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A DISEASE RESEMBLING PARATUBERCULOSIS (JOHNE'S DISEASE) IN ROE DEER (CAPREOLUS CAPREOLUS L.)

AN AETIOLOGICAL AND PATHO-ANATOMICAL STUDY

By

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Mammals in wild life are usually regarded as being relatively resistant to infections with acid-fast bacteria. Diseases in wild living ruminants caused by such bacteria are seldom seen. Weak animals are most easily infected. If an infection is established, it appears that in many cases the disease will have an acute course.

Diseases caused by acid-fast bacteria in animals belonging to the family Cervidae (deer) are now and then mentioned in the literature. *Bischofberger & Nabholz* (1964) describe an investigation on a large scale of the occurrence of tuberculosis in wild animals. All wild animals in certain areas of two Swiss cantons showing symptoms of disease were shot and examined in order to find an explanation of new infections in previously tuberculosis-free cattle herds. Animals killed during the hunting season were examined by a veterinarian and altered organs were examined further. In one of the cantons bovine tuberculosis was diagnosed in, among other animals, eight out of 55 roe deer during the years 1956—57 and in the other canton in ten out of 977 roe deer during the years 1958—63.

Bischofberger & Nabholz also give an account of cases of tuberculosis in wild living roe deer during the years 1914—1958 described mainly by German and Swiss workers. During this period 49 cases of tuberculosis had been reported. In three ani-

mals it was caused by tubercle bacilli of the human type, in two of the avian type and in 13 of the bovine type. Typing was not done in the other 31 animals.

The above mentioned workers also refer to cases of tuberculosis in other deer than roe deer described in German and Swiss literature during 1936—1950. Bovine tuberculosis was confirmed in two such animals and in a third the bacillus was not typed.

Schweizer (1964) demonstrated tuberculosis of bovine type in six wild living roe deer.

Topley & Wilson (1964) report, according to English investigations, three cases of tuberculosis in deer, all of the bovine type.

The American literature has, like the English, few reports on the incidence of tuberculosis in deer. *Ferris et al.* (1961) mention tuberculosis in two deer which apparently did not live in the wild state. Typing was not done. *Quinn & Towar* (1963) describe the results of tuberculin tests of animals belonging to the family of deer in a private park in Michigan. The tests were done after tuberculosis had been diagnosed in a dead animal. Tuberculosis caused by bovine tubercle bacilli was found in several cases of the tuberculin reacting animals.

Reports of paratuberculosis (Johne's disease) in deer are very scanty in the literature. The observed cases have usually come from zoological gardens or deer parks. The description of the disease is as a rule incomplete, especially with regard to the histopathology of the lesions and to the cultural and biological properties of the acid-fast bacteria.

Looking through the literature I have found only one case of paratuberculosis in deer — *Vance* (1961) — besides those included in the survey of the literature presented by *Katic* (1961).

M'Fadyean mentioned already in 1907 that Johne's disease was revealed in a killed deer belonging to a group of animals in a park where chronic cases of diarrhoea occurred.

Bourgeois (1940) describes a case of paratuberculous enteritis in a red deer (Edelhirsch). The animal was bought about ten months old for a park. Wasting was noted after three months, despite normal appetite. Diarrhoea did not occur. The animal was killed and emaciation was observed at autopsy. The mucous membrane of the small intestine was thickened and pinkish in some places and a few petechial haemorrhages were found in the large intestine. The mesenteric lymph nodes were enlarged and had moist cut surfaces. Numerous short acid-fast bacteria, either

solitary or in clumps, were found on microscopic examination of smears from the mucous membrane of the small and large intestine as well as from the mesenteric lymph nodes. Other laboratory tests are not mentioned.

Bourgeois (1944) also describes paratuberculous enteritis in a Japanese deer (Pseudaxis sika)*) living in a park. It was put under observation when it started to become thin at about 18 months of age. The faeces were at times somewhat loose, and short acid-fast bacteria in clumps were found on microscopic examination. The animal was killed and postmortem examination showed poor nutritional state, a high degree of anaemia, pinkish mucous membrane in some parts of the small intestine, and enlarged mesenteric lymph nodes. Paratubercle bacilli were demonstrated by microscopy of the mucous membranes and contents of the small intestine and caecum, but not from the colon and rectum. Large numbers of paratubercle bacilli were seen in smears from the mesenteric lymph nodes. Further investigations are not mentioned.

Dorofeev & Kalachev (1949) have also observed Johne's disease in a red deer. Support for the diagnosis cannot be ascertained in the report.

Johne's disease in European red-deer (Cervus elaphus) has also been observed by *Vance*. The animal, belonging to a park where cattle had previously been reared, showed suspicious symptoms of paratuberculosis. Acid-fast bacteria were demonstrated in the liquid faeces. Complement fixation test on a blood sample indicated a suspicion of Johne's disease. The animal died seven months after the onset of symptoms. Postmortem examination showed slight macroscopic changes. Thickening and corrugation of the intestinal mucosa was absent. Patchy erosions were present in the mucous membrane of the jejunum and ileum. The mucous membrane of the caecum, colon and rectum was considered somewhat thickened and had petechial haemorrhages. The posterior mesenteric lymph nodes were obviously enlarged. Histopathological examinations were not done. Numerous acid-fast bacteria with the same morphology as the paratubercle bacillus were seen in smears from the small and large intestine and from the enlarged mesenteric lymph nodes. Culture from the mesen-

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*) Latin names given only when the authors referred to have used such names.

teric lymph nodes resulted in growth of an organism which was indistinguishable from Johne's bacillus.

Paratuberculosis in reindeer — also belonging to deer — is apparently dealt with only by Russian workers. According to *Pilokarpov*, cited by *Poddoubski* (1957), the disease often runs an acute course with high mortality. The histopathological lesions are, according to *Poddoubski*, somewhat different from those found in cattle. Typical lesions are found in the intestine but especially in the mesenteric lymph nodes where specific granulomas replace the normal lymphoid tissue. Specific lesions containing paratubercle bacilli are also seen in the liver and ovaries.

All the observed cases of paratuberculosis in deer, except reindeer, have been connected with animals in parks, and in no case necroses have been observed. Paratuberculous cattle are considered as the source of infection. The disease has not been diagnosed with certainty in the roe deer (Capreolus capreolus L.). It may therefore be of interest to give an account of a paratuberculosis-like disease in wild living roe deer in Sweden caused by an acid-fast bacterium. Cultural, biological and other properties of this bacterium have been studied more closely, as well as the lesions caused by it.

MATERIAL

During the years 1955—1964 a total of 1,302 whole carcasses of roe deer were received for examination. In five animals lesions were present from which acid-fast bacteria were demonstrated by direct microscopy. Postmortem changes in one of them were so advanced that further tests were considered futile. The other four animals were either found dead or in extremis and then killed by the police. The years 1956, 1958, 1959, and 1961 are represented by one such roe deer carcass each. Their respective numbers are 101, 68, 16, and 94, also used as identification for the respective cultural strains. The animals came from different provinces of the country and the localities were widely separated.

METHODS

At *autopsy* the observed macroscopic lesions were noted.

Bacterioscopy. Smears from the intestinal mucous membrane and the lesions were stained by the Ziehl-Neelsen method and examined under the microscope.

Histopathological examinations. Sections for histological examination were stained with haemalum-eosin and by the Ziehl-Neelsen method.

Cultural examinations. Changed tissue was ground in a mortar and treated for 10 min. in 5 % sulphuric acid. It was then washed once in physiological saline and inoculated on two tubes of Löwenstein-Jensen medium with 0.75 % gycerine, two tubes of the same medium with 6 % glycerine and into one tube of Besredka medium. This method and these media are used routinely here to identify tubercle bacilli.

Another part of the changed tissue was treated with 5 % solution of oxalic acid and also in other respects according to the method recommended by *Taylor* (1950). The sediment was inoculated on five tubes containing Finlayson's medium modified by *Taylor* (1950) — Taylor/Finlayson's medium. The tubes were kept incubated at 37.5°C and were examined weekly as regards growth. In most cases they were observed for five months.

The same media were used for the subcultures from the primary cultures, as well as the medium of *Dunkin* (1928) and the Taylor/Finlayson medium without Mycobact. phlei. Subcultivation on the Taylor/Finlayson medium was done using tubes partly with, and partly without, black-paper coverings.

Biochemical examinations. Qualitative *nitrate reduction test* was performed using bacteria from subcultures about four weeks old, according to the method mentioned by *Boisvert* (1961) which in broad outline conforms to that described by *Virtanen* (1960). Reading was done after five min. The test was performed without bacteria, too, and for comparison also with laboratory strains of the human (2 strains), bovine (4 strains), and avian (8 strains) types of tubercle bacilli, as well as Mycobact. johnei (4 strains) and Mycobact. phlei (2 strains)*). Cultures four weeks old were used here, except for those of Mycobact. phlei which were one week old. *Neutral red test* according to *Dubos & Middlebrook* (1948) was performed from subcultures using the method given by *Fiehl* (1959). For comparison were again used laboratory strains of the human (1 strain), bovine (2 strains) and avian (4 strains) types of tubercle bacilli, as well as Mycobact. johnei (2 strains) and Mycobact. phlei (2 strains). In all cases three-week-old cultures were used and concerning Mycobact. phlei also bacteria from cultures four days old. The reaction was carried out at room temperature and was read after 30 min.

