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Short communication

A longitudinal study of the *Mycobacterium avium* subspecies *paratuberculosis* infection status in young goats and their mothers

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ABSTRACT

The dynamics between *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection and the immune response of goats naturally exposed to MAP were studied in a herd where the clinical expression of paratuberculosis had been observed. Four generations of goats were observed over a 33-month period: mothers of three different generations (G1, G2, G3) and their daughters, generation 4 (G4). A MAP infection status was defined according to the combined results of an IFN-γ assay, antibody response, faecal culture and post-mortem examination. Goats were defined as non-infected (NI), infected and non-shedder (INS), infected and shedder (IS) or atypical (A). Twenty-nine percent of goats were NI, 66% were infected and either shedding (14%) or not shedding (52%) MAP, and 5% were atypical. IFN-γ responses were detected first, followed by faecal shedding and antibody responses. The results showed that in goats naturally exposed to MAP, IFN-γ responses were regularly detected earlier in non-shedders than in young infected shedder goats and were stronger in shedder than in non-shedder goats. They were also higher in the mother goats than in their daughters. Goats shedding MAP or with positive antibody response at the beginning of their pregnancy are more likely to have an infected daughter positive to an IFN-γ assay by the age of 15 months.

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1. Introduction

Paratuberculosis is a chronic and contagious gastrointestinal disease of wild and domesticated ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Ruminants are infected in the first few months of life usually via the faecal-oral route; *in utero* exposure has also been reported (Clarke, 1997). Following infection, mycobacteria remain silent for a long period in the intestine or in associated lymph nodes, mainly in macrophages. Once infected intestinal macrophages have lysed, mycobacteria disseminate to different sites, including the uterus, the mammary gland or the immune foetus (Clarke, 1997; Whittington and Windsor, 2009; Sweeney et al., 1992; Seitz et al., 1989).

Cell-mediated immunity (CMI) is considered to be the earliest defence mechanism for clearing MAP infection. If the cellular immune control of the bacteria is weak, the infection irreversibly progresses and triggers antibody production often associated with bacterial shedding and clinical disease. Under experimental conditions, it is common to observe a Th1 response associated with the production of gamma interferon (IFN-γ), followed by a Th2 response associated with humoral immunity, and the release

Abbreviations: A, Atypical; ANOVA, Analysis of variance; CFU, Colony-forming unit; CI, Confidence interval; CMI, Cell-mediated immunity; ELISA, Enzyme linked immunosorbent assay; HEYM, Herrold's egg yolk medium; HPC, hexa-decylpyridinium chloride; IFN-γ, Gamma interferon; IS, Infected and shedder; INS, Infected and non-shedder; MAP, *Mycobacterium avium* subspecies *paratuberculosis*; NI, Non-infected; OD, Optical Density; OR, Odds Ratio; PBS, Phosphate-Buffered Saline; PPDa, Purified Protein Derivative from *Mycobacterium avium* subspecies *avium*; S/P, Sample/Positive; ZN, Ziehl-Neelsen.

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of mycobacteria from intestinal macrophages (Arsenault et al., 2014; Stabel, 2000).

The disease remains a challenge for farmers in terms of economic loss and decrease in production, despite major efforts to control the spread of the infection. The importance of paratuberculosis is related to the zoonotic capacity of MAP as a causative agent of Crohn's disease, an inflammatory bowel disease in humans (Naser et al., 2014). Moreover, in dairy cattle, economic losses have been observed both in animals showing clinical signs of paratuberculosis and in MAP-infected animals with no clinical signs (Nielsen and Toft, 2008). Consequently, farmers are asking for diagnostic tools that work at early subclinical stages of the infection. In ruminants, a field diagnosis is usually performed on animals over 2 years of age by immunological tests designed to detect MAP antibodies, specific DNA detection by PCR, MAP isolation from faecal or tissue culture, microscopic observation by Ziehl-Neelsen staining, or by the detection of pathological lesions following examination of the intestine or lymph nodes (Whittington and Sergeant, 2001; Nielsen and Toft, 2008). Early diagnosis of MAP infection is difficult for several reasons: the presence of subclinical but infected young animals, the absence of specific clinical signs, and the poor sensitivity of the ante-mortem diagnostic tests at the subclinical stage after MAP infection (Whittington and Sergeant, 2001; Nielsen and Toft, 2008).

In goats, the disease causes chronic enteritis, expressed as progressive weight loss with associated clinical signs, and possibly diarrhoea, though this is rare (Clarke, 1997; Windsor, 2015). Under field conditions, the dynamics of immune responses to infection are poorly understood, and goat herds still face high economic losses associated with reduced production, weight loss and, subsequently, disease and death. A better knowledge of these processes is needed to improve management of the disease.

