

MORPHOPATHOLOGY OF CAPRINE TUBERCULOSIS. II. GENERALIZATION OF TUBERCULOSIS

Estudio morfológico de la tuberculosis caprina. II. Tuberculosis generalizada

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Recibido: 27-9-90

Aceptado: 17-10-91

RESUMEN

La forma generalizada de la tuberculosis se encontró en 24 de las 35 cabras diagnosticadas de tuberculosis pulmonar. Las lesiones de generalización precoz se observaron en bazo (7 animales), hígado (12 animales), intestino (7 animales), corazón (1 animal) y riñón (1 animal), mientras que la fase de generalización tardía sólo se demostró en un caso en riñón. Los ganglios linfáticos de los órganos afectados siempre presentaron las mismas lesiones. Los ganglios linfáticos superficiales estaban afectados en 17 animales. Microscópicamente todos los animales demostraron granulomas tuberculosos característicos de la enfermedad.

Palabras claves: Tuberculosis generalizada. Cabra.

SUMMARY

Generalization of tuberculosis was found in 24 of 35 goats in which pulmonary tuberculosis was diagnosed. Lesions of early generalization were found in spleen (7 animals), liver (12 animals), intestine (7 animals), heart (1 animal) and kidney (1 animal), whereas lesions of late generalization were found only in one case in kidney. The lymph nodes of the affected organs and frequently the superficial lymph nodes (17 animals)

showed lesions. Microscopically all animals presented the tubercular granulomas which characterised this disease.

Key words: Generalization tuberculosis. Goat.

INTRODUCTION

Generalization of tuberculosis can occur early or late in the course of the disease. In the first case it occurs through the evolution of primary complex towards early generalization; the germ spreads along a lymphohematogenous route back to the lung and other organs including the liver, spleen and intestine (DAVENAS and DABRIGEON, 1955; FRANCIS, 1972; SCHLIESSER, 1973; PERRIN *et al.*, 1984; AMARA and BEN SAID, 1986). Organic-chronic tuberculosis corresponds to a process of reinfection, a decrease in organic defenses or the exacerbation of the germ's virulence of primary complex (KITZ and SCHULZ, 1985; PRITCHARD, 1988).

Late generalization (or the rupture phase) occurs when the immunity that the animal has acquired breaks down, and the germs use the lymphohematogenous route for organic dissemination (JUBB *et al.*, 1985).

The purpose of this study is to establish the anatomopathological characteristics of the tuberculosis in goat that affects organs other than lung and their correlation with the CTID test as a diagnostic method, and to highlight the zoo-

notic, sanitary and economic importance of the disease in the region of Murcia (Spain).

MATERIAL AND METHODS

The study was carried out in 35 goats of the Murciano-Granadina breed which were positive to the CTID test and were examined for pulmonary tuberculosis (BERNABÉ *et al.*, 1991b). Of these animals 24 were shown to have disseminated tuberculosis. Details of the procedures and criteria for classifying the phases of the disease are reported in that paper.

RESULTS

Intradermal reaction to avian tuberculin was poor or average in these animals while reaction to bovine tuberculin varied from moderate to intense. *Microscopic examination* of sections stained with H-E revealed tubercular granulomas, and the Ziehl-Neelsen technique showed in all cases only one acid-fast bacillus within giant Langhans-type cells.

FIGURE 1. Spleen with nodules of millet-size and 2-3 cm diameter in an early-generalization stage.

