MORPHOPATHOLOGY OF CAPRINE TUBERCULOSIS. I. PULMONARY TUBERCULOSIS

Estudio morfológico de la tuberculosis caprina. I. Tuberculosis pulmonar

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RESUMEN

Las lesiones tuberculosas fueron encontradas en los pulmones de 35 cabras procedentes de distintos rebaños de la Región de Murcia (España). Los animales fueron seleccionados por dar positiva la prueba de la intradermorreacción tuberculínica comparativa. El análisis bacteriológico reveló la presencia de *Mycobacterium bovis* en 10 animales y *Mycobacterium tuberculosis* en un animal. Además de las lesiones pulmonares se encontraron lesiones en otros órganos y en diferentes gánglios linfáticos, dependiendo del grado de generalización de la enfermedad. La fase de complejo primario se encontró en 5 animales (14.28%); de generalización precoz en 12 animales (34.29%); de tuberculosis orgánica crónica en 14 animales (40%); de generalización tardía en 5 animales (14.28%). La tuberculosis caprina tiene una alta incidencia y distribución en la Región de Murcia y es un importante factor de riesgo en la transmisión de la enfermedad al hombre.

Palabras clave: Tuberculosis, Cabra, Pulmón.

SUMMARY

Tuberculous lesions were found in the lung of 35 goats from the Region of Murcia (Spain). The animals

were selected because of a positive reaction to de CTID test. Bacteriological analysis allowed the isolation of *Mycobacterium bovis* (10 animals) and *M. tuberculosis* (1 animal). In addition to lung lesions were found in other organs and different types of lymph nodes, depending on the degree of generalization of the disease. A primary complex was found in 5 animals (14.28%); early generalization was found in 12 animals (34.29%); organic-chronic tuberculosis was found in 14 animals (40%); and late generalization in 5 animals (14.28%). These data reveal that caprine tuberculosis has a high incidence in the Region of Murcia, thus indicating a potential risk of transmission of the disease to man.

Key words: Tuberculosis, Lung, Goat.

INTRODUCTION

Tuberculosis is a chronic-course infectious-contagious disease affecting humans, pets and other animals both in the wild and in captivity, as well as birds and poultry (McFADYEAN, 1888; SCHLIESSER, 1973). It is caused by bacteria of the species *Mycobacterium tuberculosis, M. bovis* and *M. avium*.

Farm animals and pets affected most frequently by the disease are cattle and chickens, followed by swine, goat, horse, sheep, dog and cat (HUTYRA *et al.* 1973; SCHLIESSER, 1973; CUEZVA, 1973; MEISSNER, 1973; PRITHCARD, 1988). MEISSNER (1973) reports that cattle are responsible for the spread of tuberculosis to other wild and domestic animals. THOREL (1984) and JUBB *et al.* (1985) suggest that goats offer little resistance both bovine and avian type tubercle bacilli. BELLER and EHRENREICH (1941) report that goats are highly receptive to an artificial infection with human type tubercle bacilli, which may give rise to tuberculous mastitis (McFADYEAN, 1917; JOUBERT, 1975) thus constituting a serious health hazard for man as a result of consumption of milk and fresh cheese (GRIFFITH, 1913; GOLDEN, 1921).

HUTYRA *et al.* (1973) and SCHLIESSER (1973) report that infection in goats mainly takes place through the repiratory tract, and may occur as a result of contact with sick goats or tuberculous cattle. Infecction the digestive system is relatively rare (PALLASKE, 1961), though it is found in young animals having consumed goat's milk contaminated by tuberculous mastitis (SOLIMAN *et al.*, 1953) or infected cow's milk (DAVENAS and DABRIGEON, 1955)

Clinical diagnosis of this disease is not easy to establish (SAVEY, 1980), and an anatomopathological diagnosis is necessary (AMARA and BEN SAID, 1981; PRITCHARD, 1988). The comparative tuberculin intradermo-reaction test (CTID) has the same value and limitations as in cattle (THOREL, 1984).