In vitro sensitivity tests for isonicotinic acid hydrazide (isoniazid). Strains 101 and 68 were tested for this and were also compared to two

*) The two strains of Mycobact. phlei were received from The Central Veterinary Laboratory, Weybridge, Surrey, and The Agricultural Research Council Field Station, Compton, Berks., respectively. The strains of tubercle bacilli and of Mycobact. johnei used in the different tests originate from this institute.

strains each of Mycobact johnei and Mycobact. avium. Strain 101 was in the sixth passage and the others were in the second passage of subculture. All the strains were subcultivated on the Taylor/Finlayson medium for seven weeks. Bacterial suspensions were made in Ringer solution from these cultures and were standardized to an opacity containing varying concentrations of isoniazid. A stock solution of 1:100 with Ringer solution and used for inoculation on media.

For the sensitivity test the Taylor/Finlayson medium was used containing varying concentrations of isoniazid. A stock solution of 1.25 g isoniazid in 100 ml distilled water was sterilized by Seitz filtration and added to the medium in amounts to give concentrations of 1.25, 2.5 and 5 μg isoniazid per ml medium. The medium with varying concentrations of isoniazid was transferred in 5 ml amounts to tubes and inspissated in the autoclave at the same angle of inclination. Tubes without isoniazid were used as controls.

Five tubes of each concentration of isoniazid and five control tubes were inoculated with one loop of the different diluted bacterial suspensions. These were incubated at 37°C and examined every week for 10 weeks.

Biological examinations. Guinea pigs, rabbits, hens, calves, a goat (kid) and a roe deer (fawn) (Capreolus capreolus L.) were inoculated with bacterial suspensions from subcultures, six to eight weeks old, on the Taylor/Finlayson medium. The strains had been passaged at the most three times after primary isolation. The doses are given as mg wet weight.

Guinea pigs: Weight about 300 g. All four strains were inoculated separately by intramuscular injections. Three guinea pigs were infected with each strain. The dose was 0.1 mg bacteria in 1 ml Ringer solution per animal. Observation time: 3 months.

Rabbits: Weight about 2,000 g. Three rabbits were infected with strain 101. Doses: 0.5 mg, 0.1 mg and 0.01 mg bacteria in 1 ml Ringer solution. Two rabbits were inoculated with strain 68. Doses: 0.1 mg and 0.01 mg bacteria in 1 ml Ringer solution. Route of infection: Intravenous. Observation time: 12 months.

Hens: Weight ranging between 1,100 and 1,600 g. The hens did not react to avian or bovine tuberculin. Three hens were infected with 0.1 mg and three with 0.01 mg bacteria of strain 101. One hen was inoculated with 0.1 mg bacteria of strain 68 and another with 0.01 mg. The doses were given in 1 ml Ringer solution by intravenous injections. Observation time: 12 months unless death ensued earlier.

Calves: Age four weeks. The animals showed no reactions neither to avian nor to bovine tuberculin. Complement fixation tests on blood sera using Mycobact. johnei antigen were negative. Only strain 101 was used in this infection experiment. One calf was given 100 mg bacteria in 20 ml Ringer solution by intravenous injection and another, also intravenously, 10 mg bacteria in 10 ml of the same solution. Observation time: 33 and 26 months, respectively.

Goat: Age six weeks. 10 mg bacteria of strain 101 in 5 ml Ringer solution was given intravenously. Observation time: 6 weeks (died).

Roe deer (Capreolus capreolus L.): Estimated age eight to ten weeks, male. The same dose of strain 101 as for the goat was given intravenously. Observation time: 9 weeks (died).

After inoculation the experimental calves were subjected to *allergy* tests on six and four occasions, respectively. The Swedish avian and bovine tuberculin normally used on cattle in this country were used intradermally, as well as johnin received from The Royal Veterinary College and Hospital, Streatley, Berks. The doses were in all cases 0.1 ml and the reactions were measured after 72 hrs.

Before the allergy tests, blood samples were taken from the calves for *serological* examinations as regards complement fixing antibodies against Mycobact. johnei. Examinations and the production of antigen were performed according to the method described by *Hole* (1953, 1956). Sera from the experimental animals were titrated to the highest dilution which gave total inhibition of haemolysis (serum titer).

All experimental animals were autopsied either subsequent to spontaneous death or after killing at the end of the observation period. Macroscopic lesions were noted. Bacterioscopical, histopathological and cultural examinations, using the methods and media described above, were carried out on material from organs with, and in some cases without, lesions.

RESULTS

Two of the four roe deer subjected to closer examination were females with an estimated age of 6—7 years, and the other two were males with an estimated age of 4—5 years. Fourty-three % of the 1,302 roe deer carcasses examined during the ten-year period 1955—64 were females and 57 % were males. The age distribution was 60 % adults (over 12 months) and 40% young animals.

At *autopsy* the most obvious changes of the five roe deer, from which acid-fast bacteria were demonstrated, were greatly enlarged mesenteric lymph nodes and emaciation. Mesenteric lymph nodes were as a rule up to a good walnut size with greyish-white lardaceous cut surfaces and infiltrated with large or small greyish-yellow necroses similar to caseation (Fig. 1). The contents from all sections of the intestines in all animals had a liquid consistency. No macroscopic changes were noted in the intestinal mucosa of any of the animals. Retropharyngeal, iliac, and most of the body lymph nodes were in all five animals moderately en-

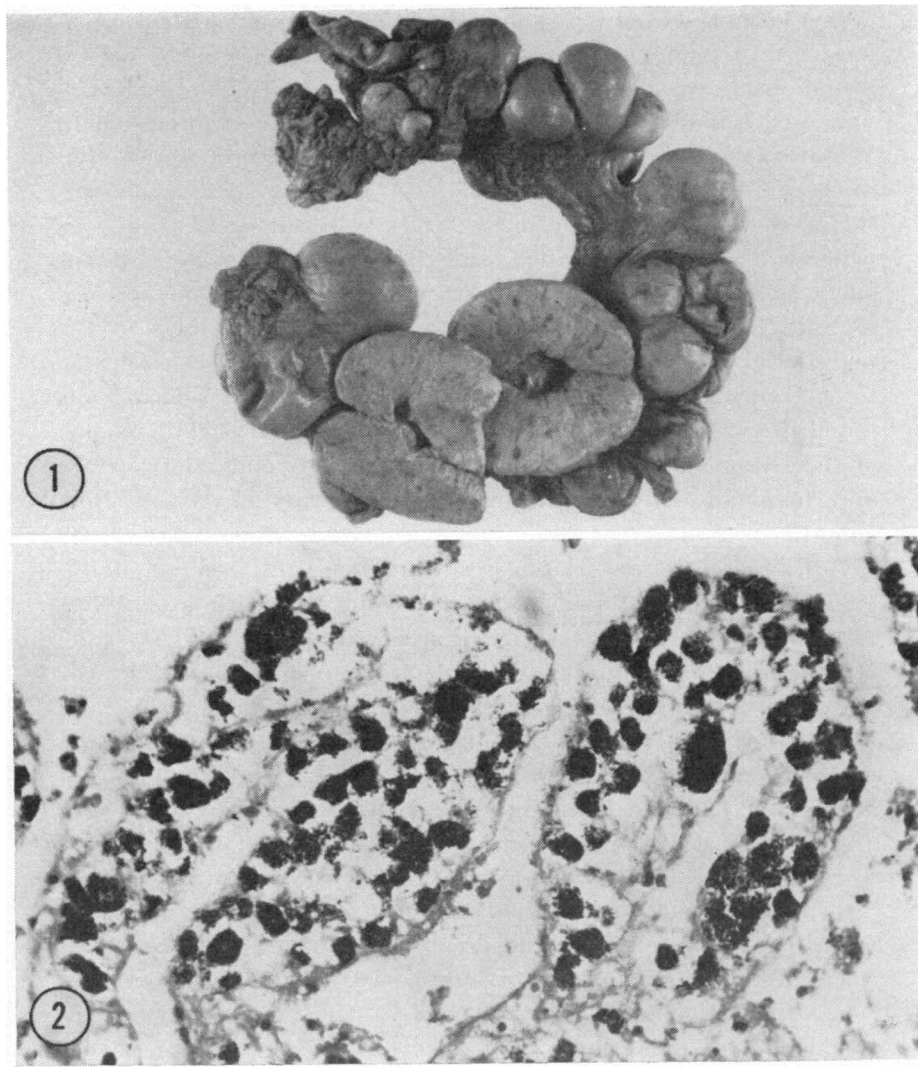

Figure 1. Mesenteric lymph nodes; roe deer, spontaneous case. Enlarged with lardaceous cut surfaces and irregularly demarcated necrotic areas.

Figure 2. Ileum; roe deer, spontaneous case. Numerous intracellular acid-fast bacteria (stained black) in the villi. Ziehl-Neelsen. × 300.

larged and presented the same macroscopic picture as the mesenteric lymph nodes, although the necrotic changes were not so pronounced. Bronchial, mediastinal, and hepatic lymph nodes were also somewhat enlarged with a similar appearance. Small, about pea size, poorly demarcated consolidated areas were found in the lungs in three of the animals (101, 68 and 16). No macroscopic changes were noted in the livers and kidneys. The spleens were enlarged with rounded edges and bulging granulated cut surfaces. One animal (16) had also pale well demarcated pea size nodules in the spleen and similar but smaller nodules in the marrow of the long bones, especially in the epiphyses, but also in the red marrow. Serous atrophy of the bone marrow fat was noted in two animals (16 and 94). The mammary gland in one of the females (94) had a lardaceous greyish-yellow cut surface and the gland cisterns contained small amounts of a yellow flocculent secretion.