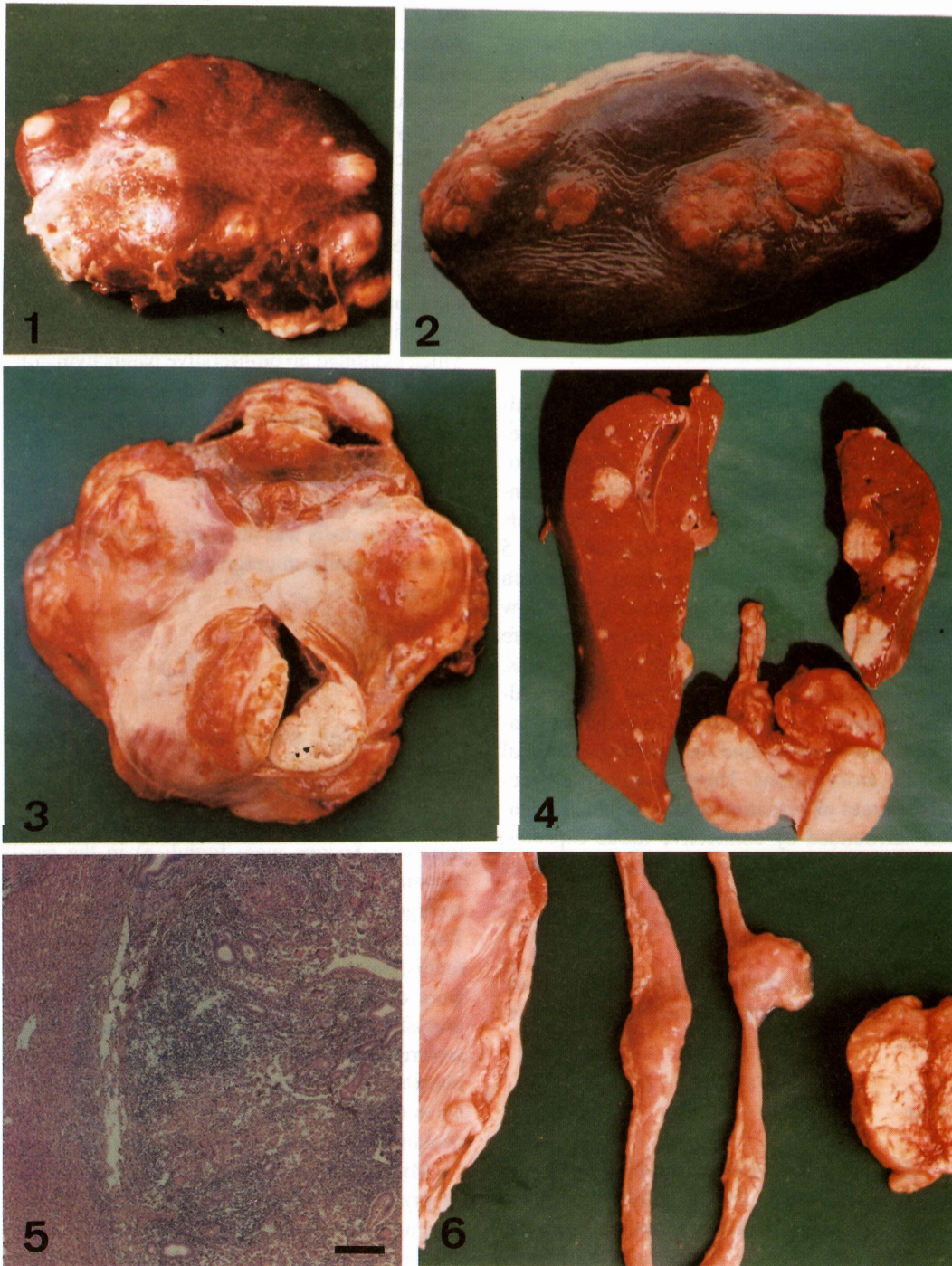
FIGURE 2. Spleen with large and irregular nodules in an early generalization stage.

FIGURE 3. Spleen with large-nodule tuberculosis. Early generalization.

FIGURE 4. Liver with miliary and pea-sized nodules of early generalization. Splenic and hepatic lymph nodes are also affected.

FIGURE 5. Proliferative portal lesions in the early-generalization stage. H-E. Bar= 100 µm.

FIGURE 6. Focal saccule-like enlargement of intestinal diameter in a primary complex.



Superficial lymph nodes

Gross lesions were observed in retropharyngeal, prescapular and/or subiliac lymph nodes that were enlarged and exuded a clear liquid after cut in 16 goats. Whitish-yellow cheesy masses, which squeaked when cut, were noted in retropharyngeal and/or prescapular lymph nodes in 3 animals, sternal lymph nodes in 4 animals, and in the subiliac lymph node in 1 animal (BERNABÉ *et al.*, 1991b, Table 1).

