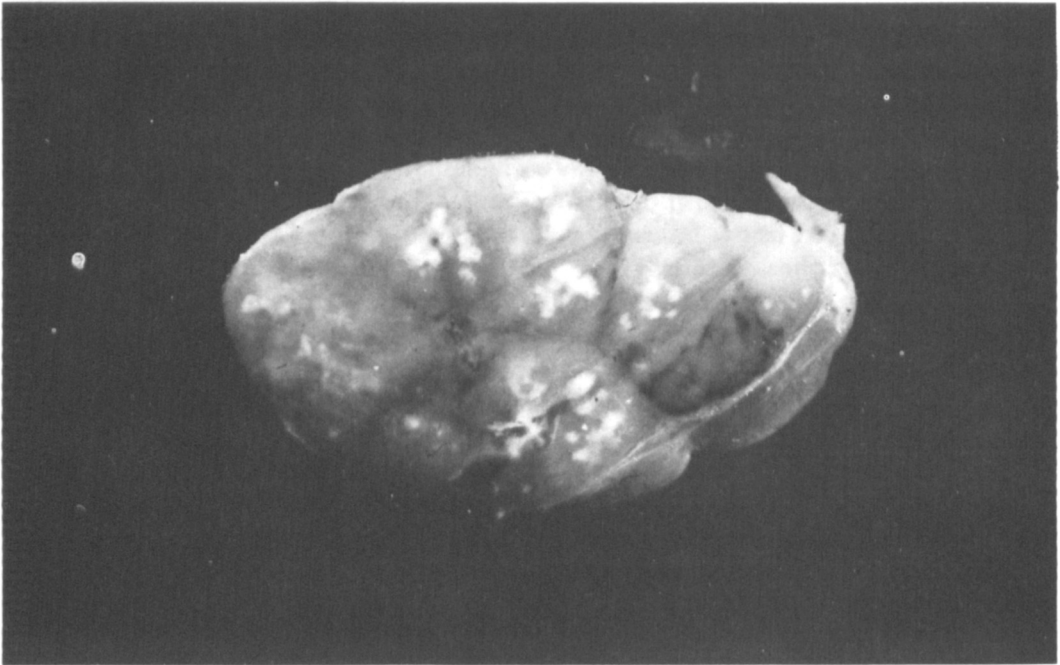


Fig. 1. Mesenteric lymph nodes, showing numerous caseous foci.



Fig. 2. Liver, showing numerous discrete tubercular lesions.



Fig. 3. Liver, showing diffuse tubercular lesions.

(Fig. 3). With one exception lesions in the liver were accompanied by lesions in the gut lymph nodes; the one pig had liver lesions associated with the massive lesions in the sub-maxillary lymph nodes. Nine of the twelve condemned carcasses but none of the condemned viscera had lesions in the lungs.

Miliary lesions in lungs varied from a few to many thousands and each lesion was small (less than 5 mm in diameter) and translucent. Lesions were found in only four of the spleens and in appearance they resembled those seen in the lungs; 100 lesions were counted in one spleen. No lesion was seen in kidneys, bones or brains.

In none of the organs was any caseation recorded. Smears stained by the modified Ziehl-Neelsen method were examined, but acid-fast bacteria were not seen in liver, lung or spleen, and they were often hard to find in smears of head or mesenteric lymph nodes.

#### *Cultural examination*

The results of attempted isolations are given in Table 1 (for the carcass) and Table 2 (for the viscera examination). Because of the way in which the examinations were carried out, not all tissues sampled were cultured. The storage of the samples resulted in many of the cultures being overgrown by fungi. Several of the samples that were expected to be positive (active lesions, or acid-fast bacteria seen in smears) failed to yield mycobacteria. Conversely, tissues which were not expected to contain mycobacteria were positive. No lymph node in the carcass or abdominal group showed signs of disease, yet 4/12 of the former and 5/12 of the latter were positive for the organism. From none of the condemned viscera without lesions were mycobacteria isolated. The first eight isolates were all identified as *M. avium* type 2 and so later isolates were not typed.