Microscopic examination of smears from the mucosa of duodenum, ileum, jejunum, caecum and colon showed in all animals large amounts of acid-fast bacteria in clumps. Numerous such clumps were also found in smears from lesions in the mesenteric, body, retropharyngeal, iliac, bronchial, mediastinal, and hepatic lymph nodes, as well as from all spleens and from nodules in the bone marrow of animal 16 and from the mammary gland of animal 94. The acid-fast bacteria measured about 0.5 μ in width and 1—3.5 μ in length. The majority of the bacteria, however, were 1—2 μ in length. The organism was Gram-positive.

The interpretation of *the histological picture* of the intestines was difficult because of widespread postmortem changes. Ziehl-Neelsen stained slides from different parts of the intestines confirmed nevertheless the bacterioscopical findings from the intestinal mucous membranes. The acid-fast bacteria were found mainly in the lamina propria and especially in the top parts of the villi (Fig. 2). Lymph nodes showing macroscopic changes and nodes without such changes but from which acid-fast bacteria had been demonstrated microscopically, presented largely the same microscopic picture: Proliferation and diffuse infiltration of reticuloendothelial cells with abundant, pale cytoplasm and relatively large, light-coloured, pleomorphic nuclei, i. e. epithelioid cells (Fig. 3). These cell infiltrations constantly showed various stages of nuclear regression. In some cases there was focal necrosis, characterized by strongly basophilic nuclear fragments.

Table 1. Acid-fast bacteria isolated from roe deer (Capreolus capreolus L.). Spontaneous cases.

Organ	No. 101 male		No. 68 female		No. 16 male		No. 94 female	
	1	2	1	2	1	2	1	2
Retropharyngeal lymph node			+	—	+	—		
Bronchial „ „	+	—	—	—	+	—	+	—
Hepatic „ „			+	—	+	—		
Iliac „ „	+	—						
Prescapular „ „			+	—	+	—	+	—
Popliteal „ „			+	—	+	—		
Ileocaecal „ „			+	—	+	—		
Jejunal „ „	+	—	+	—	+	—	+	—
Ileum			+	—				
Caecum			—	—				
Colon			—	—				
Spleen	+	—	+	—	+	—	+	—
Mammary gland							+	—
Bone marrow			+	—	+	—		

1: Taylor/Finlayson's medium.
2: Löwenstein-Jensen's medium containing varying amounts of glycerine, and Besredka's medium.
+: Colonies consisting of acid-fast bacteria.
—: No growth.
No cultures made where + or — is lacking.

The lungs of all animals except 94 showed parasitic pneumonia (Dictyocaulus viviparus) and all had single or multiple miliary epithelioid cell granulomas. Also the livers, except 68 which had advanced postmortal changes, showed multiple epithelioid cell granulomas. All spleens showed the same microscopic picture as the livers. Necrobiotic or necrotic processes were found only in animal 94. The bone marrow of animals 16 and 94 also had miliary nodules consisting of epithelioid cells. Giant cells were very rarely found in the organs mentioned. Such cells of the Langhans type were more numerous in the mammary gland of animal 94 in which the parenchyma was infiltrated with them and with epithelioid cells (Fig. 4). The epithelioid cells in the organs named were more or less filled with acid-fast bacteria.

Results from the *primary cultural examinations* appear in Table 1. The time taken before colonies could be observed with the naked eye varied between four and 14 weeks. Colonies would grow only on media containing killed Mycobact. phlei. Morpho-

Table 2. The effect of isoniazid on strains 101 and 68 and on two strains each of Mycobact. johnei and Mycobact. avium.

Strain	µg/ml	Growth after weeks of incubation									
		1	2	3	4	5	6	7	8	9	10
101	0	—	—	+++	+++	+++	+++	+++	+++	+++	+++
	1.25	—	—	++	++	+++	+++	+++	+++	+++	+++
	2.5	—	—	—	+	++	++	+++	+++	+++	+++
	5.	—	—	—	—	+	+	++	++	+++	+++
68	0	—	—	+++	+++	+++	+++	+++	+++	+++	+++
	1.25	—	—	+	++	+++	+++	+++	+++	+++	+++
	2.5	—	—	—	++	++	++	++	++	+++	+++
	5.	—	—	—	—	+	+	++	++	+++	+++
Mycob. johnei a	0	—	—	+++	+++	+++	+++	+++	+++	+++	+++
	1.25	—	—	++	++	++	+++	+++	+++	+++	+++
	2.5	—	—	+	++	++	++	++	+++	+++	+++
	5.	—	—	—	++	++	++	++	++	+++	+++
Mycob. johnei b	0	—	—	+++	+++	+++	+++	+++	+++	+++	+++
	1.25	—	—	++	++	++	++	++	++	+++	+++
	2.5	—	—	—	++	++	++	++	++	+++	+++
	5.	—	—	—	++	++	++	++	++	+++	+++
Mycob. avium a	0	—	+	+++	+++	+++	+++	+++	+++	+++	+++
	1.25	—	+	++	++	++	++	++	++	+++	+++
	2.5	—	—	++	++	++	++	++	++	++	+++
	5.	—	—	—	+	+	+	+	+	++	++
Mycob. avium b	0	—	+	+++	+++	+++	+++	+++	+++	+++	+++
	1.25	—	+	++	++	++	++	++	++	++	++
	2.5	—	—	++	++	++	++	++	++	++	++
	5.	—	—	+	+	+	+	+	+	++	++

+++: > 100 colonies.
++ : 25—100 colonies.
+ : < 25 colonies.
— : no growth.

logically the colonies were dry, dull and greyish-white in colour. The colonies consisted of acid-fast bacteria which were on the whole shorter than those seen in direct smears from the different organs.

At *subcultivation* growth was obtained from primary cultures on the Taylor/Finlayson and Dunkin media, but never on any medium which lacked Mycobact. phlei. The colony appearance corresponded to that in the primary cultures, whether they were exposed to light or not.

Nitrate reducing ability was examined in strains 101 and 68. Both were in this respect completely inactive like the four strains of Mycobact. johnei and the four bovine tubercle bacillus strains. Two of the eight avian tubercle bacillus strains were completely inactive, five showed very faint activity and one strain showed a reducing ability of medium strength. The human tubercle bacillus strains and the strains of Mycobact. phlei reduced strongly. Control tubes without bacteria showed negative reactions.

Neutral red reactions of strains 101 and 68 were positive like the human, the two bovine and the four avian tubercle bacillus strains, as well as the two strains of Mycobact. johnei. Strain 68 showed a stronger positive reaction as regards colour intensity than the others. Both strains of Mycobact. phlei were completely negative.

Sensitivity to isoniazid is illustrated in Table 2. The different bacterial strains were inoculated on five tubes each of medium without isoniazid and on five tubes each with the mentioned concentrations of isoniazid. Intensity of growth or of growth inhibition was on the whole similar within the respective series of five tubes and these have for that reason been dealt with as one in the table.

The table shows that the avian tubercle bacillus strains grew earlier than the other strains. After incubation for three weeks, the isoniazid concentrations of 2.5 and 5 μg per ml appeared markedly, and about equally, active against strains 101 and 68 and the two strains of Mycobact. johnei. This activity must, however, be considered as transient. A certain inhibition of growth also occurred with the avian tubercle bacillus strains. As regards the latter, it was observed that on the medium containing isoniazid there were partly typical spherical avian colonies and partly unpigmented flat colonies with irregular edges. Quite typical, pigmented and spherical, avian colonies developed gradually from the centre in some of the flat colonies. A similar type of growth by avian tubercle bacillus colonies on the Löwenstein medium containing isoniazid has been observed by *Plum* (1952).

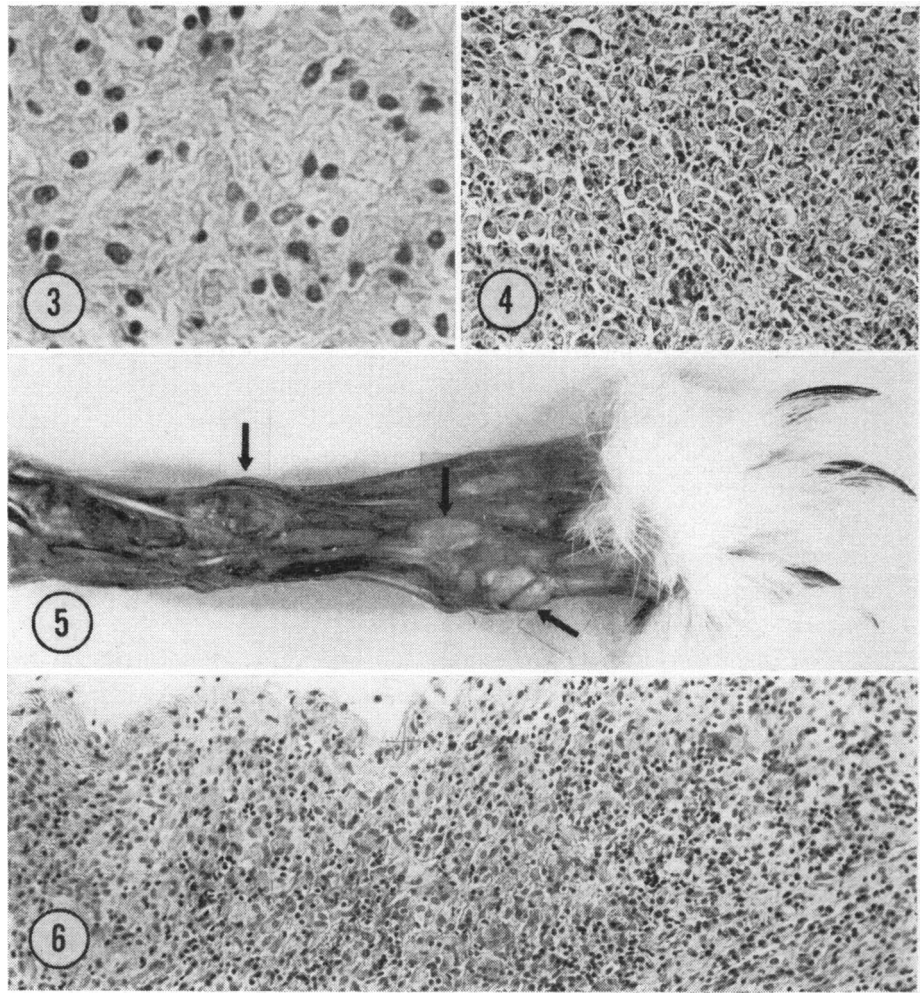

Figure 3. Retropharyngeal lymph node; roe deer, spontaneous case. Infiltration of cells with abundant, pale cytoplasm and relatively large light-stained nuclei (epithelioid cells). The picture also shows nuclear regressions. Haemalum-eosin. × 500.