Caprine tuberculosis has been reported in France (DAVENAS and DABRIGEON, 1955; THOREL, 1980; PERRIN *et al.*, 1984), Germany, USA (BILLON, 1944), England, Ireland, Uganda (SOLIMAN *et al.*, 1953; LESSLIE *et al.*, 1960), Tanganyka (MILNE, 1955), Bulgaria (SAVOV, 1974), Italy (TRADATI *et al.*, 1976) and India (KAKKAR *et al.*, 1977).

Given that goats constitute a considerable source of transmission of tuberculosis, and that traditionally —even today— goats are considered to be particularly resistant, the purpose of this study is to establish the anatomopathological characteristics of pulmonary tuberculosis in goats and their correlation with the CTID test as a diagnostic method, and to highlight the zoonotic, sanitary and importance of the disease in the Region of Murcia.

MATERIALS AND METHODS

A total of 34 Murciano-Granadina goats aged between 3 and 11 years, and one kid aged 3-4

TABLE 1

DISTRIBUTION OF TUBERCULOSIS INFECTION WITH MACRO AND MICROSCOPIC LESIONS IN 35 GOATS CORRELATED WITH TUBERCULIN TEST REACTION

Animal N.º	Age (year)	Lung	Mediastinal Lymph node	Spleen	Liver	Intestine	Mesenteric lymph node	Other Lymph nodes	Other Organs	Tuberculin test bovine	avian	Type of Tuberculosis
1	4		TB	TB						+3	+4	2
2	4	TB	TB							+1	+4,5	1
3	4	TB	TB		TB		TB	i.s		+13	+4	3
4	5	TB	TB		+/+	PTB	+/+			+5	+3	3
5	5	TB	TB		TB		PTB	PTB	i.s	+11	+7	3
6	4		TB			PTB				+6	+1	1
7	3	TB	TB	TB	PTB	PTB	+/+			+12	+2	2
8	3	TB	TB		+/+	PTB		TB2,3				2
9	8	TB	TB	TB	TB					+5	0	4
10	4	TB	TB		PTB	PTB		TB1	K			4
11	4	TB	TB			PTB		TB3				4
12	3	TB	TB	TB	+/+	PTB	TB	TB2/i.s		+10	+2	3
13	4	TB		TB	+/+	PTB	TB	i.s				4
14	3	TB			PTB	PTB	PTB	i.s.		+3	+2	3
15	6	TB			PTB	PTB		i.s		+5	0	1
16		TB	TB		PTB	PTB	PTB	i.s		0	+1	3
17	3	TB	TB	TB			i.s.			+6	+3	2
18		TB	TB			PTB	i.s	TB4/i.s				4
19		TB			TB	PTB	PTB	TB3/i.s		+6	+2	2
20	5	TB	TB		+/+	PTB	PTB	TB3/i.s		+8	+2	2
21	5	TB	TB		PTB	PTB	PTB					3
22	5	TB	TB		+/+	+/+	TB					3
23	3	TB	TB		TB	TB						3
24	7	TB										3
25	6	TB	TB			PTB	+/+	i.s				3
26	5	TB	TB		TB	PTB						3
27	7	TB	TB		PTB	TB	+/+			+10	+6	2
28	6	TB	TB		+/+	+/+	+/+	TB1		+9	+5	2
29	8	TB	TB		PTB	PTB	+/+			+10	+5	3
30		TB	TB	TB	TB	TB	TB	i.s	H, K, D			3
31		TB	TB			TB	TB					3
32	11		TB									1
33	5	TB	TB							+10	+3	2
34	3	TB	TB							+3	+7	1
35		TB	TB		PTB	+/+	PTB					4

TB: Tuberculosis lesion. PTB: Paratuberculosis lesion. +/+: TB and PTB lesion. TB1: TB in Retropharyngeal lymph node. TB2: TB in Prescapular lymph node. TB3: TB in Sternal lymph node. TB4: TB in Subiliac lymph node. H: TB in Heart. K: TB in Kidney. D: TB in Diaphragm. i.s: increased size. Type of Tuberculosis: 1 = Primary complex; 2 = Early generalization; 3 = Organic-Chronic; 4 = Late generalization.

