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Vaccination of kids under one month of age with a killed vaccine and reduction in the frequency of faecal shedding of *Mycobacterium avium* subspecies *paratuberculosis*

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ABSTRACT

A study was conducted under field conditions in two French dairy goat herds in order to evaluate the efficacy of a killed MAP vaccine in goats vaccinated before one month of age, by measuring production, epidemiological and pathogenetic effects. In each herd, half of the replacement kids were vaccinated, the other half were used as controls. Samples of blood/sera and faeces were collected from each goat at intervals of approximately every seven months for 31 months, to evaluate immune responses and faecal shedding. Goats culled during the study were submitted to 'post-mortem' examinations (gross lesions, culture on tissues and ZN staining). The milk production of each goat was recorded for the first four years of lactation. Compared to controls, the average annual milk production levels of vaccinates was significantly higher. Vaccination was associated with a reduction in the frequency of faecal shedding. The percentages of shedding goats detected by faecal culture dropped significantly to 0% versus 4% at 15.5 months PV, 3% versus 16% at 23 months PV and 8% versus 20% at 30.5 months PV, in vaccinates and in controls, respectively. The stimulation of both the cell-mediated and humoral immune systems by vaccination was evident from the elevated proportion of positive reactors for both tests in vaccinates compared to controls. To conclude, this study confirmed the results previously obtained in goats on the efficacy of a killed vaccine in controlling paratuberculosis by reducing faecal shedding of MAP.

1. Introduction

Paratuberculosis is a chronic enteric disease of ruminants caused by infection with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) (Clarke, 1997). Animals are usually infected with MAP at an early age via the faecal–oral route (Sweeney, 1996) and infected animals often remain subclinically infected for years before the onset of clinical signs (Clarke, 1997). In goats, clinical signs occur in animals over two years of age and are not pathognomonic: the main signs are reduction in milk production and weight loss, both associated with premature culling, and eventually diarrhoea. The gross pathological lesions associated with MAP infection are thickening of the intestinal mucosa and hypertrophy of the mesenteric lymph nodes, associated with frequent caseous and/or calcified lesions. Field diagnosis of paratuberculosis in ruminants is usually performed on faeces or intestinal tissues by MAP isolation from culture, specific DNA detection by PCR, microscopic observation by Ziehl-Neelsen staining, and detection of

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pathological lesions following histology examination of the intestine or lymph nodes. Detection of cellular and humoral immune responses is performed by immunological tests for the detection of interferon-gamma (IFN-γ) or antibodies to MAP, respectively (Bakker et al., 2000; Huda et al., 2003; Whittington and Sergeant, 2001). However, detection of MAP-infected animals is difficult due to the lack of sensitivity of the majority of diagnostic tests (Corpa et al., 2000b), particularly in the early stages of infection (Manning and Collins, 2001). Consequently, procedures to control paratuberculosis based on the identification and culling of infected animals ('test and cull') have been relatively unsuccessful and vaccination was therefore found to be a successful alternative for controlling paratuberculosis (Corpa et al., 2000b).

Vaccination was initiated in 1926 in France by Vallée and Rinjard. They vaccinated cattle with a live non-virulent strain of MAP mixed with liquid paraffin, olive oil and pumice powder. This method was based on establishing a state of sensitivity, which protected the animal as long as bacteria persisted in the fibrocaseous nodule formed at the site of vaccination. Since this first description, different live (Larsen et al., 1964; Saxegaard and Fodstad, 1985) and killed (Larsen et al., 1978; Sigurdsson, 1960) vaccines have been administered to the various species of domestic ruminants. They have demonstrated their usefulness in reducing both the number of animals with clinical disease and the number of animals shedding MAP (Bastida and Juste, 2011; Chiodini et al., 1984; Merkal, 1984). The effect of vaccination is not to prevent the infection of the animals but to modify the inflammatory response, limiting the progression of the granulomatous lesions in infected animals (Garcia-Marin et al., 1999). Immunity to all mycobacterial infections is dependent on cell-mediated responses and must be elicited by a vaccine to be protective. Humoral response has little protective value in mycobacterial infections (Chiodini, 1996).

Mineral oil adjuvants are routinely added to the vaccine formulation. Some also contain an irritant (such as pumice powder) in order to increase the local inflammatory response, and therefore to enhance the immunogenicity of the vaccine. The goal of these vaccines is to establish a focus of inflammation where the antigens can permanently stimulate the host's immune system. According to this principle, it would not be necessary to revaccinate animals. Paratuberculosis vaccines are recommended for exclusive use in infected herds in very young animals on the grounds that this is necessary to prevent infection. The vaccines are inoculated subcutaneously.

