Paratuberculosis in sheep from Serra da Estrela Region, Portugal

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INTRODUCTION

This work is part of a National Project (AGRO 786), started in September 2004 and with the duration of three years, aiming the study of Paratuberculosis incidence in sheep herds in one of the best Portuguese cheese production region, Serra da Estrela.

Several diagnostic parameters were used and correlated with each other: clinical signs, serological results, pathological lesions at necropsies, histopathological tests, Ziehl-Neelsen staining, immunohistochemical technique, direct PCR and bacteriological culture.

MATERIALS AND METHODS

Thirteen sheep herds were selected and submitted to an epidemiological inquiry focused on the herd size, animal movement, clinical history and sanitation measures, type of bedding, manure management and human involvement. ELISA and AGID tests were performed in 2 562 animals. Live and and *post mortem* examination were done and several tissue samples of 33 ewes and 1 ram, 30 of them previously submitted to screening ELISA and AGID tests, were collected for histopathological and immunohistochemical analysis. Bowel tissues with lesions were subjected to direct detection of *Mycobacterium avium* subsp. *paratuberculosis* by PCR, according to the ADIAVET kit, and also cultured in selective media for bacteriological isolation. From 144 sero-reacting animals, faeces were sampled, stained by Ziehl-Neelsen method and cultured in selective media for bacteriological isolation.

RESULTS

The serological survey revealed that 234 samples (9.1%) were positive in ELISA and 30 (1.2%) in AGID. From the 13 herds, only two of them did not show positive serological results.

From 34 animals submitted to necropsies, 27 presented clinical signs compatible with the disease. Typical lesions of paratuberculosis were observed in 28 animals (Figs. 1 and 2): mucosa folds, lymphangiectasia lesions, lymphangitis and lymph nodes enlargement. However, macroscopic valvular lesions were noticed only in a very low percentage of animals.

Histophatological lesions were present in 21 animals and in 18 of them acid-alcohol resistant bacilli were observed (Fig. 3). The predominant microscopic lesion was granulomatous lymphadenitis, followed by the granulomatous inflammation in the intestine and the ileocecal valve mucosa and submucosa. Only two animals were tested by immunohistochemistry and showed positive results (Fig. 4).

From 28 PCR positive samples collected at necropsies, *Mycobacterium avium* subsp. *paratuberculosis* was isolated in 20 of them. The results are expressed in Fig. 5.

Acid-fast bacilli were found in 26 faecal samples but, until now, *Mycobacterium avium* subsp. *Paratuberculosis* was only isolated in 1 of them.

Mycobacterium avium subsp. *paratuberculosis* isolates are being molecular typed by single nucleotide polymorphisms of *gyrA* and *gyrB* genes.

Fig. 1. Macroscopic exam. Mesenteric lymph nodes enlargement, thickened of lymphatic vessels and atrophy of mesenteric fat.

Fig. 2. Macroscopic exam. Diffuse thickening of the mucosa, which is folded into transverse rugae.

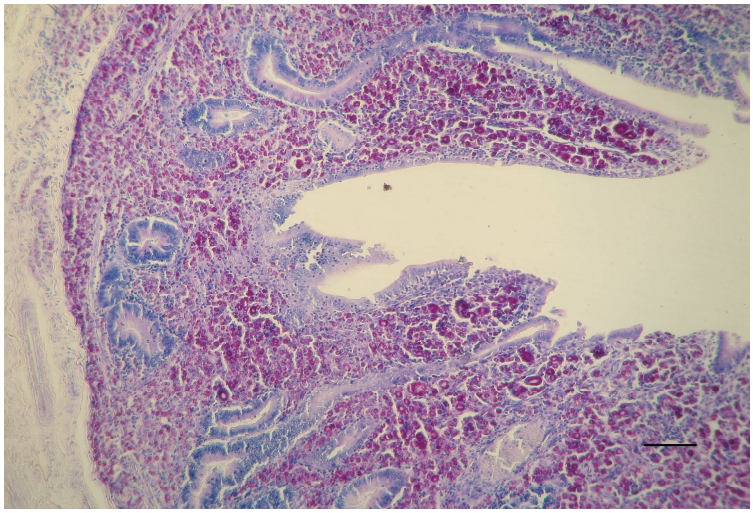

Fig. 3. Ziehl-Neelsen staining. Diffuse giant cells infiltrate with multiple alcohol-acid-resistant bacteria, in the lamina propria of the intestinal villi (Bar = 50μm).