months (animal n.º 30) were used for this study (Table 1). All the goats were from the Region of Murcia (districts of Jumilla, Calasparra, Caravaca, Mula, Alcantarilla and Campos del Río), and their origin as follows: 19 animals were selected from two herds comprising a total of 251 goats, all of which were subjected to the CTID test (THOREL and GAUMONT, 1977) using avian and bovine tuberculin; 77.2% of these animals reacted positively according to the criteria of DEDIE (1973). The other 16 animals were taken from other herds which had also reacted positively to avian and bovine tuberculin (Table 1). Clinical signs in most cases consisted of respiratory distress and a hoarse cough together with generalized weight loss and decreased milk production.

After external examination animals were anaesthetised with penthotal and sacrificed by puncture of the jugular. Following necropsy, samples were taken for processing using routine light microscopic techniques. Samples for the structural study were stained according to the haematoxylin-eosin (H-E), van Giesson and Ziehl-Neelsen procedures.

Criteria for classifying the different phases of tuberculosis are those proposed by KITT and SCHULZ (1985) in cattle, due to the resemblance of lesions between goat and cattle.

The bacterial analysis carried out at the laboratorio de Sanidad y Reproducción Animal in León was based on samples of lung, mediastinal lymph nodes, liver and kidney of four animals. Samples were tested by direct focal impression and stained by Ziehl-Neelsen prior to being ground and macerated. After decontamination treatment samples were centrifuged and inseminated in special culture medium (STONEBRINK, 1958). In addition Guiea-pigs were experimentally inoculated with the macerated matherial from the sampled organs. Metabolic tests using growth cultures identified the bacteria as Myc*obacterium bovis* in all samples except two in which perhaps the presence of *Bacillus subtilis* and other bacteria precluded the growth of *M. bovis*. Of 20 animals analysed at the laboratory de Sanidad in El Palmar (Murcia), *M. bovis* was isolated in 6 and human-type *M. tuberculosis* in one (individual n.º 33, Table 1), using the above mentioned techniques as well as gas chromatography, thin-layer chromatography and various biochemical tests (nicotinic acid production, nitrate reduction and sensitivity to T.C.H), which allowed the two types to be differentiated.

RESULTS

Microscopic analysis of section stained with H-E revealed tubercular granulomas; the Ziehl-Neelsen technique identifying in all cases an acid-fast bacillus within giant-type cells (Fig. 1). In two lung samples and one mediastinal lymph node sample numerous bacterial forms were found in cells epithelioid cells or in clusters within caseous matter.

FIGURE 1. Giant Langhans-type cell with an acid-fast bacillus (arrow). Ziehl-Neelsen. Bar= 9.2 µm.

