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Faecal shedding detected earlier than immune responses in goats naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*

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ABSTRACT

Paratuberculosis was diagnosed in a goat herd that participated in a sanitation program against *Mycobacterium avium* subsp. *paratuberculosis*. The aim of this study was to characterise the development of gamma interferon (IFN-γ) and antibody responses as well as the occurrence of faecal shedding. Faecal culture appeared surprisingly sensitive as about 18% and 40% of the goats were positive at 9 and 15–17 months of age, respectively, and shedding was often seen prior to peripheral immune responses. Peripheral IFN-γ responses were not related to protection as clinical and high shedding goats often had high responses. An IFN-γ response usually preceded a humoral response. However, positive antibody titers could sometimes be seen simultaneously with, and even prior to, IFN-γ responses. In conclusion, faecal culture appeared as sensitive as IFN-γ testing. Furthermore, the antibody ELISA and the IFN-γ assay may perform equally well in an infected herd if surveillance is conducted annually.

1. Introduction

Paratuberculosis, a chronic granulomatous enteritis of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) (Chiodini et al., 1984; Johne and Frothingham, 1895), can be diagnosed by faecal culture, detection of pathological lesions, serology or by measuring the gamma interferon (IFN-γ) response (Bakker et al., 2000; Whittington and Sergeant, 2001). It is of major importance to be able to make an early diagnosis of the infection, due to the substantial economic losses caused by the enteric disease (Benedictus et al., 1987) and the possible link between MAP and Crohn's disease in humans (Chamberlin and Naser, 2006; Olsen et al., 2009). An early diagnosis of paratuberculosis is however difficult, because of the slow disease progression and the poor performance of diagnostic tests in the subclinical stages (Bakker et al., 2000; Whittington and Sergeant, 2001).

Animals are usually infected at an early age via the faecal-oral route, but there is also evidence that in utero infection may occur (Chiodini et al., 1984; Clarke, 1997; Whittington and Windsor, 2009). The infection is controlled by cellular immune responses, including IFN-γ production which is considered critical in the defence against mycobacteria (Coussens, 2001; Stabel, 2000, 2006; Storset, 2003). Infected animals often remain subclinically infected for years before the onset of clinical symptoms, and some may even eliminate the bacteria (Chiodini, 1996; Chiodini et al., 1984; Clarke, 1997). The immunity eventually switches to a less protective humoral response, and the infected animals start to shed more bacteria and develop clinical disease (Coussens, 2001; Stabel, 2006; Storset, 2003). In line with this, bacterial culture or serum antibody assays have been assigned relatively low sensitivity in the early stages of the disease (Bakker et al., 2000; Whittington and Sergeant, 2001), while tests like the IFN-γ assay are believed to have the greatest potential for detecting subclinical paratuberculosis (Billman-Jacobe et al., 1992; Stabel, 1996). Despite emerging evidence of a more complex picture, these characteristics are still commonly used to describe the development of paratuberculosis.

Paratuberculosis is a prevalent disease in goats (Djønne, 2003). Still, studies of disease development in this species are far less numerous than in sheep and cattle, and studies with naturally infected goats are particularly scarce. Goats in Norway appear more susceptible to paratuberculosis than sheep and cattle. Caprine paratuberculosis has been endemic in certain parts of Norway. In these regions vaccination against paratuberculosis has been compulsory (Djønne, 2003), but asymptomatic, vaccinated goats still shed MAP in the faeces. In total, only 15 sheep and cattle herds have been diagnosed with paratuberculosis since 1996, and all the cases can be attributed to imported cattle or contact with infected goats (Kampen and Djønne, 2008).

A sanitation program intending to eliminate paratuberculosis, caseous lymphadenitis and caprine arthritis and encephalitis in goats has been initiated in Norway (Leine, 2006). The IFN-γ assay was chosen for monitoring the goats for MAP-infection after

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sanitation, as evaluation of the IFN-γ assay in Norwegian goats had been promising and showed that false positive results were less prevalent than in cattle (Storset et al., 2005). In one goat herd, paratuberculosis was diagnosed 3 years after the commencement of a sanitation program. The aim of this study was to characterise the development of IFN-γ and antibody responses as well as the occurrence of faecal shedding in this herd. Our emphasis was on the IFN-γ assay, and we wanted to see if this test had advantages compared to the antibody ELISA.