Table 1. *The examination of carcasses from 12 pigs totally condemned because of miliary tuberculosis*

	Animals with gross lesions in lymph nodes or organ		Animals with no gross lesion in lymph nodes or organ	
	No. of animals from which <i>M. avium</i> was isolated	No. of animals from which <i>M. avium</i> was not isolated	No. of animals from which <i>M. avium</i> was isolated	No. of animals from which <i>M. avium</i> was not isolated
Liver	5	7	—	—
Lungs	3	6	0	3
Spleen	0	2	0	10
Lymph nodes				
Head	3	0	4	5
Alimentary tract	6	5	0	1
Respiratory	—	—	2	10
Abdominal	—	—	5	7
Carcass	—	—	4	8

Table 2. *The examination of condemned viscera from 41 pigs with tuberculosis*

Tissues	Number submitted	No. with lesions		No. with no lesion	
		Total	<i>M. avium</i> Isolated	Total	<i>M. avium</i> Isolated
Liver	9	9	3	0	—
Lung	4	0	—	4	0
Spleen	3	2	0	1	0
Lymph nodes					
Head	15	15	9	0	—
Alimentary tract	25	13	12	12	0
Respiratory tract	3	0	—	3	0
Abdominal	2	0	—	2	0

#### *Histological examination*

Lesions in lymph nodes were typical of tuberculosis, the necrotic or calcified central part of the lesion being surrounded by a fibrous tissue capsule. The tubercles consisted mostly of lymphocytes, eosinophils, some epithelioid and occasional giant cells. Acid-fast bacteria were readily visible and in a few sections the red colour predominated. In the liver eosinophils and lymphocytes made up the bulk of the reaction, with a marked fibrous reaction (Fig. 4). Epithelioid cells were occasionally seen and were often the only distinguishing feature between tuberculosis and 'milk-spot liver'. In very few sections were acid-fast bacteria seen. Lung lesions were mostly encapsulated and contained numerous eosinophils and lymphocytes with few epithelioid cells and no giant cells.

#### DISCUSSION

There seems little doubt that in this case the hens at the breeding farm were the source of infection for the pigs; six months after the last of these hens had been killed tuberculosis ceased to be a problem on the farm. It is unfortunate that the

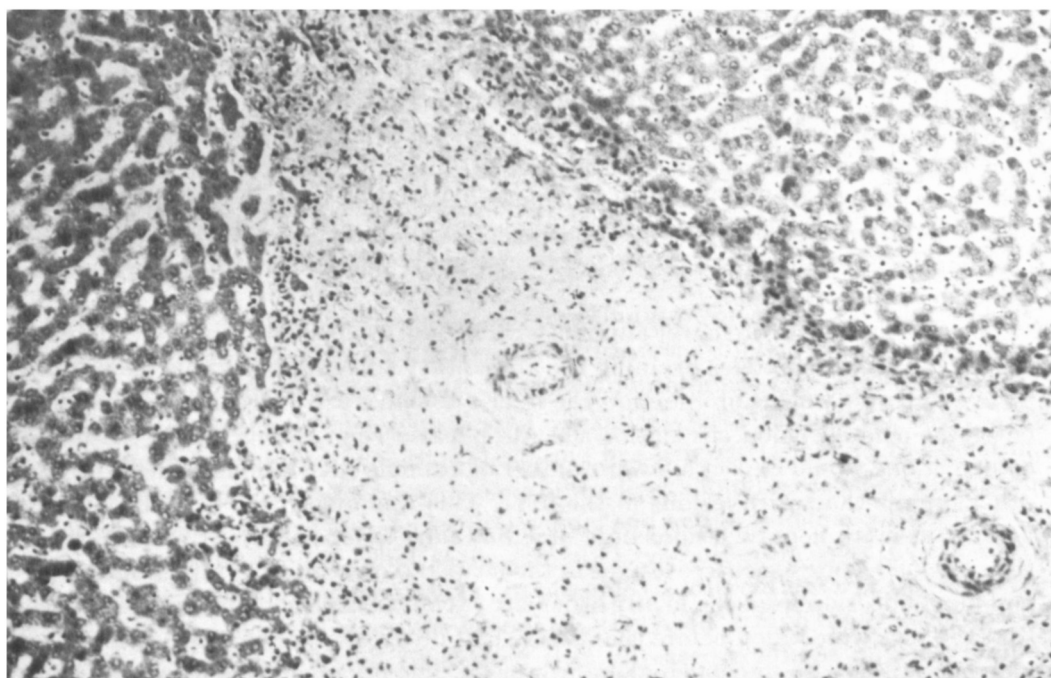


Fig. 4. Histological section of liver with a tubercular lesion (magnification  $\times 100$ ) showing a marked reaction.