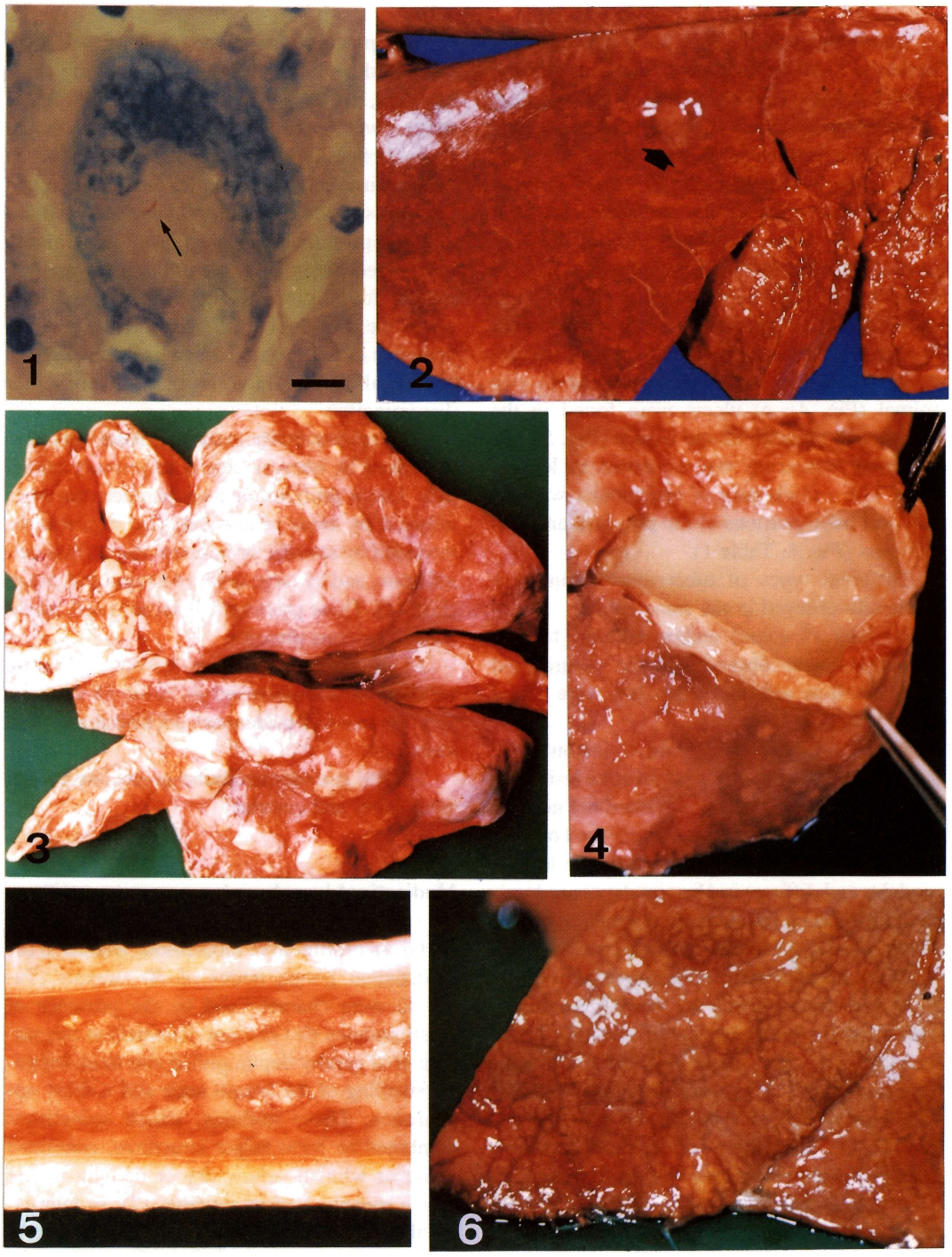
FIGURE 2. Principal lobe of the lung with walnut-sized nodule (arrow). Primary complex.

FIGURE 3. Pulmonary parenchyma with large-nodule tuberculosis.

FIGURE 4. Organic-chronic tuberculosis with cavernae in principal lobes.

FIGURE 5. Organic-chronic tuberculosis with ulcers in trachea.

FIGURE 6. Late generalitation with caseous lobar tuberculous peumonia.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Lung and pleura

Gross lesion in the lung took the following forms. a) small yellowish-white nodular formations of 0.3-0.4 cm, located throughout the lung. These nodules, which squeaked when cut, and contained a yellowish-white doughy substance, were found in four animals; b) 0.5-2.3 cm diameter nodules with similar characteristics were found in the more ventilated areas of the lung (dorsal portion of the principal lobes) in 18 animals (Fig. 2); c) a small number of 4-5 cm diameter nodular formation were found in similar locations in three animals (Fig. 3); d) cream-white filled cavernae were observed in one animal (Fig. 4); e) ulcers were found in the trachea and bronchii of one animal (Fig. 5); f) a yellowish colouring was observed in lobular areas with a thickening of interlobular walls in the most basal portions of the principal and apical lobes (Fig. 6, Table 1).

Different types of tubercular lesion were frequently obseved in the same animal.

Nodular (2 cases) and/or villous (1 case) formations were observed in the pleura, and whitish-yellow subpleural plaques were found in one case.

Three types of microscopic lesion were found: a) predominantly *proliferative* lesions in 11 animals, two of which exhibited the nodular manifestation of tubercular pleuritis and one the villous form; b) *proliferative-exudative* lesions (nodular and diffuse) in 15 animals; c) predominantly *exudative* lesions in 6 animals, in one of which a caseous pleuritis was observed.