Figure 4. Mammary gland; roe deer, spontaneous case. Diffuse infiltration of epithelioid cells and giant cells of the Langhans type. Haemalum-eosin. × 150.

Figure 5. Hind leg; experimental rabbit. Inflammatory swellings of tarsal and phalangeal joints and extensor tendon sheaths (arrows).

Figure 6. Tendon sheath; experimental rabbit. Diffuse infiltration of epithelioid cells, lymphocytes and plasma cells in the tendon sheath wall. Haemalum-eosin. × 150.

Experimental transmission

The *guinea pigs*, inoculated with all roe deer strains, were killed, and showed no macroscopic changes suggesting tuberculosis at the end of the observation period.

The *rabbits*, infected with strains 101 and 68, showed no disease symptoms during the observation period. They all increased in weight by 600—800 g and were killed.

All five rabbits showed joint and tendon sheath changes regardless of differences in the infection doses. The changes were primarily present in the carpal and tarsal regions and in the phalangeal joints. Sometimes they could be present in both fore and hind legs. Knee, elbow and shoulder joints were affected inconstantly. The lesions appeared as larger or smaller bulges of the joint capsules and tendom sheaths which sometimes appeared yellowish. They contained greyish-yellow, sticky and granulated material (Fig. 5). No macroscopic changes were observed in the internal organs.

Acid-fast bacteria were demonstrated in smears from affected joint capsules and tendon sheaths. These conformed morphologically with the bacteria used for inoculation of the rabbits.

Histopathological examination showed a considerable thickening of the joint capsules and the walls of the tendon sheaths (Fig. 6). Proliferation of epithelioid cells could be seen close to the synovial membrane, and regressive changes could be observed in some areas. Lymphoid cells and plasma cells were quite abundant in the periphery. Livers, lungs, spleens and intestines showed no histopathological changes.

Acid-fast bacteria were isolated from the affected joint capsules and tendon sheaths in all rabbits. Growth was observed in all cases after from four to six weeks' incubation, but only on medium containing killed Mycobact. phlei. From the rabbits infected with strain 101 a few colonies of acid-fast bacteria were in the same manner found in cultures from the livers, spleens, lungs, and kidneys of two rabbits and from the caecum in one of them. Growth on subcultures occurred only in the presence of killed Mycobact. phlei. The colonies and acid-fast bacteria were morphologically similar to those isolated from the roe deer.

The *hens*, infected with strain 68 (0.1 and 0.01 mg), died after 15 and seven weeks, respectively. One of the three hens inoculated with 0.1 mg of strain 101 died after six weeks, the two others were killed after 12 months. The hens inoculated with 0.01 mg

of strain 101 died after five, 16, and 35 weeks, respectively. All, except one of the experimental birds which died, showed loss of weight varying from 100 to 500 g. The two hens which were killed had a weight gain of 200 and 300 g.

At autopsy all the hens showed the same picture. The livers and most of the spleens were enlarged. They were infiltrated with multiple miliary greyish-white nodules resembling those of tuberculosis (Fig. 7). Macroscopic lesions were not observed in lungs, kidneys and intestines. In one of the hens, inoculated with 0.01 mg of strain 101 and which died 35 weeks after inoculation, some half hemp seed size nodules were found in the red bone marrow of the middle part of the long bones. The nodules resembled those of tuberculosis.

Acid-fast bacteria were demonstrated microscopically from all changed livers and spleens as well as from the above mentioned nodules in the red bone marrow. These conformed morphologically with those demonstrated from the experimental rabbits.

Histologically all livers and spleens showed multiple miliary granulomas mainly consisting of epithelioid cells. In the periphery of the granulomas eosinophilic leucocytes and plasma cells were found (Fig. 8). Some of the granulomas in the hens killed after one year had central necrosis. Giant cells were not observed. No microscopic lesions could be seen in the lungs, kidneys, intestines, and bone marrows of the two hens killed after 12 months (strain 101), nor in the kidneys and intestines of the other hens. The lungs from the hens which died had solitary or multiple miliary granulomas like those found in the livers and spleens. Two of the hens infected with 0.01 mg of strains 101 and 68, respectively, had some granulomas with complete central necrosis. The above mentioned macroscopic nodules in the red bone marrow consisted of epithelioid cell granulomas with central necrosis. Such granulomas without necrosis were also found in the red bone marrow of the two hens inoculated with 0.1 mg and 0.01 mg, respectively, of strain 101 and the hen inoculated with 0.1 mg of strain 68.

Mainly short, acid-fast rods were culturally recovered from all livers and spleens, but only on the Taylor/Finlayson medium. In these cases growth could be observed after from four to six weeks. Acid-fast bacteria were similarly isolated from the lungs, kidneys and bone marrow of all hens except those which were killed after 12 months, as well as from the intestinal mucosa

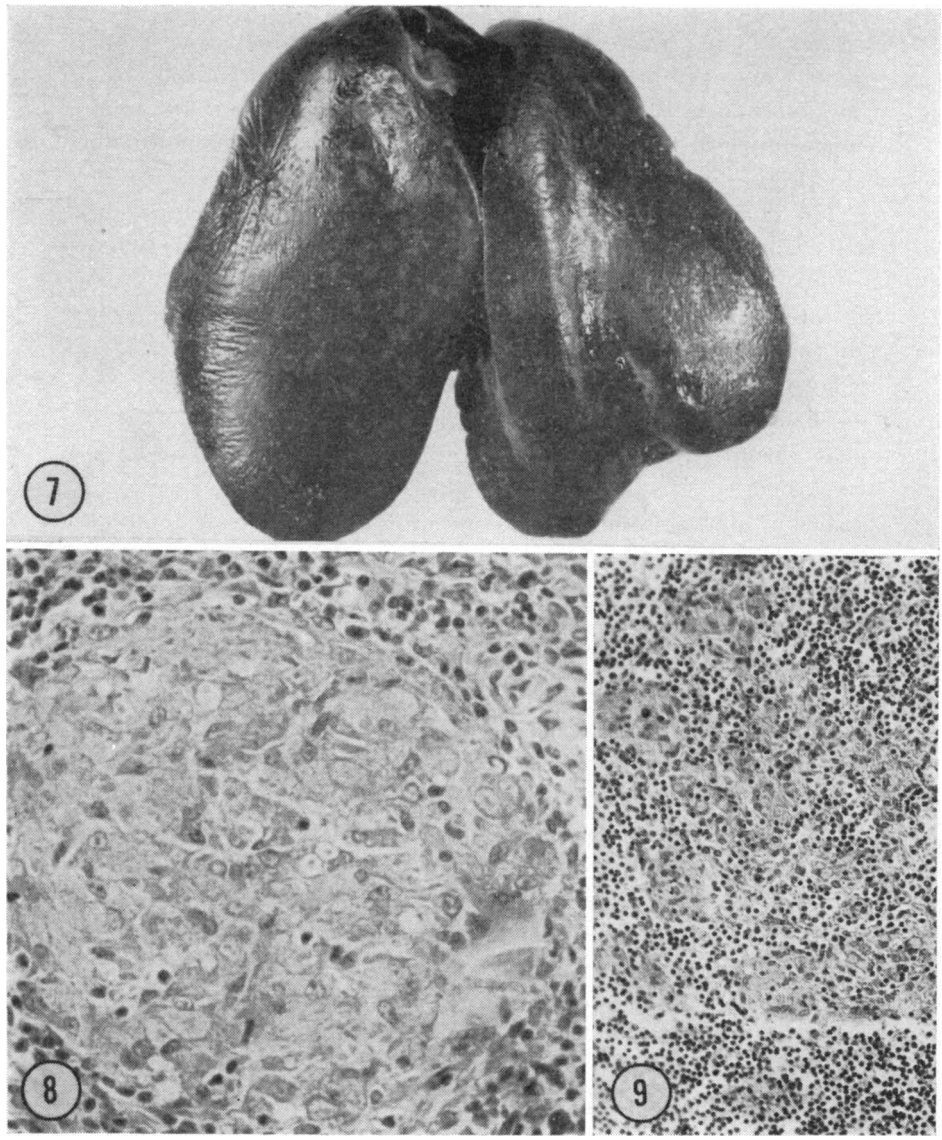

Figure 7. Liver; experimental hen. Infiltrated with greyish-white miliary nodules.

Figure 8. Spleen; experimental hen. Epithelioid cell granuloma. Haemalum-eosin. × 500.

Figure 9. Mesenteric lymph node; experimental goat. Patchy infiltration of epithelioid cells. Haemalum-eosin. × 150.