Spleen

Macroscopic lesions were observed in 6 animals (BERNABÉ *et al.*, 1991b, Table 1). Lesions consisted of: a) 0.3-0.4 cm diameter nodules in three animals; b) large, irregular, rounded nodules (2-3 cm diameter) in two animals (Figs. 1, 2); and c) very large nodules (up to 5 cm diameter) in one animal (Fig. 3) which squeaked when cut and contained whitish-yellow dry or doughy matter. Villous outgrowths were found in the capsule of one of the above animals.

Microscopic lesions consisting of fibrocalcified granulomas were observed in the six goats with gross lesions and one additional animal with no gross lesions. These contained characteristic areas of caseation with central calcification surrounded by connective tissue capsules, which was incomplete in five animals, though swollen in another. Lesions in the capsular serosa were similar to those observed in the pulmonary serosa.

In three cases, deposits of amyloid substance were observed peripherally to splenic corpuscle.

Liver

Tubercular lesions were observed in 12 goats (BERNABÉ *et al.*, 1991b, Table 1) but were not grossly apparent in 4 of them. In 5 animals miliary nodules were distributed uniformly

throughout the liver; two other animals exhibited 0.5 cm diameter nodules surrounded by a connective tissue capsule (Fig. 4), while an eighth animal had a small number of large nodules (up to 5 cm diameter) containing yellowish doughy matter which squeaked when cut.

Microscopic analysis revealed two distinct lesions: a) parenchymatous proliferative lesion in 11 goats consisting of perfectly formed granulomas, which in 7 of them were surrounded by smaller or resorptive granulomas; b) additional portal proliferative-exudative lesion found in 2 of these 7, and in one other goat which exhibited no proliferative parenchymatous lesions. The portal lesion was distinguished by a typical cell infiltrate, which subsequently underwent necrosis due to caseation (Fig. 5). Partially calcified caseous masses appear in interlobular bile duct lumina.

Distomatosis was observed in 2 animals, and paratuberculous microgranulomas were found in 6 others.

Intestine

Microscopic tubercular lesions were observed in 7 of the goats studied (BERNABÉ *et al.*, 1991b, Table 1), although gross evidence was present in only 3: two adults and one kid. The following lesions were found: a) in the small intestine of the kid, a focal saccule-like dilation/enlargement of intestine diameter (Figs. 6, 7). The mucosa proved thickened when cut, was reddish in colour and showed erosions which were overlaid with whitish doughy matter; b) greyish-white shiny nodules of varying sizes protruding from the luminal surface of the small intestine; c) whitish 0.3-0.4 cm diameter nodules in the cecum, which squeaked when cut and contained a yellowish doughy material.

Microscopic analysis differentiated tubercular granulomas in the mucosa (Fig. 8), submucosa and subserosa (Fig. 9). Those found in the mucosa consisted of an infiltrate into the lamina

propia made up of epithelioid cells and some giant cells and others with central necrosis by caseation, subsequently giving rise to the destruction of the epithelial lamina and the forming of ulcers. Tubercular granulomas were sometimes clearly delimited by connective tissue capsules. These lesions were observed in 5 animals. Perfectly formed granulomas with partially calcified centers were observed in 2 animals. Similar lesions were observed in the subserosa of another 2 animals.

Intestinal paratuberculosis was found in 22 goats. Combined tuberculosis-paratuberculosis lesions was noted in 3 animals. Two cases of coccidiosis and 7 cases of roundworm infection (unidentified parasitic nematode) were also recorded.

Mesenteric lymph nodes

Gross tubercular lesions were only observed in the mesenteric lymph nodes of 8 animals. Lymph nodes showed irregular swelling, with whitish or whitish-yellow nodules which squeaked when cut. In two cases, parasites of *Linguatula serrata* were found when the lymph node was cut.

Microscopic tubercular lesions were observed in the mesenteric lymph nodes of 12 animals (BERNABÉ *et al.*, 1991b, Table 1), of which 8 exhibited clearly defined calcified tubercular granulomas surrounded by smaller (resorptive) granulomas, which were subsequently incorporated into large central masses. Granulomas were poorly defined in 4 animals. The lesions observed in 2 cases were negative for acid-fast organisms following Ziehl-Neelsen staining.