The predominantly *proliferative* form (Figs. 7, 8) was charecterised by small granulomas with a small amount of central necrosis due to secondary caseation. These calcified granulomas were surrounded by epithelioid cells, a few giant Langhans-type cells, lymphocytes and connective capsule, for which the degree of development depended on the phase of the disease. Circular granulomas were observed in the pleura, together with villous formation the form of papillary outgrowths of fibroblasts an epithelioid cells.

The *proliferative-exudative* form was characterised by large necrotic, and in some cases calcified, masses due to caseation (Fig. 9). These masses were enlarged by the inclusion of smaller peripherally located granulomas composed of a diffuse infiltrate of epithelioid cells, giant Langhans-type cells and lymphocytes, surrounded by proteinaceous sustance, presumably coagulated plasm (Fig. 10). This form was generally found close to the bronchii, and may affect the bronchial wall, giving rise to the appearance of large cavernae and subsequently to bronchial ulcers, facilitating intracanilicular dissemination.

The predominatly *exudative* form was characterised by necrosis due a to primary caseation. Large portions of lung parenchyma were affected by areas of non-calcified necrosis surrounded by specific or non-specific diffuse cell infiltrate, and by large areas of coagulated plasm (Fig. 11). These processes, if accentuated, might lead to caseous pleuritis.

In addition tubercular lesions, parasitic pneumonia was observed in 10 of 35 animals, and two cases of pulmonary adenomatosis were identified.

Pleural granulomas found in just one animal were small and circular.

Mediastinal lymph nodes

Gross proliferative tuberculous lesions in the mediastinal lymph nodes of 30 animals (Table 1) were characterised by the presence of small nodules (0.1-0.5 cm diameter) at the surface or at greater depth, giving rise to an irregular swelling of the lymph node and a shiny appearance. These nodules, which sometimes combined to form larger structures, contained white or whitish-yellow dry or doughy matter which squeaked when cut (Fig. 12). In four cases the whole lymph node was affected.

Microscopic analysis revealed these nodules to be granulomatous formations with necrotic calcified centres surrounded in 11 animals by a well-developed connective capsule; in 8 animals, the capsule was poorly developed. Other nodules contained cells clusters without necrotic area.

Exudative lesions were observed in two cases, and were characterised by the appearance of a diffuse infiltrate of epithelioid cells and giant cells of the Langhans type which subsequently underwent primary caseation not accompanied by calcification.

In one case, granulomas were found in the lymph node serosa. They were perfectly formed, with a necrotic calcified centre surrounded by epithelioid cells, some giant cells, lymphocytes and a strong connective capsule.

In addition to these lesions, an amyloid substance formed by small deposit among lymphocytes was found in the cortex of three lymph nodes.

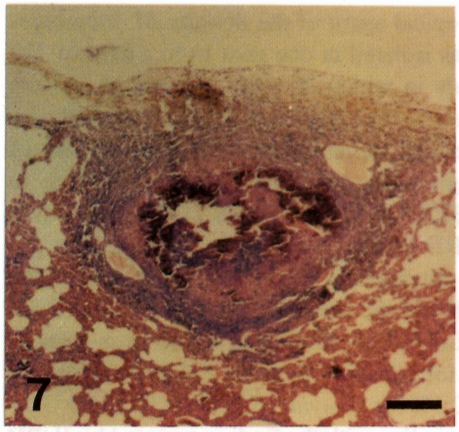
There is no direct relationship between the age of the animals and the frequency and location of the different signs.

DISCUSSION

Incidence of tuberculosis in goats. This is the first report of anatomopathological findings in naturally acquired tuberculosis in Murciano-Granadina goats in the Region of Murcia. The results obtained, taken in conjuntion with those obtained using CTID, confirm that tuberculosis is one of the most important disease in goats in this Region. It has hitherto been virtually unnoticed, ignored or confused with other infectious or parasitic disease frequent in goats. The high number of animals that reacted to bovine and avian tuberculin (195 animals), and the geographic dispersion of herds, suggest that the disease is so widespread that the problems it poses are therefore more serious than previously thought.