(duodenum and ileum) of two hens infected with strain 101 (0.1 mg and 0.01 mg, respectively) and of one hen infected with strain 68 (0.01 mg). All primary cultures were subcultivated and growth was obtained only on the Taylor/Finlayson and Dunkin media. The colonies in the primary cultures and subcultures conformed with those obtained from the roe deer.

The *calf*, infected with 100 mg of strain 101, developed normally and showed no symptoms of disease except on two occasions. Diarrhoea lasting three days occurred 22 months after the infection and again four months later lasting one day. Short acid-fast bacteria were demonstrated by microscopy of the faeces on both occasions. The animal was killed.

The *calf*, infected with 10 mg of the same strain, also developed normally and showed no symptoms of disease. The animal was killed.

At autopsy neither macroscopic nor microscopic lesions were observed in the intestines and mesenteric lymph nodes or other organs with their associated lymph nodes. Cultures were made from different intestinal sections (jejunum, ileum, ileocaecal valve, caecum, colon) and from the ileocaecal, jejunal, colic, bronchial, hepatic, retropharyngeal, and prescapular lymph nodes, and the spleens in both experimental animals. Acid-fast bacteria were recovered, however, only on the Taylor/Finlayson medium, from the ileocaecal, bronchial, and retropharyngeal lymph nodes of the animal infected with 100 mg bacteria. Colonies were observed after from seven to 12 weeks' incubation. Subcultivation produced growth only on the Taylor/Finlayson and Dunkin media. The colonies consisting of acid-fast bacteria conformed morphologically with those which by cultivations were obtained from the roe deer.

Results from the allergy tests and serological examinations can be seen in Tables 3a and b. The avian tuberculin caused stronger reactions than the bovine tuberculin and the johnin. One could possibly say that both animals reacted positively to avian and very slightly, or negatively, to bovine tuberculin and johnin. According to Table 3a the serological reactions which are relatively constant, may possibly indicate the presence of complement fixing antibodies to Mycobact. johnei in the blood serum of this animal. The results of the serological examinations according to Table 3b cannot, however, be interpreted in the same way.

Table 3 a. Tuberculin, johnin and serological tests of the calf infected with 100 mg of strain 101.

Kind of test	Months after infection					
	5	8	12	15	20	31
Tuberculin, avian	6	3.5	3	3.5	5	4.5
Tuberculin, bovine	0.5	1	2	1	1	1
Johnin	0.5	1	1	1.5	1	1
Complement fixation	1:20	1:20	1:5	1:20	1:20	1:10

Table 3 b. Tuberculin, johnin and serological tests of the calf infected with 10 mg of strain 101.

Kind of test	Months after infection			
	3	8	13	24
Tuberculin, avian	7.5	5	5	4.5
Tuberculin, bovine	4	2	1.5	0.5
Johnin	4	1.5	1.5	1
Complement fixation	0	1:20	0	0

The figures given in the allergy tests express the increase in skin thickness in mm 72 hours after injection.

The *goat,* infected with strain 101, showed at the end of the sixth week after inoculation symptoms of pneumonia and died after two days.

The autopsy showed a nutritional state below average. The lungs were enlarged and hepatized and some bronchi contained a sticky exudate. Bronchial, mediastinal and mesenteric lymph nodes were somewhat enlarged and hyperplastic. No other macroscopic lesions were present.

Histopathological examinations showed acute interstitial pneumonia with necrotic tendency, and multiple miliary epithelioid cell granulomas in the liver, kidneys, spleen, and bone marrow of the long bones. The lung lymph nodes and especially the mesenteric lymph nodes showed patchy infiltrations of epithelioid cells (Fig. 9). Hyperplasia of the lymphatic tissue occurred in the ileum. Focal infiltrations of large mononuclear cells and miliary epithelioid cell granulomas were present in the submucosa of the ileum and moderate, mainly lymphocytic, hyperplasia

in the villi. A few giant cells of the Langhans type were seen in the liver.

Under the same conditions as for the previously mentioned experimental animals cultures of acid-fast bacteria were growing after from four to six weeks' incubation from the lungs, liver and spleen, as well as from the bronchial, mediastinal, hepatic, jejunal, ileocaecal, colic and prescapular lymph nodes. The colony appearance and conditions of the subcultures were completely analogous with those previously mentioned.

The *roe deer,* inoculated with strain 101, developed normally during the first six weeks after the infection. Decreased condition, forced breathing and reduced appetite were observed in the seventh week. The condition considerably deteriorated in the beginning of the ninth week. The animal was laying and had breathing difficulties and reduced appetite. After a few days of improvement a couple of days followed with profuse diarrhoea and the animal died.

Microscopic examination of the fluid faeces showed masses of short acid-fast bacteria in clumps.

Autopsy of this experimental animal gave the same result as those of the roe deer sent in for examination. Besides emaciation the most obvious finding was greatly enlarged mesenteric lymph nodes which had greyish-white lardaceous cut surfaces with greyish-yellow necroses (Fig. 10). Similar changes, although less pronounced, were present also in the hepatic, bronchial and mediastinal lymph nodes. The body lymph nodes were enlarged with greyish-white cut surfaces. Focal consolidations were present in the lungs, and the spleen was somewhat enlarged and had a hyperplastic cut surface. No macroscopic lesions were present in the intestinal mucosa, and no other organs showed lesions.

Masses of short acid-fast bacteria in groups and clumps were demonstrated by microscopy of smears from the bone marrow, different sections of the intestinal mucosa and from the above mentioned organs with lesions.

The histopathological examinations showed the same changes as in the spontaneous cases. The villi were swollen due to heavy diffuse infiltration in the propria mucosae by large cells with pale cytoplasm and relatively light-coloured nuclei (epithelioid cells) and also by plasma cells, lymphocytes, and leucocytes (Fig. 11). Giant cells were not demonstrated. Mesenteric (Fig.

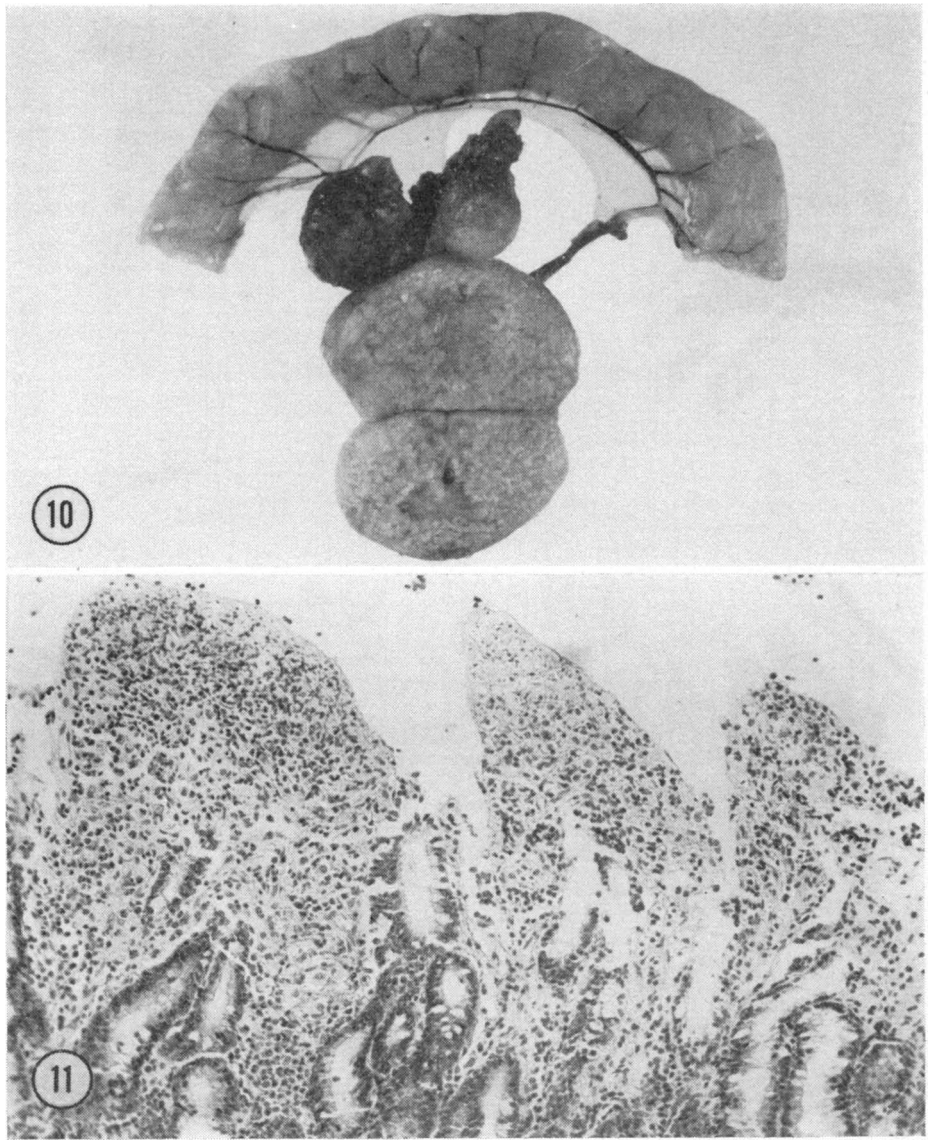

Figure 10. Mesenteric lymph node; experimental roe deer. Enlarged, necrotic areas.

Figure 11. Small intestine; experimental roe deer. Swollen villi. Diffuse infiltration of epithelioid cells, lymphocytes and plasma cells. Haemalum-eosin. × 150.