Heart

Two types of gross lesion were found in the epicardium: 1) small greyish-white nodules go-

ing deep into the myocardium and 2) small (miliary) nodules protruding into the pericardial cavity (Fig. 10). These nodules sometimes combined to form larger focal plaques. This latter type of nodule was also observed to spread over the parietal pericardium.

Microscopic analysis revealed circular granulomas immediately below the epicardium in only one case (Fig. 11); serosal lesions in the heart were similar to those described in other organs.

In most of the animals studied sarcosporidiosis was observed in cardiac muscle fibers and in some Purkinje fibers, although no inflammatory reaction was apparent.

Diaphragm

Diaphragmatic lesions were only observed in the serosa of one animal (BERNABÉ *et al.*, 1991b, Table 1) involving both the thoracic and the abdominal portions (Fig. 5). These lesions were typically proliferative and consisted of nodules appearing as superficial villous outgrowths of serosa.

Kidney

Although there was no gross evidence of lesions, microscopic lesions were observed in the kidney of two animals (BERNABÉ *et al.*, 1991b, Table 1). These subcapsular lesions were proliferative and were characterized by a central area of caseous necrosis. This central area was generally calcified and surrounded by a connective tissue capsule (Fig. 12). In one case, smaller peripheral granulomatous forms were also observed.

DISCUSSION

There is a clear-cut correspondence between gross microscopical observations and the iden-

tification of this bacteria in stained sections. The identification of etiological agents was carried out by bacteriological analysis in some animals (data of many of them are unfortunately lacking) for *M. bovis*. There is also a correspondence between a marked skin reaction to bovine tuberculin (CTID test) and a diagnosis based on gross and microscopical observations, as reported by GÖTZE (1951) and SIGURDSON (1956).

Enlargement of *superficial lymph nodes* (prescapular, sternal and subiliac lymph nodes) was found in 8 animals. In 6, the cheesy matter that filled the nodes revealed the presence of the disease, whereas in other animals the enlargement may be related to a response of the node to the CTID test (THOREL and GAUMONT, 1977).

Lesions to the *spleen*, 20% of total lesions to all organs (BERNABÉ *et al.*, 1991 b), corresponded to primoinfection processes: miliary tuberculosis in two cases; slow-early tuberculosis in three cases and large-nodule tuberculosis in one case. Tuberculous perisplenitis in the form of capsular outgrowths was found in one of the above animals. Microscopic analysis revealed lesions similar to those observed in mediastinic lymph nodes, showing characteristics typical of miliary tuberculosis, slow-early tuberculosis and large-nodule tuberculosis, with calcified centres and fairly clear limits, accompanied in three cases by peripherically splenic corpuscle amyloidosis.

Lesions to the spleen are thought to be the

result of an early generalization process, while in cattle they are indicative of congenital infection according to JUBB *et al.* (1985).

Liver lesions (34.3% of total lesions in all organs) corresponded to miliary tuberculosis (5 animals), slow-early tuberculosis (2 animals), and large-nodule tuberculosis (1 animal). Lesions in the parenchyma (phases of early generalization) were found in 7 of these 8 animals and in the intralobular bile duct walls of 3 of them, 2 of which also exhibited early generalization lesions. Microscopic analysis revealed that the lesions were proliferative and thus characteristic of early generalization. The mixed parenchyma-portal process resembles that described by NIEBERLE and COHRS (1961).

The presence of paratuberculous microgranulomas in 7 goats confirmed the existence of a mixed tuberculosis-paratuberculosis process, without reference to other cases in which both diseases occurred simultaneously (23 cases, 65% of total) a phenomenon which will be dealt with more fully in a forthcoming paper (BERNABÉ *et al.*, 1991a).

Lesions in the *intestine* are attributed to organic-chronic pulmonary tuberculosis (2 individuals), and to infection through the digestive system (one kid). The microscopic analysis revealed lesions characteristic of primary tuberculosis: tubercular granulomas in the mucosa (3 animals), in the submucosa (2 animals), in the serosa (1 animal) and even together in all three laminae (1 animal). Intestinal lesions accounted for 20% of all tubercular lesions in organs. The

FIGURE 7. Thickened intestinal mucosa.