One limitation to the widespread use of paratuberculosis vaccination is the development of abscesses at the injection site. Another complication following vaccination is that sensitisation with vaccines causes interference with immunological tests used for the diagnosis of natural MAP infection and tuberculosis due to *Mycobacterium bovis* infection (Begg and Griffin, 2005).

A commercial vaccine (heat-killed vaccine) is currently registered in Spain for sheep and goats, but in France the use of paratuberculosis vaccines is restricted to goats under one month of age, in order to decrease interferences with tuberculosis diagnosis. As there were no scientific reports demonstrating the efficacy of a killed MAP vaccine used under these conditions, a field trial was undertaken from 2006 to 2010 to determine the efficacy of such a vaccine for the control of paratuberculosis in French dairy goats.

The present study was conducted under field conditions in two French dairy goat herds where MAP is endemic and clinical expression of paratuberculosis observed. The objective of this study was to evaluate the efficacy of a killed MAP vaccine in goats vaccinated under one month of age, by measuring production, epidemiological and pathogenetic effects, as described by Bastida and Juste (2011).

2. Materials and methods

2.1. Experimental design

The study was carried out from 2006 until 2010 in two French dairy goat herds (Alpine breed) with clinical paratuberculosis and enrolled in the milk recording scheme. The two herds were officially free of tuberculosis. In each herd, replacement kids (mainly females) born in 2006 were ear tagged for individual identification and randomly allocated to treatment groups (vaccinated, $n = 120$ and unvaccinated controls, $n = 134$). The vaccinated kids were not separated from the control kids. The trial goats in each herd were subjected to the usual farm management practises, along with the rest of the goats on each farm.

In the vaccinated group, kids under one month of age were injected once with a killed MAP vaccine (GUDAIR, CZ Veterinaria). A subcutaneous injection of 1 mL vaccine was administered in the neck behind the ear, with a new needle for each kid, in accordance with the recommendations for use of the vaccine. The vaccine used is a heat-inactivated vaccine containing 2.5 mg of the culture of strain 316F of MAP combined with an immunological adjuvant consisting of highly refined mineral oil. Control kids were left unvaccinated.

Samples of blood/sera and faeces were collected on each goat, approximately every seven months for 31 months. Blood samples were collected from the vena jugularis into siliconated (ELISA test) or heparin (IFN-γ test) glass tubes. Faeces were sampled directly from the rectum, and gloves were changed for each goat. No samples were collected before vaccination. Samples were taken in compliance with the French Animal Experimental law (Act 87–848 of 19/10/87) by a trained staff member, in accordance with animal welfare and pain reduction guidelines.

Goats culled during the study (2006–2010) due to clinical signs of paratuberculosis, or for any other reasons, were submitted to 'post-mortem' examinations.

The milk production levels of each goat were measured by collecting milk samples from lactating goats 11 times per year in keeping with the milk recording scheme. Annual milk production levels were recorded for each lactating goat during the first four years of lactation (2007–2010).

2.2. Analyses

In some cases, the quantities of the samples collected were insufficient to carry out all of the tests and the results were recorded as 'missing value' (MV).

Faecal shedding of MAP was detected in faecal samples by culturing, as previously described (Mercier et al., 2009) and by polymerase chain reaction (PCR).

In brief, faecal samples were decontaminated with HPC (hexadecylpyridinium chloride) and inoculated onto four Herrold's egg yolk medium (HEYM) slants, supplemented ($n = 3$) or not ($n = 1$) with 2 mg/L of mycobactin (Institut Pourquier). Incubation was carried out at 37 °C, for up to 5 months. Samples were checked for growth of MAP every month, starting 1 month post inoculation. Visible MAP colonies on tubes with mycobactin were identified by Ziehl-Neelsen (ZN) staining. Faecal samples were considered positive if at least one MAP colony was present on at least one of the three tubes supplemented with mycobactin.

A real-time PCR assay targeting the *IS900* sequence present in MAP was carried out on faecal samples. DNA extraction and amplification were carried out according to the manufacturer's instructions with Qiagen kits and an LSI kit (TaqVet M.paratuberculosis, Laboratoire Service International). A faecal sample was considered positive if the threshold cycle