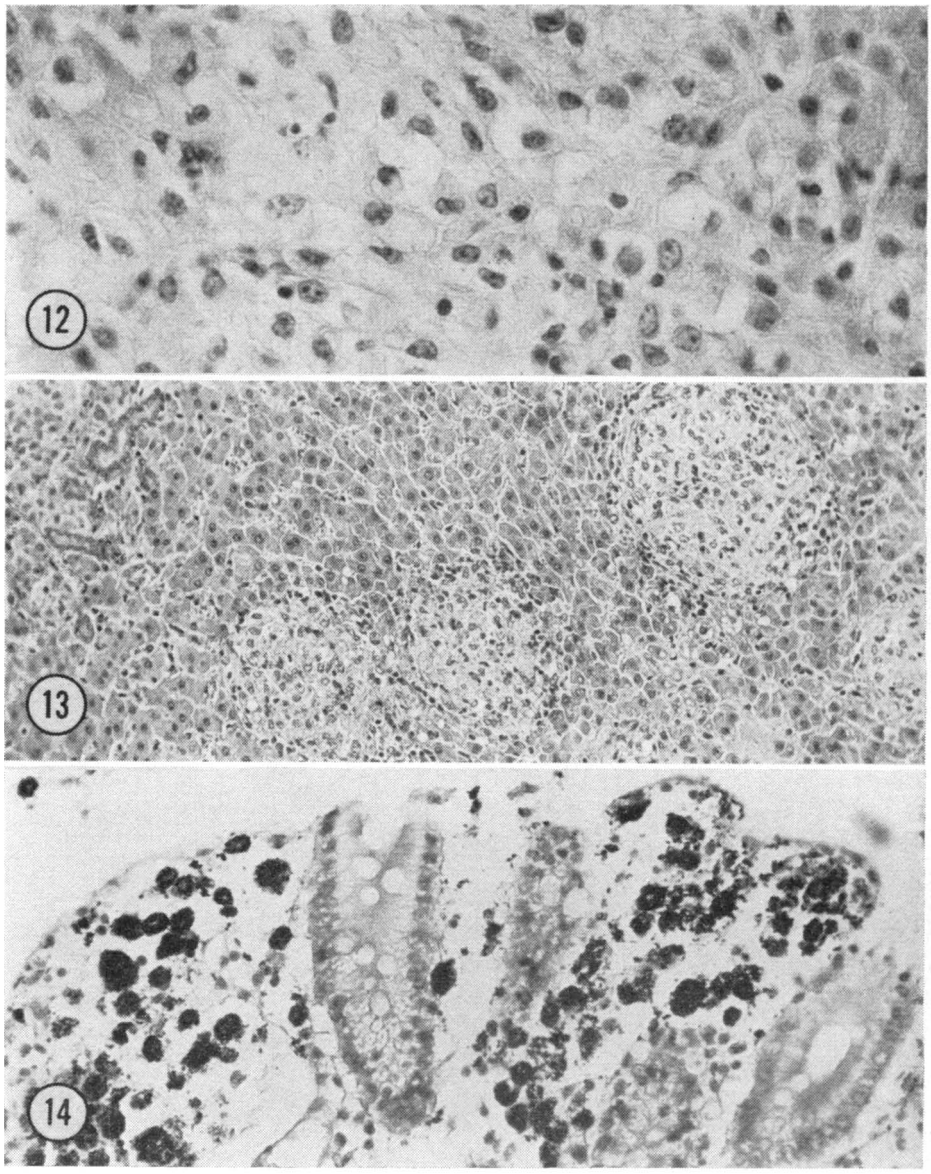

Figure 12. Mesenteric lymph node; experimental roe deer. Diffuse infiltration of epithelioid cells. Haemalum-eosin. × 500.

Figure 13. Liver; experimental roe deer. Multiple epithelioid cell granulomas. Haemalum-eosin. × 150.

Figure 14. Ileocaecal valve; experimental roe deer. Intracellular acid-fast bacteria (stained black) in propria mucosae. Ziehl-Neelsen. × 300.

12), hepatic, bronchial, mediastinal, retropharyngeal, and prescapular lymph nodes showed proliferation and infiltration by reticuloendothelial cells with abundant, pale cytoplasm and relatively large, light-stained nuclei. The cell infiltrations could show complete necrosis. Multiple miliary foci of epithelioid cells occurred in the bone marrow, spleen and liver (Fig. 13). The lungs showed advanced interstitial pneumonia with numerous epithelioid cells as well as giant cells of the Langhans type and focal necroses. In all organs examined the epithelioid, and allied cells were filled with acid-fast bacteria (Fig. 14).

Acid-fast bacteria were recovered after five weeks' incubation on the Taylor/Finlayson medium from the ileocaecal, jejunal, colic, bronchial, mediastinal, hepatic, and prescapular lymph nodes, as well as from different sections of the intestinal mucosa, the lungs and bone marrow. In both primary cultures and subcultures the bacteria behaved in the same manner as those which were isolated from the spontaneous cases and the other experimental animals, i. e., they would only grow on media containing killed Mycobact. phlei. Colony appearance and bacterial morphology conformed to previous observations.

DISCUSSION

The acid-fast bacteria which were isolated from the roe deer, have been examined in varying degrees. Strain 101 and 68 have been examined more closely. All four strains had similar morphological and cultural properties, and the animals from which they were isolated showed largely the same macroscopic and microscopic lesions. It may therefore be assumed with great probability that the other two strains have conformity to the strains 101 and 68 also as regards other properties.

The general properties of the isolated acid-fast bacteria entitle them to be included in the Mycobacteria.

Several workers have demonstrated tuberculosis in the wild living roe deer as already mentioned in the introduction. Human, bovine or avian tubercle bacilli were established as the cause of infection. The acid-fast bacteria isolated from roe deer in the present work can be excluded from being identical with human or bovine tubercle bacilli on account of the results from the experimental transmissions in laboratory animals. However, the tissue changes in the experimental hens indicate that Mycobact.

avium cannot be excluded. If such is the case, the roe deer must have been infected through food contaminated by tuberculous birds. The autopsies showing the largest and apparently oldest lesions in the mesenteric lymph nodes support this view.

Whereas bovine tuberculosis in cattle is eradicated, the avian type of tuberculosis still occurs in different parts of this country. Not infrequently, at this institute, tuberculosis is diagnosed in birds other than hens, such as in the pheasant, peacock, tawny owl, woodcock, duck, crane, starling, black grouse and sparrowhawk. In addition, avian tuberculosis sometimes occurs in swine.

However, the cultural properties of the acid-fast bacteria isolated from the roe deer do not conform to those usually attributed to the avian tubercle bacillus. The latter grows readily on media used in routine diagnosis of tubercle bacilli and with colonies of the smooth type, whereas the acid-fast bacteria demonstrated in the roe deer were difficult to cultivate. They required both in primary cultures and subcultures not only medium containing killed Mycobact. phlei, but generally also a longer, in some cases a much longer, incubation period than Mycobact. avium. The colonies were of the rough type in both primary cultures and subcultures.

Christiansen et al. (1946) and *McDiarmid* (1948) observed in wood-pigeons (Columba palumbus L.) lesions resembling tuberculosis which contained masses of acid-fast bacteria. *Christiansen et al.* did not succeed in cultivating these bacteria. *McDiarmid* (1948) isolated avian tubercle bacilli from all but two pigeons examined, in spite of masses of acid-fast bacteria in smears from the lesions of the latter. *McDiarmid* (1961) mentions that in wood-pigeons a rough variety of the avian tubercle bacillus sometimes occurs besides the ordinary smooth type. The rough variety is difficult to cultivate, and it will grow in primary culture only on medium used for cultivating Mycobact. johnei. But after passage in domestic fowl it reverts to the smooth type which readily grows on ordinary avian media. At the same time it would regain its pathogenicity for chickens which were not killed by intravenous inoculation from the primary isolation. In other words, the avian tubercle bacillus seems to change character in the wood-pigeon which is an unnatural host. This opinion, however, is not relevant in the examination of our material. We have never had any difficulty in isolating avian tubercle bacilli on media without killed Mycobact. phlei, when examining

material from wild birds with tuberculous lesions or from swine and cattle with avian tuberculosis. The growth on primary cultures has always been of the smooth type.

Our experience under Swedish conditions is that the avian tuberculosis in cattle is restricted to certain organs, especially the mesenteric lymph nodes and the uterus. *Thordal-Christensen* (1952), however, has described a case of generalized avian tuberculosis in a cow. He had no difficulties in isolating avian tubercle bacilli from, for instance, the milk. Rabbits and hens inoculated intravenously with material from the primary cultures developed generalized tuberculosis.

Other workers, such as *Engbæk* (1961), have shown that attenuated avian tubercle bacilli given intravenously into hens in relatively large doses, did not cause death or macroscopic lesions indicating tuberculosis. Tubercle bacilli were not recovered on the Löwenstein-Jensen medium from internal organs. The same attenuated avian strain given in the same infection dose intravenously into rabbits produced, however, lesions in joints and tendon sheats, but did not cause death. The avian tubercle bacilli were recovered on the Löwenstein-Jensen medium from these lesions. The tubercle bacilli were recovered on the same medium also from internal organs which did not present macroscopic tuberculous lesions.

The two roe deer strains inoculated into hens turned out to be virulent for these, even though the death ensued rather late for most of them or not at all. The positive neutral red reactions also report the view that the experimental examinations have been made with virulent bacteria. The neutral red test as performed in the present work, is a reaction used to test the virulence of tubercle bacilli or organisms closely related to them.