Aetiological agents. According to the bacterial diagnosis, *M. bovis* was isolated in 10 goats from different herds, and was the main aetiological agent of the disease. *M. tuberculosis* was isolated in one goat from a herd of 222 animals in which 29 were selected at random and tested for CTID and 25 reacted positively. These data suggest that there is a high risk of contagion for the human being according to JOUBERT (1975), THOEN (1988) and PRITCHARD (1988), which may account for a positive intradermal of the tuberculin test in one member of the breeder's family. We agree with SCHLIESSER (1973) that *M. bovis* is responsible for infection in goats (97.9%), compared with 2.1% for *M. avium* (GRIFFITH, 1936). This is also in line with the finding reported by JUBB *et al.* (1985) in cattle «in areas of high incidence, the disease is almost exclusively caused by *M. bovis*». The involvement of *M. avium* cannot be asserted with certainty, although the presence of numerous bacterial forms in epithelioid cells and cheesy matter in histological section of lung and lymph node parenchyma taken from two animals with tuberculosis, which were not diagnosed bacteriologically, might confirm the finding reported by JUBB *et al.* (1985).

Stages and signs of the pulmonary tuberculous disease. Lesions found in caprine tuberculosis are similar to those found in cattle (NIEBERLE, 1938; NIEBERLE and KOHRS, 1961; PALLAKE, 1961; KITT and SCHULZ, 1985; JUBB *et al.*, 1985). In lung there are macroscopic findings of lesions in the form of nodules of 0.3-0.4 cm (2 cases) or of a variable size (1 case) in the principal lobe and the caudodorsal portions. The simultaneous presence of adenopathy in tracheal and/or mediastinal lymph nodes suggests the ocurrence of *primary complex*, in accordance with the terminology proposed by KITT and SCHULZ (1985) in cow. Tuberculous granulomas were found in the pharynx and in satellite (retropharyngeal) lymph node in 2 cases, but this is not indicative of the route of infection reported by JUBB *et al.* (1985) in cattle.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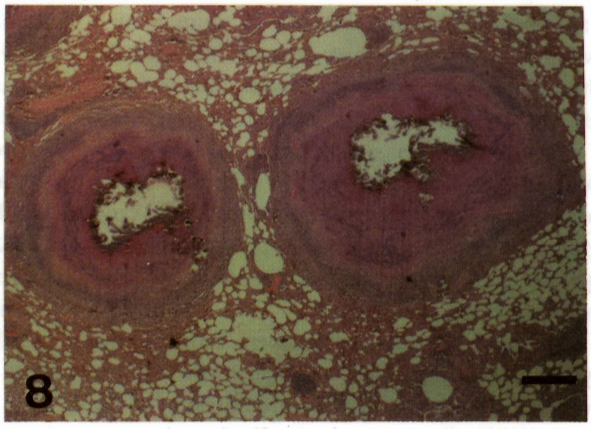
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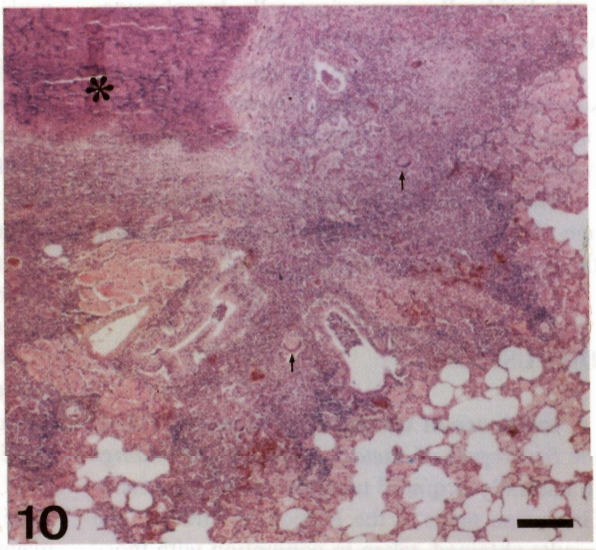
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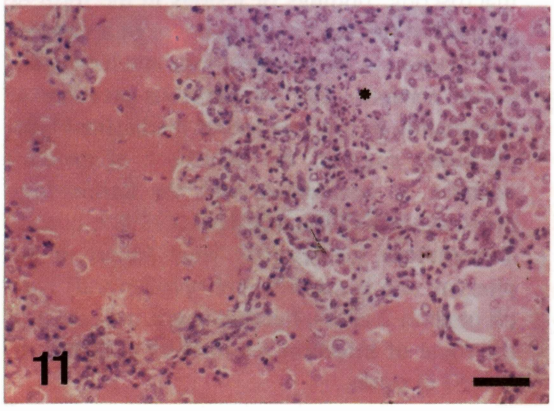
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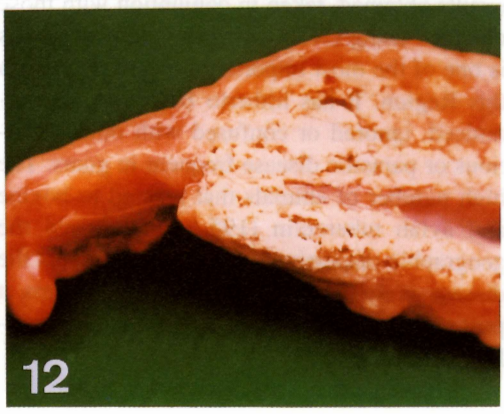
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Microscopical analysis revealed in five animals subpleural or intraparenchymal proliferative tuberculous granulomas with central calcification, characteristic of the primary complex. In three of these animals, lesions were incomplete or had passed unnoticed, since this type lesion is poorly developed and difficult to detect (two cases showed no lung lesion, and the third showed no lymph node lesion). These facts might explain the scarce incidence and detection of the primary complex in goat, in accordance with the repor by PALLASKE (1961); in cattle, it seems to be also difficult to detect (JUBB *et al.*, 1985).