hens were not examined. All strains typed were *M. avium* type 2 and this constitutes further evidence for a common source of infection. Because of the short life of the bacon pig, lesions of tuberculosis are usually limited to the lymph nodes of head and neck, or the alimentary tract. The fact that 26 pigs had widespread lesions requiring total condemnation would suggest a continuous exposure to a massive source of infection. The results of tuberculin testing of the sows ruled them out as a source of infection. Lesslie *et al.* (1968) discussed ways in which infection could persist within a herd and considered that the disease might be maintained in some herds by pig-to-pig transmission; but this outbreak ceased following the deaths of the hens, and the consequent cessation of continuous introduction of infection. This finding is in agreement with that of Szabo *et al.* (1975). Investigations by us into 60 outbreaks of tuberculosis suggest that the normal picture on a farm is for a single animal to become infected but to show no sign of disease until it has been slaughtered. Even when sows are diseased, no 'open' lesion has been seen (Windsor, unpublished observation). It was surprising that none of the sows on this farm was positive to the tuberculin test, considering the number of bacon pigs with lesions. It can only be assumed that the sows had no access to the hens' droppings. These findings add further weight to the case against pig-to-pig transmission.

Chaussé (1915) stated that 80% of pigs are infected via the tonsil, although usually lesions are first observed in the sub-maxillary lymph node. Transmission to the pulmonary lymph nodes occurred after haematogenous spread to the lungs.

Lesions in liver and spleen were thought to develop later than in the lungs unless there was a heavy bacteraemia. The present study suggests a different pathogenesis. Fifty-three pigs were examined, and whereas 36 had lesions in mesenteric lymph nodes 18 had lesions in head lymph nodes; there were 21 with lesions in the liver of which nine also had lesions in the lungs and four in the spleen. This suggests that the organism only gains entry either through the tonsil or the lining of the intestine. The absence of visible lesions in the intestinal mucosa can be explained as the 'primary incomplete syndrome' which is common in pigs. If the weight of challenge is sufficient, *M. avium* travels – probably via the portal circulation – to the liver.

The fact that there are fewer animals with lesions in the liver than there are with lesions in the lymph nodes draining the alimentary tract supports this view. With an even greater weight of infection the liver's defences are overcome and bacteria enter the general blood circulation via the hepatic vein. If this line of reasoning is correct one would expect a fewer number of animals with lesions in the lung and other organs than with lesions in the liver. This was indeed the case, because of 21 animals with hepatic lesions only nine had lung lesions and four had lesions in the spleen. Despite extensive lesions in the head and mesenteric lymph nodes, liver and lung, no lesion was seen in any other site except the spleen; this suggests either that the pigs were killed before the disease had progressed, or that *M. avium* finds many pig tissues an uncongenial environment. The organism was recovered from carcass and abdominal lymph nodes on a surprising number of occasions. Since the tissues were bulked for cultural examination it was not possible to determine exactly which nodes were infected. However, lesions were not detected in histological sections of lymph nodes that showed no gross abnormality. Szabo (1977) has also isolated mycobacteria from lymph nodes in which no change was detectable, either by the naked eye or in histological sections. Furthermore, such animals were negative to the tuberculin test. Even in old sows with extensive lesions in liver, lung and spleen, carcass lymph nodes were not seen to be diseased (Windsor, unpublished observation).

The Meat Inspection Regulations 1963 state the following.

#### Schedule 1

##### *Part III. Additional instructions where tuberculosis is suspected*

10. Where the inspector has reason to suspect that any part of the carcass or offal of any animal is infected with tuberculosis, he shall, in addition to carrying out the provisions of Parts I and II of this Schedule –

(a) in the case of any carcass, require the carcass to be split, examine the vertebrae, ribs, sternum, spinal cord and, if he considers it necessary the brain, and expose, and if a lesion in a kidney is visible or suspected, incise, the kidney...