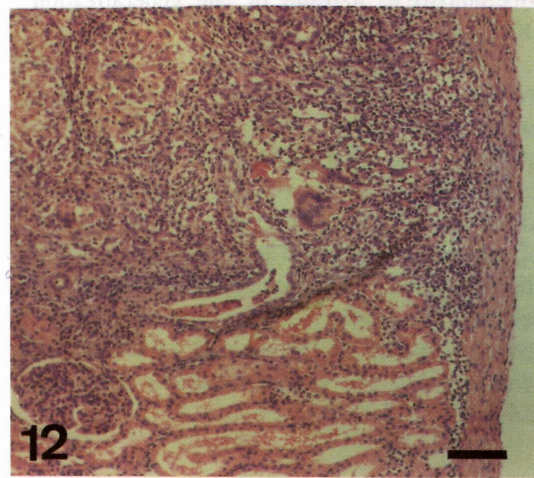
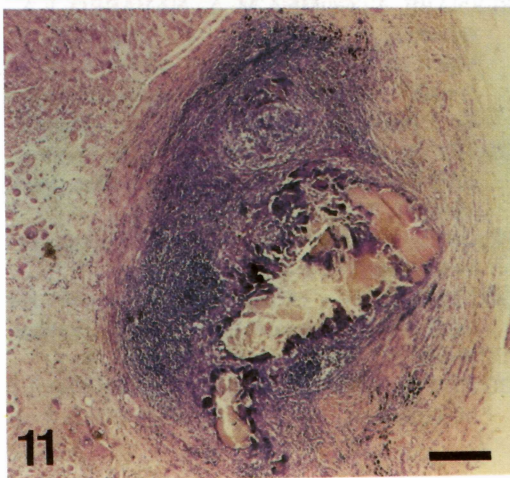
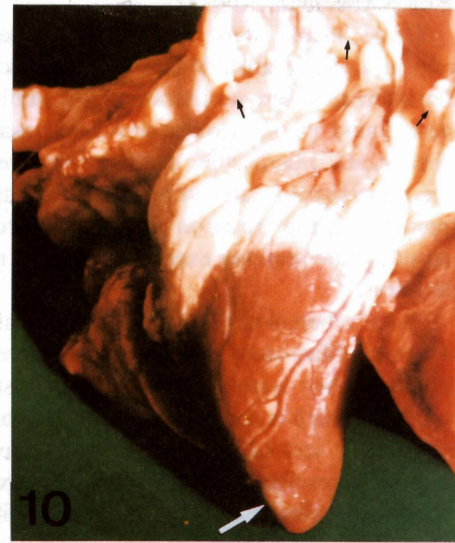
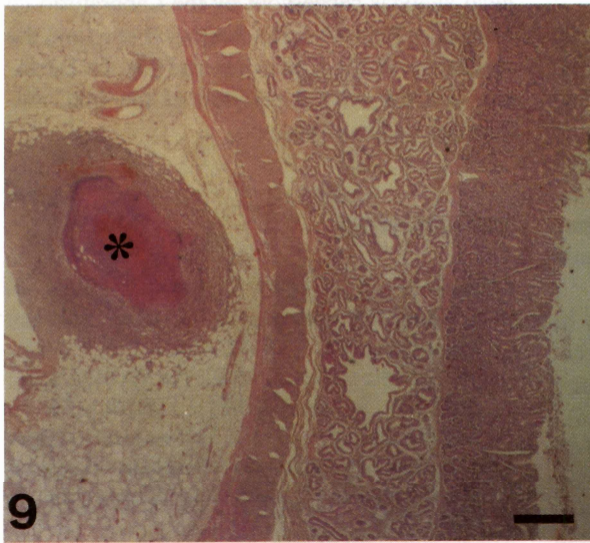
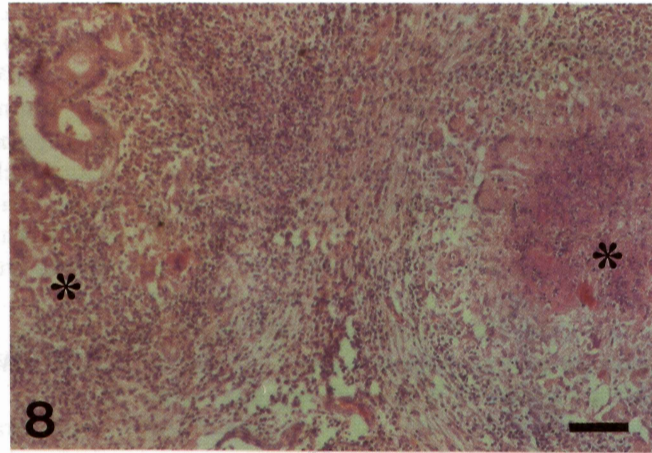
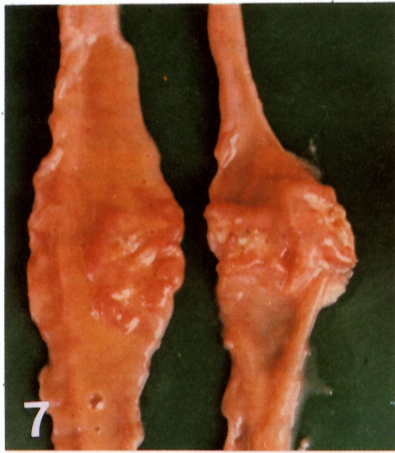
FIGURE 8. Tuberculous granuloma of intestinal mucosa (*): H-E. Bar= 50 µm.