Although the acid-fast bacteria were passed in hens, they could be recovered only on the Taylor/Finlayson medium, and the colonies were always of the rough type. This was also the only colony type which appeared on cultures from the other experimental animals. The relatively common ring formation in cultures of avian tubercle bacilli was not seen in any of the cultures from the roe deer or the experimental animals.

With my knowledge of the cultural properties of avian tubercle bacili, I can find no resemblance between these properties and the corresponding properties of the acid-fast bacteria isolated from the roe deer or the experimental animals.

The histopathological changes in the roe deer and affected experimental animals do not exactly indicate tuberculosis. The nodules of epithelioid and allied cells were poorly or not at all demarcated as is usually the case in tuberculosis. The microscopic picture of tuberculosis in birds is characterized by early caseation, abundance of foreign body giant cells which together with epithelioid cells often radially surround the necrosis. Necroses certainly occurred in the livers and spleens of some of the experimental hens, but neither giant cells of any kind nor radially arranged epithelioid cells around the necroses were observed.

Joint and tendon sheat lesions which occurred in the experimental rabbits, inoculated with the same bacterial suspensions as the experimental hens, can be caused by attenuated avian tubercle bacilli as previously mentioned. These lesions are by no means specific for the avian tubercle bacilli. Several other species of mycobacteria can under certain conditions also cause such changes. Common for most of them, however, is that they can be cultivated from the lesions on medium without the addition of other killed acid-fast bacteria. This was not the case concerning the lesions of the rabbits inoculated with the roe deer strains 101 and 68.

Topley & Wilson (1964) write that a dose of 5 mg avian tubercle bacilli inoculated intravenously into calves may cause death in two or three weeks. The same bacilli inoculated into goats will seldom give rise to progressive disease, according to the authors. These properties do not correspond with the experimental results in the present investigations.

The roe deer strains which were inoculated into experimental animals, accordingly, did not show all the biological properties one expects from virulent avian tubercle bacilli. For instance, rabbits inoculated intravenously did not die.

The acid-fast bacteria which caused the disease in roe deer apparently belong to the group of mycobacteria which is difficult to grow on artificial media. Among others, Mycobact. johnei belongs to this group.

Paratuberculosis (Johne's disease) has occurred in certain herds in this country. To my knowledge the disease did not, however, occur amongst cattle in the areas where the diseased roe deer were found.

The acid-fast bacteria isolated from the roe deer and the ex-

perimental animals show morphological and, particularly, cultural properties which generally are attributed to Mycobact. johnei.

The clinical course in the experimental roe deer does not, however, resemble the course of paratuberculosis in cattle. Diarrhoea did certainly occur, but only at a late stage. *Couturier* (1960) mentions the same observation in paratuberculous stonebucks (Capra aegarus ibex L.) in a park. Hypertrophic mesenteric lymph nodes were the only macroscopic lesions in these animals.

The macroscopic lesions in the roe deer received for examination and those in the experimental one did not agree with the changes found in paratuberculous cattle. The typical corrugation of the intestinal mucous membrane in cattle was absent in all roe deer. Necroses with caseation and calcification in mesenteric lymph nodes have not been observed in cattle. However, the typical macroscopic intestinal lesions in cattle do not always occur in paratuberculous sheep and goats. Several workers, *Stamp & Watt* (1954), *Cross & Hughes* (1955), *Groth* (1964), *Holmboe & Slagsvold* (1934), *Levi* (1948), and *Harding* (1957), have on the other hand observed necroses in mesenteric lymph nodes of paratuberculous sheep and goats. They also describe certain cases with necroses in other nodes than the intestinal lymph nodes, and state that macroscopic intestinal lesions were not always present. *Jarmai* (1922) has also described the occurrence of necroses in the mesenteric lymph nodes and liver of an antelope (Connochetes albojubatus Thom) with paratuberculous enteritis in a zoological garden.

The macroscopic lesions in the roe deer have accordingly some resemblances to the lesions which occur in certain cases of paratuberculosis in, for instance, sheep and goats.

The histopathological changes in the lymph nodes of the spontaneous and experimental roe deer cases were mainly found in the peripheral parts. Such a localization often occurs in paratuberculosis. The cell picture of the lymph nodes, intestines and other organs of the roe deer, the presence of acid-fast bacteria in cells with abundant, pale cytoplasm (epithelioid cells) and the poor demarcation of the lesions from the surrounding tissue also indicate paratuberculosis. It possibly indicates such a form of paratuberculosis which in certain cases can occur in sheep and goats.

Lesions in joints and tendon sheaths of rabbits can be ex-

perimentally produced by different mycobacteria, among them also by Mycobact. johnei. *Olikaeva* (1940), among others, have demonstrated such lesions in joints and pleura 12 months after intravenous injection of paratubercle bacilli.

The acid-fast bacteria were not pathogenic for guinea pigs, but showed pathogenic properties for hens. The occurrence of the observed lesions in the hens does not conform to the view generally held on the pathogenicity of Mycobact. johnei. Cases are, however, described where paratuberculous lesions were experimentally produced in hens. *Mohler* (1939) infected chickens with paratubercle bacilli in paraffin oil. Pin-point lesions in the livers and spleens somewhat similar to tuberculosis were observed at autopsy. Microscopic examination showed considerable numbers of acid-fast rods which morphologically corresponded to paratubercle bacilli. *Schaaf & Beerwerth* (1960) observed submilliary paratuberculomas in the livers and spleens of hens which had been inoculated intravenously with paratubercle bacilli.

The lesions in the experimental hens are not unlikely to be of a paratuberculous nature on the basis of the histopathological structure, the methods required to recover the bacteria, and the fact that paratuberculous lesions in certain cases have been experimentally produced in hens.

The fact that paratuberculosis could not be established in the two experimental calves may not exclude Mycobact. johnei as the agent in concern. It is possible that the predisposing factors which further the establishment and development of the disease were lacking. The animals were, for instance, not exposed to the stress which pregnancy and calving constitute. It is also worth noting that the acid-fast bacteria could be recovered — but only on the Taylor/Finlayson medium — from one of the animals nearly three years after the inoculation, which to some degree speaks in favour of Mycobact. johnei. This bacterium is known to be an organism resistant to destruction within the animal body.

The value of allergy tests and serological examinations in the diagnosis of paratuberculosis has been disputed. The results given in Table 3a may not exclude an infection with Mycobact. johnei. The results may in that case be taken as an indication of a preclinical stage of paratuberculosis.

The microscopic structures of the lesions in different organs of the roe deer and the experimental goat, show great similarity

to those which can occur in paratuberculosis of, for instance, sheep and goat.

The nitrate reducing ability of the examined strains showed, with the exception of the bovine strains of tubercle bacilli, greatest similarity to the simultaneously examined strains of Mycobact. johnei.

The isoniazid sensitivity tests showed after three weeks' incubation that strains 101, 68 and those of Mycobact. johnei were nearly equally sensitive, and that they were more sensitive than the examined strains of Mycobact. avium. The described growth type of the avian colonies was observed neither in strains 101 and 68 nor in the two strains of Mycobact. johnei.

In conclusion, I am of the opinion that the disease described here in wild living roe deer and the acid-fast bacteria isolated from these cases, exhibit the greatest similarity with paratuberculosis (Johne's disease) and Mycobact. johnei, respectively. The disease has occurred in a generalized form. The previous view that the infectious agent of paratuberculosis, at any rate in cattle, is restricted to the intestines and associated lymph nodes has now been confuted by many workers. Mycobact. johnei has also been isolated from the tissue of the mammary gland, *Doyle* (1954) and *Schaaf & Beerwerth,* and from milk, *Schaaf & Beerwerth,* of paratuberculous cows.

A rather remarkable observation is, however, that the isolated bacteria proved to be highly pathogenic for hens and to a certain extent for rabbits. I have not been able to find any information in the literature on inoculations into birds and rabbits with paratubercle bacilli isolated from diseased animals with necrotic lesions. It may be suggested that the bacteria isolated from the roe deer possibly constitute a variety of the classic bovine Mycobact. johnei. Two such varieties are known at the present time, namely the pigmented and the Icelandic. Both these varieties can, in my cases, be excluded among other things on account of their growth properties. According to *Taylor* (1951, 1953), they do not grow in primary cultures and first subcultures on Dunkin's medium, which is in contrast to the acid-fast bacteria isolated from the roe deer.

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SUMMARY

A disease in wild living roe deer (Capreolus capreolus L.) caused by acid-fast bacteria is described.

The morphological and cultural properties of these bacteria agree closely with corresponding properties of Mycobact. johnei.

Enlarged lymph nodes, especially mesenteric lymph nodes with greyish-yellow necroses, were the most prominent macroscopic lesions. No intestinal lesions were present.

The histopathological picture of the lymph nodes resembled mostly lesions which can occur in paratuberculous sheep and goats. The epithelioid cells contained masses of acid-fast bacteria. Such rods were also demonstrated in the intestinal villi.

The acid-fast bacteria could be isolated from the mesenteric lymph nodes, spleens, bone marrow, mammary gland and also from the lymph nodes of other organs.

The organism did not produce tuberculosis in guinea pigs. Intravenous injections into hens and rabbits resulted in miliary nodules resembling those of tuberculosis in the livers and spleens of the hens, and in joint and tendon sheath lesions in the rabbits. Microscopically the lesions mostly resembled those in paratuberculosis.

One of the strains which was inoculated intravenously into two calves caused no lesions. The results of the allergy tests and serological blood tests in one animal indicate that infection with Mycobact. johnei cannot be excluded.