2. Material and methods

2.1. The paratuberculosis-infected herd and animals

Norwegian dairy goats from a herd diagnosed with paratuberculosis three years after sanitation for MAP were examined. Sanitation and establishment of a new herd was performed by "snatching" of the kids at birth to prevent all contact with other goats and the environment (Nord et al., 1998). A number of kids were also purchased form neighbouring herds following "snatching" or from herds that had performed sanitation for paratuberculosis. The kids were raised in a separate building, excluded from potentially contaminated pastures and fed milk from cows, since paratuberculosis is very rarely diagnosed in Norwegian cattle (Kampen and Djønne, 2008). Additionally all the adult goats were culled, and the barn, the pens and the outdoor areas were cleaned and disinfected. The disease had never been documented in the herd prior to sanitation, but limited testing had been carried out. Up until sanitation, all the goats had been vaccinated against paratuberculosis with the Gudair™ vaccine (CZ Veterinaria, Spain), which comprises heat-killed MAP cells (Reddacliff et al., 2006; Rosseels and Huygen, 2008). Following establishment of the new herd, vaccination was terminated. The new herd was monitored for paratuberculosis by annual IFN-γ testing. After the diagnosis of paratuberculosis, the goats were examined repeatedly for 15 months by IFN-γ testing, antibody ELISA testing and faecal culture. Table 1 shows at what age the testing was performed in the different generations. At the beginning of the study, the herd consisted of approximately 160 goats. The goats born in the year of sanitation were termed generation 1 goats while the goats born 1, 2 and 3 years after sanitation were termed generations 2, 3 and 4 goats, respectively. Goats were culled during the study due to clinical symptoms and positive test results for paratuberculosis, but also for reasons not related to the disease. Procedures and animal management protocols were approved by the Norwegian Animal Research Authority in accordance with the Animal Experimental and Scientific Purposes Act of 1986.

2.2. Animals used for test evaluations

The specificity of the IFN-γ assay and the antibody ELISA test was evaluated using a total of 10,506 samples from 108 herds for the IFN-γ assay and 4171 samples from 74 herds for the antibody ELISA. All the goats came from Norwegian herds in the sanitation program where MAP-infection had not been diagnosed after establishment of the new herd. The blood samples were collected from 0.5 to 7 years old goats.

To evaluate the effect of transport temperature and time on IFN-γ responses, 20 goats vaccinated against paratuberculosis from two different herds and four naturally infected goats from the herd in this study, were sampled.

2.3. Clinical examination, haematological and biochemical analysis

Clinical examination of all goats in the herd was carried out shortly after the disease was diagnosed. Haematological (Lyche et al., 2004) and biochemical analysis (aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, glutamate dehydrogenase, creatine kinase, total protein, albumin, globulin, urea, creatinine, total bilirubin, β-hydroxybutyrate, glucose, inorganic phosphate, calcium, magnesium) was performed on blood from seven clinically affected adult goats and six young goats without symptoms.

2.4. Interferon gamma assay

Blood was collected from the vena jugularis into heparin tubes and arrived at the laboratory within 24 h. Nine hundred and ninety microliter of blood per well were incubated with 10 μg/ml purified protein derivative from MAP (PPDj) (National Veterinary Institute, Norway) in 24-well plates (Corning incorporated) for 24 h at 37 °C in humidified air with 5% CO_2. Blood with no antigen added was used as controls. Plasma was harvested and stored at −20 °C until examined. IFN-γ production was assessed using a capture ELISA for bovine IFN-γ according to the manufacturer's instructions (Bovine IFN-γ EASIA, Invitrogen) (Storset et al., 2005). Plates were read at 450 nm, and results were calculated as ΔOD: (OD PPDj stimulated wells − OD control wells). ΔOD values between 0.2 and 1.0 were classified as weak positive, while ΔOD values ⩾ 1.0 were classified

Table 1
Sampling time and the results of faecal culture, IFN-γ- and antibody ELISA testing. The age at sampling is indicated for each of four generations. Each testing is assigned a number (lower row).

Generation	1						2						3						4			
Months of age	9	22	36–37[c]	38	41	44–45[e]	51	10	24–25[c]	26	29	32–33[e]	39	12	14	15–17[d]	20–21[e]	27–29[f]	2	3–5[d]	8–9[e]	15–17[b,f]
IFN-γ*	0/92	0/46[a]	32/59	25/50	15/42	14/37	7/18	0/40	12/30	9/27	8/21	9/19	8/12	0/25	0/26	4/25	9/23	8/15	1/42	0/40	1/39	8/34
Antibody ELISA*	Nd	Nd	29/59	19/50	12/40	8/37	1/17	0/40	8/30	10/26	6/20	3/17	3/12	0/26	2/25	4/25	8/23	4/15	Nd	0/40	0/39	3/34
Faecal culture*	Nd	Nd	31/59	Nd	Nd	8/33	Nd	Nd	11/30	Nd	Nd	8/16	Nd	Nd	Nd	10/ 25	16/22	2/14	Nd	0/42	6/33	12/34
Testing nr	1	2	3	4	5	6	7	2	3	4	5	6	7	3	4	5	6	7	4	5	6	7

Nd = not done.
* No. of test positive goats/no. of goats tested.
[a] All goats were not tested.
[b] Three goats were tested at 13 months of age.
[c] Blood testing was performed 3 weeks before faecal culture.
[d] Blood testing was performed 7 weeks after faecal culture.
[e] Blood testing was performed 5 weeks before faecal culture.
[f] Blood testing was performed 6 weeks before faecal culture.