(c) in the case of the carcass of any pig, examine in detail the following lymphatic glands (being glands not already examined by him in accordance with the provisions of Part II of this Schedule), namely, the superficial inguinal, supramammary, cervical, prepectoral, prescapular, subdorsal, sublumbar, iliac, precrural and, if he considers it necessary, the popliteal.

#### 'Schedule 2

##### Indications of unfitness for human consumption

1... Tuberculosis (generalized). Tuberculosis with emaciation...

3. The inspector shall in determining for the purpose of this Schedule whether tuberculosis



is generalized take into account the sum of the evidence of disease and the character of the lesions throughout the carcass and, in particular, shall regard evidence of any of the following conditions as evidence of generalized tuberculosis:

- (a) miliary tuberculosis of both lungs with evidence of tuberculosis elsewhere;
- (b) multiple and actively progressive lesions of tuberculosis;
- (c) widespread tuberculous infection of the lymphatic glands of the carcass;
- (d) diffuse acute lesions of tuberculosis of both the pleura and peritoneum associated with an enlarged or tuberculous lymphatic gland of the carcass;
- (e) active or recent lesions present in the substance of any two of the following: spleen, kidney, udder, uterus, ovary, testicle, brain and spinal cord or their membranes, in addition to tuberculous lesions in the respiratory and digestive tracts.

What, then, are the implications of these findings for meat inspectors? This investigation has raised several problems. The first is the difficulty in differentiating 'milk-spot liver' from tuberculosis. In the slaughterhouse the decision must be made on the carcass – histological and biological tests cannot be used. The conclusion from this study is that if the pig has caseous lesions in the head or mesenteric lymph nodes then any lesions in the liver should be considered, for the purposes of meat inspection, to be tubercular, and action taken accordingly.

The second question is, what is the role of *Corynebacterium equi*? Lesslie *et al.* (1968) were of the opinion that it was not possible to differentiate visually between tubercular and corynebacterium lesions in the head lymph nodes. However, Roberts & Hamilton (1968) considered that all caseous lesions in head lymph nodes were caused by *M. avium* and that previous workers had failed to isolate the organism because of faulty technique. No biological test was used in the present study, which relied on cultural techniques. For this reason little emphasis has been placed on individual isolations, or failures.

It is likely that our results underestimate the absolute number of tissues that were infected at the time of sampling. Despite this, 12 of 18 head lymph nodes with lesions were positive for *M. avium* on culture. Because carcasses cannot be held while cultural examinations are undertaken we consider that, for purposes of meat inspection, caseous lesions in head lymph nodes should be treated as tuberculous.

Thirdly, Schedule 1, Part III, paragraph 10 (a) of the Meat Inspection Regulations 1963 requires that pig carcasses be split where tuberculosis is suspected. In many slaughterhouses with production lines, splitting large number of carcasses can present serious problems. The results of this study suggest that such action is not necessary since no lesion was found in bones. Furthermore it is laid down in the Regulations that numerous carcass lymph nodes be incised and examined. More than a thousand carcass lymph nodes were examined during this study and not a single lesion was detected on gross appearance or in histological section. Such examinations in slaughterhouses are therefore unnecessary.

The final problem is the judgement of the carcass. Schedule 2, paragraph 3 (a) states that miliary tuberculosis of both lungs with evidence of tuberculosis elsewhere is reason enough for total condemnation of the carcass. It is argued above that this study has underestimated the number of infected tissues examined. Five of 12 carcasses in this study yielded *M. avium* from either abdominal or carcass lymph nodes or both. Marks & Birn (1963) discuss the possible public health

significance of *M. avium*, which can cause disease in man. There are two possible lines of action on these grounds for condemnation:

(1) Total condemnation if the pig has lesions in either head or mesenteric lymph nodes, together with miliary lesions in liver and lung or spleen.

(2) A more strict interpretation which will result in a greater number of losses – total condemnation of the carcass if there are lesions in the head or mesenteric lymph nodes, together with miliary lesions in the liver.

Little has changed since Francis (1958) wrote that in one year 1% of three million pig carcasses in an English slaughterhouse were found to be affected with tuberculosis on routine examination, whereas a detailed examination of 100 pigs in the same slaughterhouse showed seven to be affected. One thing is certain – that many cases of tuberculosis are not recognized in the slaughterhouse.

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