A goat which was inoculated in the same manner and with the same strain as the calves died after six weeks. Miliary epithelioid cell granulomas in the liver, spleen, kidneys, bone marrow of long bones and in the submucosa of the ileum, as well as patchy infiltration of epithelioid cells in, among others, the mesenteric lymph nodes were observed on microscopic examinations.

By intravenous infection the disease could be reproduced in a roe deer (fawn) (Capreolus capreolus L.). The animal died nine weeks after the infection, and during the last two days before death it had a profuse diarrhoea. Masses of short acid-fast bacteria in clumps were present in the faeces. Nor in the experimental animal did macroscopic intestinal lesions occur. Enlarged villi infiltrated with epithelioid cells containing acid-fast bacteria were demonstrated by histological examination.

The acid-fast bacteria could be recovered, but only on the Taylor/Finlayson medium, from all experimental animals except the guinea

pigs. Concerning the experimental calves, acid-fast bacteria were recovered from only one of them and then nearly three years after the infection.

The acid-fast bacteria did not reduce nitrate. They showed positive neutral red reactions and were sensitive to isoniazid in a concentration of 2.5 μg per ml medium after three weeks' incubation.

The possibility that the isolated acid-fast bacteria and the lesions caused by them might be avian tubercle bacilli and avian tuberculosis has been discussed by the author. He does not, however, find any relevant reason for such an assumption. The author considers that the bacteria and the lesions exhibit the greatest similarity to Mycobact. johnei and paratuberculosis (Johne's disease).

On account of the organism's pathogenicity for hens and rabbits, and necroses in the course of the disease, the author suggests that these bacteria possibly constitute a variety of the classic bovine Mycobact. johnei, different from the pigmented and the Icelandic varieties.

ZUSAMMENFASSUNG

Eine der Paratuberkulose ähnliche Krankheit beim Reh (Capreolus capreolus L.). Eine ätiologische und pathologisch-anatomische Studie.

Die morphologischen und kulturellen Eigenschaften der Bakterien ähneln denen von Mycobact. johnei.

Die hervortretendsten makroskopischen Veränderungen waren vergrösserte, zum Teil nekrotisierte Lymphknoten — vor allem die Mesenteriallymphknoten. In den Därmen wurden keine Veränderungen festgestellt.

Histopathologisch erinnerten die in den Lymphknoten verkommenden Veränderungen an die, welche bei Paratuberkulose von Schafen und Ziegen auftreten können. Die epitheloiden Zellen enthielten reichlich mit säurefesten Stäbchen. Auch in den Villi der Dünndärme kamen die Bakterien intrazellulär vor.

Abgesehen von den Mesenteriallymphknoten konnten die säurefesten Bakterien auch von anderen Organlymphknoten sowie von Milz, Knochenmark und Euter isoliert werden.

Bei Meerschweinchen riefen diese Bakterien keine tuberkulösen Veränderungen hervor. An Hühnern und Kaninchen führten die intravenösen Injektionen zur Bildung von miliären — an Tuberkulose erinnernde — Herde in Leber und Milz der Hühner sowie zu Gelenk- und Sehnenscheidenveränderungen bei Kaninchen. Diese Veränderungen ähneln histologisch denen bei Paratuberkulose.

Einer der isolierten Stämme wurde an 2 Kälber intravenös verimpft. Die Ergebnisse von Allergie- und serologischen Blutuntersuchungen lassen den Verdacht einer Infektion mit Mycobact. johnei bei einem der Tiere nicht ausschliessen. Im übrigen konnten bei beiden Tieren nach einer Beobachtungszeit von nahe drei Jahren keinerlei Veränderungen festgestellt werden.

Eine Ziege wurde mit dem gleichen Stamm ebenfalls intravenös infiziert. Sie ging nach sechs Wochen ein. In der histologischen Untersuchung wurden miliäre Epitheloidzellgranulome in Leber, Milz, Nieren, Knochenmark der Röhrenknochen und in der Submucosa der Ileums sowie herdförmige Infiltration mit epitheloiden Zellen u. a. in den Mesenteriallymphknoten festgestellt.

Nach intravenöser Infektion konnte die Krankheit an einem Rehlamm (Capreolus capreolus L.) reproduziert werden. Neun Wochen nach der Infektion starb das Tier. Die zwei letzten Tage vor dem Exitus hatte es eine profuse Diarrhöe. In den Fäzes kamen massenweise säurefeste Stäbchen in Haufen vor. Makroskopisch zeigte das Versuchstier keine erkennbaren Darmveränderungen. Histologisch liessen sich jedoch geschwollene, mit epitheloiden Zellen infiltrierte Villi feststellen. Die epitheloiden Zellen enthielten säurefeste Bakterien.

Von den Versuchstieren konnten die säurefesten Bakterien — mit Ausnahme von den Meerschweinchen — auf Taylor/Finlaysons Nährboden wieder gezüchtet werden. Bei den Versuchskälbern liess sich die Reisolierung nur von einem der Tiere vornehmen, und zwar nahe drei Jahre nach der Infektion.

Die säurefesten Bakterien reduzierten Nitrat nicht. Die Neutralrot-Reaktionen waren positiv und für Isoniazid in einer Konzentration von 2,5 μg/ml Nährboden waren sie nach drei Wochen Inkubation empfindlich.

Der Verfasser erörtert die Möglichkeit, dass es sich bei den von ihm isolierten säurefesten Bakterien und den durch sie hervorgerufenen Gewebsveränderungen um aviäre Tuberkelbazillen bzw. aviäre Tuberkulose handelt. Für diese Annahme kann er jedoch nicht genügend Anhaltspunkte finden, sondern seines Erachtens nach haben die Bakterien und die Veränderungen die grösste Ähnlichkeit mit Mycobact. johnei bzw. Paratuberkulose (Johne's disease).

Auf Grund der pathogenen Eigenschaften der Bakterien für Hühner und Kaninchen sowie der vorkommenden Nekrosen fragt sich der Verfasser, ob es sich bei diesen um eine Variante des klassischen bovinen Typs von Mycobact. johnei handelt, die von der pigmentierten und der isländischen Variante abweicht.

SAMMANFATTNING

En sjukdom liknande paratuberkulos hos rådjur (Capreolus capreolus L.). En etiologisk och patologisk-anatomisk studie.

Bakteriernas morfologiska och kulturella egenskaper överensstämmer närmast med motsvarande egenskaper hos Mycobact. johnei.

De mest framträdande makroskopiska vävnadsförändringarna utgjordes av förstorade lymfkörtlar, framför allt förstorade mesenteriallymfkörtlar med grågula nekroser. Inga tarmförändringar förekom.

Lymfkörtlarnas vävnadsförändringar erinrade histologiskt närmast om de, som kan förekomma vid paratuberkulos hos får och get. De epiteloida cellerna innehöll massor med syrafasta bakterier. Så-

dana intracellulärt belägna bakterier förekom också i bl.a. tunntarmarnas villi.

De syrafasta bakterierna kunde förutom från mesenteriallymfkörtlar även isoleras från andra organlymfkörtlar samt från mjältar, benmärg och juver.

Mikroorganismerna framkallade icke tuberkulos hos marsvin. Intravenösa injektioner på höns och kaniner resulterade i miliära om tuberkulos påminnande härdar i hönsens levrar och mjältar och i led- och senskideförändringar hos kaninerna. Dessa förändringar påminde histologiskt närmast om de vid paratuberkulos.

En av stammarna, som inokulerades intravenöst på två kalvar, framkallade inga vävnadsförändringar hos dessa. Hos ett av djuren visade allergitester och serologiska blodundersökningar resultat, vilka icke kan utesluta en infektion med Mycobact. johnei.

Get som inokulerades på samma sätt och med samma stam som kalvarna, dog efter sex veckor. Mikroskopiskt konstaterades miliära epiteloidcellsgranulom i lever, njurar, rörbenens benmärg och i ileums submucosa samt härdformig infiltration av epiteloida celler i bl. a. mesenteriallymfkörtlar.

Sjukdomen kunde efter intravenös infektion helt reproduceras på rådjurslamm (Capreolus capreolus L.). Detta dog nio veckor efter infektionen och hade de två sista dygnen före döden en profus diarré. I tarmuttömningarna förekom massvis med korta syrafasta bakterier i hopar. Makroskopiskt iakttagbara tarmförändringar förelåg icke heller hos försöksdjuret. Mikroskopiskt avslöjades dock ansvällda villi infiltrerade med epiteloida celler innehållande syrafasta bakterier.

Från försöksdjuren, med undantag av marsvinen, kunde de syrafasta bakterierna återvinnas på Taylor/Finlaysons substrat. Beträffande försökskalvarna dock endast från ena djuret och då nära tre år efter infektionen.

De syrafasta bakterierna reducerade icke nitrat. De visade positiva neutral-rött-reaktioner och var känsliga för isoniazid i koncentrationen 2,5 μg/ml substrat och efter tre veckors inkubering.

Författaren diskuterar möjligheten av att de syrafasta bakterierna och de av dem framkallade vävnadsförändringarna skulle kunna vara aviära tuberkelbakterier resp. aviär tuberkulos. Han finner dock inga relevanta skäl för sådant antagande. Bakterierna och vävnadsförändringarna anser författaren ha största likhet med Mycobact. johnei resp. paratuberkulos (Johne's disease).

På grund av mikroorganismernas patogena egenskaper för höns och kaniner samt sjukdomens förlöpande med nekroser frågar sig författaren, huruvida dessa bakterier möjligen utgör en variant av den klassiska bovina Mycobact. johnei, avvikande från den pigmenterade och den isländska varianten.

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