Nine animals showed signs of a stage more advanced than primary complex, with lesion taking the form of nodules of 0.3-0.4 cm in two cases: *early generalization*. In the course of this phase the germ is supposed to spread along a lymphohaematogenous route back to the lung and to other organs including the liver, spleen and intestine (FRANCIS, 1947,1972). This seems to have occurred in 6 animals.

The early generalization stage is divided into other stages according to the type of proliferative lesion of lungs, presumed route of infection, etc. Of these stages, *miliary tuberculosis* was found in two goats, a low incidence already reported by PALLASKE (1961). Gross lesions consisted of 0.3-0.4 cm diameter granulomas scattered throughout the pulmonary parenchyma, mainly in the more basal areas of the principal lobes. Microscopic analysis revealed granulomas with necrotic centres that were not always calcified. *Slow-early tuberculosis* was observed in four goats; the most marked feature both macro and microscopically was the joint presence of old and young proliferative granulomas ranging from 0.5 to 2.5 cm diameter. *Large-nodule tuberculosis* was only found in one case, and was characterised by granulomas larger than those described previously, though sharing the same histological features. *Tuberculosis of serosas* is, according to KITT and SCHULZ (1985), due to the use of retrograde lymphogenous route of infection, and was observed in two animals. It took the form of villous outgrowths in the pleura of one animal and the classic «pearly» form in the other.

After the stages of early generalization there is a *postprimary period* which seems to be almost exclusive to cattle and rare in goat and pig. In this study, however, it was found in 15 cases (42.8% of all lung lesions). Of these, 8 presented proliferative lesions in other organs thus corresponding to processes derived from the provious phase. Twelve goats had lesions constituting *acinous-nodular tuberculosis*, giving a percentage coinciding with that of PALLASKE (1961). *Cavernae* were found in one goat and *tracheal* and *bronchial ulcers* in another. Most authors suggest that this phase co-

FIGURE 7. Subpleural proliferative lesion in a primary complex. H-E. Bar = 50 mm.

FIGURE 8. Miliary tuberculosis in pulmonary parenchyma. H-E. Bar = 100 mm.

FIGURE 9. Organic-chronic tuberculosis with proliferative-exudative lesion.