The present study was conducted under field conditions on a farm where MAP infection was endemic and clinical cases were observed. The objectives of this study were: (i) to determine the MAP infection status of goats, from birth to about 2 years of age along, with that of their mothers according to the combined results of an IFN-γ and antibody response, faecal shedding and post mortem examination, (ii) to evaluate the frequency, dynamics and intensity of these responses—and in particular IFN-γ—under farm conditions, due to the need to improve MAP diagnosis in the early subclinical stages, and (iii) to analyse the relationships between goat MAP infection status and that of its mother.

2. Materials and methods

2.1. Animals and herd management

Four generations of apparently healthy goats (n = 98) from a dairy herd with a history of clinical paratuberculosis (LEGTA, Melle, France) were included in the study: 50 pregnant mothers of generations 1 (G1), 2 (G2), and 3 (G3), and their 48 daughters, generation 4 (G4) (Fig. 1). This herd of about 400 tuberculosis-free Alpine and Saanen goats was regularly monitored for growth and milk production in addition to their general health. Goats were kept indoors and managed according to the farm's routine procedures for feed, dehorning, withdrawing and/or culling. Animals included in this study gave birth after oestrus synchronisation in winter (February and March), with goats giving birth for the first time at about one year of age. Newborns were removed from their mothers on the day of birth and transferred to the nursery, where they were fed with heat-treated colostrum for several days. Kids were weaned from milk at about 2 to 3 months of age, depending on their body weight, and then mixed with adult goats.

Animals that failed to complete the study, i.e. that were either found dead or withdrawn and humanely euthanised, were subjected to a post-mortem examination.

2.2. Sample collection

Blood and faeces were collected monthly over a 26-month period. G1, G2 and G3 goats were sampled from the first sampling time (Sampling time 1, S1) whereas G4 goats were sampled from the age of one month, corresponding to the fifth month of the survey (S5). Samples were not collected at months 3, 17, 18, 19 and 22 because of internal management constraints, and samples were collected from months 20 to 26 only for G3 and G4 goats (Fig. 1).

Blood was collected from the jugular vein into silicone- or heparin-coated glass tubes for the ELISA or IFN-γ assay respectively. Faecal samples were collected directly from the rectum of each goat using a disposable glove in order to avoid cross-contamination. They were then transported to the laboratory under refrigerated conditions. All samples were processed using the standard operating procedures of the testing laboratories and according to the manufacturers' recommendations.

2.3. Gamma interferon detection

The assay was started within 6 h of blood collection. Briefly, 1.5 mL of blood was incubated with 100 μL of 0.3 mg/mL of purified protein derivative from *M. avium* subspecies *avium* (PPDa) (Bovigam™, AES,

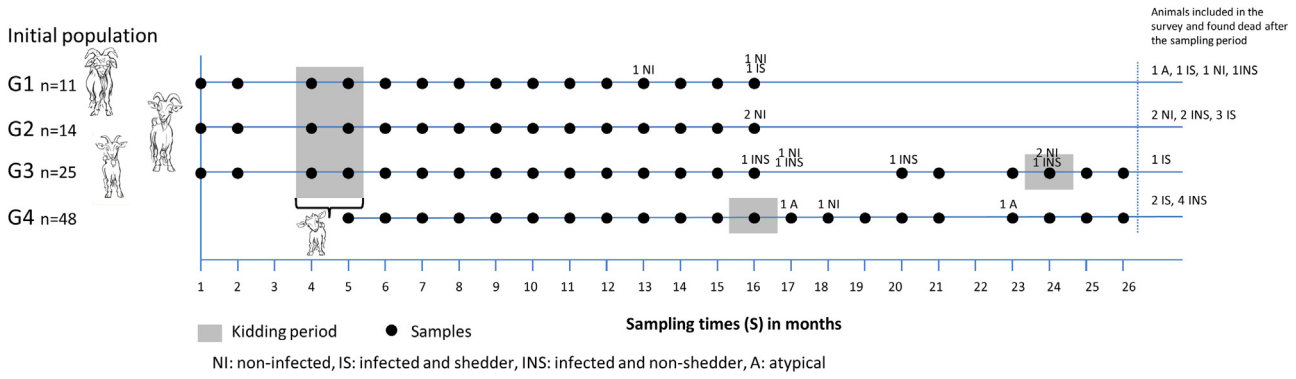

Fig. 1. Summary of sample collection during the study.

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