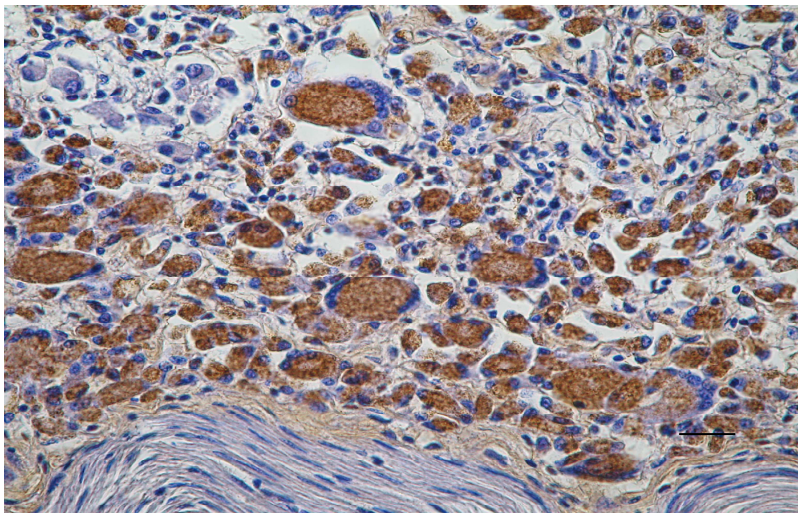

Fig. 4. Imunohistochemistry. Diffuse giant cells and epithelioid macrophages infiltrate, positive for MAP antibody, in the intestinal submucosa. (Bar = 200μm).

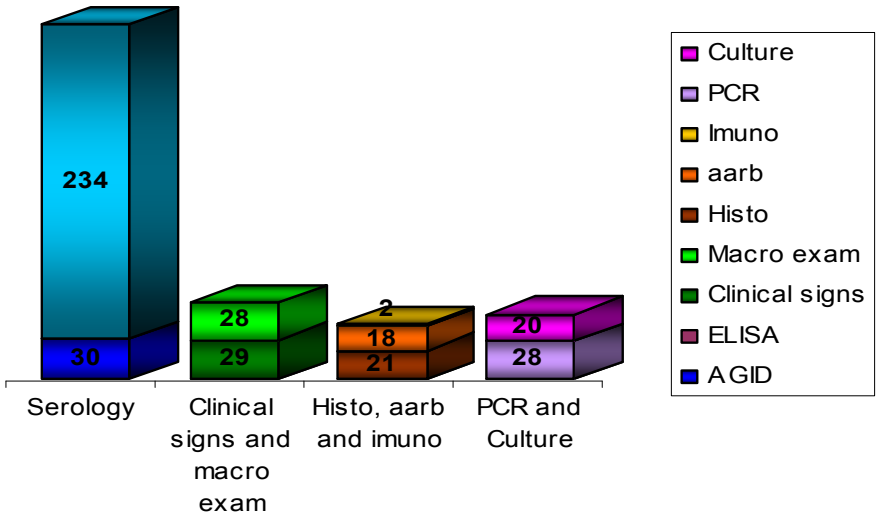

Fig. 5. Number of samples tested positive in the nine diagnostic tests used for paratuberculosis

DISCUSSION

The fact that the herds are large sized, implies higher housing density and, consequently, a higher level of exposure to the etiologic agent (Berghaus *et al*., 2005). Only in three herds beddings were removed in a weekly base and other three do that only twice or three times a year which increases the risk of disease spreading.

Transhumance and route sharing is practiced in eight herds, which promotes infection transmission between herds (Collins & Manning, 2001; Navneet *at al*., 2007). None of the herds had maternity pens and, in seven of them, lambing ewes were not placed in lambing pens, a fact that is particularly important since the animal's age is probably the parameter that most affects the infection transmission (Badiola *et al*., 2000). In seven herds, suspected animals were not isolated from other animals, increasing the risk of infection (Badiola *et al*., 2000).

ELISA technique showed higher sensibility and lower specificity than AGID, fact that has been previously referred (Badiola *et al*., 2000; Garrido, 2002). All animals submitted to necropsies were aged between two and six years, which is in agreement with what is known about this disease (Radostits *et al*., 1994; Sweeney, 1996).

The majority (76.5%) of the animals submitted to necropsy presented clinical symptoms compatible with paratuberculosis (Jubb *et al*., 1991; Wilson, 1998; Martin & Aitken, 2000; McGavin *et al*., 2000). In most of them, lymph nodes lesions were evident and

corresponded to microscopic lesions of granulomatous lymphadenitis. In agreement with other authors (Jubb *et al*., 1991; Wilson, 1998; Gracey *et al*., 1999; McGavin *et al*., 2000; Verna, 2005), diffuse thickening of the mucosa was also evident, with folds that couldn't be smoothed out by stretching, corresponding to a diffuse granulomatous cell infiltrate in the intestine and ileocecal valve mucosa and submucosa,

CONCLUSIONS

The epidemiological inquiry revealed that factors such as: the great size of the herds, poor herd management and sanitary conditions, type of bedding, the absence of suspected animals sequester, as well as common transhumance routes, favoured the spread of the disease.

All the animals submitted to necropsy had equal or superior age required for the disease incubation period to occur. It was noticed a high correspondence between the diagnosis methods of histopathology and Ziehl-Neelsen. All Ziehl-Neelsen positive cases were multibacillary lepromatous type.

A low correspondence between sero-positive animals and direct Ziehl-Neelsen stained and culture of faeces was observed.

All the activities developed in the frame of Project AGRO 786, improved the knowledge of the producers towards this disease and contributed to a better control and awareness of paratuberculosis in Portugal.

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