FIGURE 10. Microscopic proliferative-exudative lesion with caseation central (*), surrounded by diffuse infiltrate of epithelioid cells, giant Lnghans-type cells (arrow), lymphocytes and areas of intra-alveolar plasma coagulation. H-E. Bar= 100 mm.

FIGURE 11. Exudative lesion with non-calcified necrosis (*) surrounded by no-specific diffuse cell infiltrate and areas of plasma coagulation. H-E. Barr= 20 mm.

FIGURE 12. Mediastinic lymph node with large areas of caseation and calcification.

rresponds to a process of reinfection, a decrease in organic defences or the exacerbation of the germ's virulence. Macroscopical characteristics differ from those described above because of the typical location in organs and the route of infections used, which is intracanicular and/or by continuity. The lymphohaematogenous route is not used, as it is in cattle, where infection begins as primary and becomes post primary (FRANCIS, 1947, 1972; JUBB *et al.*, 1985). Acinous-nodular tuberculosis in goats is caudodorsally located in the principal lobes. The generally peribronchial micoscopic lesion was proliferative-exudative in nature though granulomas of different sizes had calcified centres, unlike those described in cattle by authors such as JUBB *et al.* (1985). The process is supposed to continue with the formation of larger nodular granulomas, which in most cases destroyed the bronchial wall giving rise to the formation of cavernae, which favours intracanicular dissemination within the organ. Thence, germs may reach the larger bronchii and trachea, giving rise at times to lesions in the mucosa and forming bronchial and tracheal ulcers. This type of open tuberculosis may, due to deglution of caseum, tead to intestinal tuberculosis which was also found in goats. If lymph nodes are affected during this phase lesions are old and correspond to the primary complex and/or the early generalization phase (KITT and SCHULZ, 1985).

And advanced stage to post-primary period is known as *late generalization*, or the rupture phase. It was observed in 6 animals which showed evidence of *caseous lobular tubercular pneumonia* characterized by typically pneumonic lesions, accompanied in three of them by the presence of 0.5-2.5 cm diameter nodules. Pneumonic lesions were located in the more ventral and less ventilated portions of the lung (anterior and principal lobes), and appeared as small focal areas of softening, with dry caseation and no sign of encapsulation. These findings are in agreement with those of KITT and SCHULZ (1985) in cattle, but differ in that no areas of emphysema were found, a fact perhaps due to the sacrifice of the animals used in this study. *Caseous pleuritis* was found in one case. Lymph nodes in this phase share similar characteristics with regard to caseation and the absence of calcification. Additionally, one case of miliary dissemination in lung and kidney was found, which has been described for this phase in cattle by NIEBERLE (1938), NIEBERLE and COHRS (1961) and KITT and SCHULZ (1985). Gross and microscopic alterations in the lung are predominantly of an exudative nature in goat in agreement with LUKE (1958) and SCHLIESSER (1973).

Microscopic analysis of *mediastinal lymph nodes* revealed two types of lesion. The first of these was a fibrocalcified parenchymatous and/or capsular lesion. Lesions to the parenchyma took the form of granulomas with strong connective tissue in 11 cases, of which the connective capsule did not perfectly delimit the granuloma in 8 animals. The capsular lesion, observed in only one case, was miliary and proliferative with calcified centres. These signs correspond to the stage of primoinfection. There are other two cases of lymph node lesion with small tuberculous granulomas that were not clearly defined but formed large necrotic and non-calcified areas. One of these cases corresponded to the gross lesions of late generalization termed «radiated caseation». Technical failures did not allow microscopical diagnosis in 8 cases.

In conclusion, this study has revealed that caprine tuberculosis has a high incidence in the Spanish Region of Murcia, and that the use of avian and bovine tuberculine may be an efficient tool for its diagnosis. Macroscopic and microscopic lesions in goats are similar those described in cattle thus making suitable their categorization using the criteria of KITT and SCHULZ (1985). Pulmonary lesions are more frequent than in other organs. These results must encourage a more detailed study of caprine tuberculosis now almost resticted to cattle, as goat may be a high

risk vehicle of transmission of tuberculosis to man.

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