FIGURE 9. Tuberculous granuloma in intestinal subserosa (*): H-E. Bar= 227.2 µm.

FIGURE 10. Tuberculous granuloma in epicardium (white arrow) and pericardium (black arrows).

FIGURE 11. Microscopic tuberculous granuloma in epicardium. Ziehl-Neelsen. Bar= 50 µm.

FIGURE 12. Microscopic tuberculous granuloma in kidney. H-E. Bar= 50 µm.



presence of the primary complex in the intestine was only confirmed in one case—a young goat at early generalization phase—, with organic dissemination in the form of miliary tuberculosis affecting visceral and parietal pericardiac serosa as well as the diaphragm. Intestinal lesions were proliferative and in two cases led to ulceration.

Tubercular lesions in *mesenteric lymph nodes* were similar to those found in mediastinal lymph nodes (BERNABÉ *et al.*, 1991b). Macroscopic and microscopic analysis revealed fibrocalcification. In 6 cases these lesions were accompanied by signs of paratuberculosis (BERNABÉ *et al.*, 1991a).

Heart lesions were rare as they appeared only in a young goat. Macro and microscopic characteristics were proliferative, and similar to those found in the serosae and subserosae of other organs. Microscopic examination revealed sarcosporidiosis in almost all animals, with no apparent organic reaction except for atrophy caused by the pressure exerted on cardiac muscle fibres.

Renal tuberculosis is observed here only in two cases. Macroscopic lesions were not recorded since they were confused with interstitial nephritis, which is common in goats. One of the microscopic lesions observed corresponded to an early generalization in the form of fibrocalcified tuberculosis, and the other to a miliary tuberculosis as a result of late generalization (KITZ and SCHULZ, 1985).

Unlike other studies of cattle and goats, no evidence was found of lesions in bone, udder, uterus, testicles or central nervous system (AMARA and BEN SAID, 1986; GÓMEZ-VILLAMANDOS *et al.*, 1987). Nevertheless, the kid probably received oral infection as a result of tubercular mastitis. The mother presented a reaction of +5 to bovine tuberculin in the CTID test, and was negative to avian tuberculin. It belonged to a herd of 155 animals, of which 106 reacted positively—all except the new-born kids—.

In conclusion, caprine tuberculosis is not only frequently found in the lungs of goats from the region of Murcia (BERNABÉ *et al.*, 1991b) but also can metastasize to other organs. The frequency and phase of the lesions within organs vary widely, thus making it necessary to study more individuals that react positively to the CTID test for a more accurate estimation of disease prevalence.

ACKNOWLEDGEMENTS

We gratefully acknowledge the bacteriological analysis carried out by the Laboratorio de Sanidad y Reproducción Animal (León) and the assistance of Mr J. Sánchez and Mr. C. De Jodar, and the critical revision of the final manuscript by Dr. J. Serrano.

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