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Effect of an inactivated paratuberculosis vaccine on the intradermal testing of goats for tuberculosis

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ABSTRACT

The effect of an inactivated paratuberculosis vaccine on the diagnosis of tuberculosis (TB) in goats was investigated in a herd with a history of clinical paratuberculosis but which was free of TB. Cohorts of animals in 2006, 2008 and 2009, were vaccinated once at 1 month of age, and 50% of the 2006 cohort served as unvaccinated controls. The goats were aged 8 months, 20 months and 3.5 years old at the time of the survey. All animals were assessed using a single intradermal injection of bovine tuberculin purified protein derivative (PPD) (SID test), or using both bovine and avian PPD (CID test). An interferon (IFN)-γ assay using both bovine and avian PPD was carried out on the 2006 cohort and was interpreted according to three different 'cut-off' points.

No unvaccinated (control) animals tested positive to any of the assays, confirming that the herd was TB-free. The SID test had a low specificity in vaccinated animals at 8 and 20 months of age, whereas the CID test demonstrated 100% specificity in animals ⩾20 months-old. The specificity of IFN-γ assay was less than maximal for vaccinated animals 3.5 years old as small numbers of false positives were detected, although this depended on the chosen cut-off point. The study findings demonstrate that the use of an inactivated paratuberculosis vaccine in goats <1 month-old in a TB-free herd does not result in false positives to a CID test for TB when performed in animals ⩾20 months-old.

Introduction

Caprine tuberculosis (TB), largely caused by *Mycobacterium bovis*, is very uncommon in dairy goats in France with as few as two positive herds identified annually (Franquet et al., 2008). These cases are typically linked epidemiologically with infected cattle herds. In contrast, paratuberculosis (paraTB) caused by infection with *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) is found in almost 65% of goat herds in France (Mercier et al., 2008). The herd infection prevalence varies according to region and the within-herd prevalence ranges from 0.7% to 17.6% (Mercier et al., 2008).

Although paraTB is mainly controlled by measures designed to delay and reduce the natural exposure of goat kids to *Map*, in herds where there is a high prevalence of infection, vaccination is used as an additional control measure as this is effective in reducing the number of clinical cases and faecal 'shedders' in goats (Leslie et al., 1988; Marly et al., 1988; Garcia Marin et al., 1999), as well as in sheep and cattle (Wentink et al., 1988; Körmendy, 1994; Lopez Cruz et al., 1999; Reddacliff et al., 2006).

Both live and killed vaccines against paraTB induce immune responses similar to those in infected animals (Garcia Marin et al., 1999; Corpa et al., 2000; Muskens et al., 2002; Reddacliff et al., 2006; Santema et al., 2009) and have been reported to interfere with the diagnosis of TB in cattle (Wentink et al., 1988; Lopez Cruz et al., 1999). In consequence, the use of vaccines against paraTB is restricted to animals <1 month old and to herds where severe clinical cases are occurring.

There are limited reports of interference in the diagnosis of TB by the use of paraTB vaccines in goats (Marly et al., 1988). Goats given an attenuated paraTB vaccine (Neoparasec, Mérial) at <1 month old, were found positive on a single intradermal (SID) test with bovine tuberculin up to 16 months later, but as this response was always less than that induced by a simultaneous intradermal injection of avian tuberculin, these animals were deemed negative on a comparative intradermal test (CID) (Marly et al., 1988). Approximately 50% of goat kids given killed or attenuated paraTB vaccines tested positive on the SID, but negative on the CID test, 1–2 years post-vaccination (Leslie et al., 1988; Garcia Marin et al., 1999). Thus given the potential confounding effect of vaccinating goats against paraTB on the diagnosis of TB in this species, this study was established to evaluate its significance using an inactivated paraTB vaccine.

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Material and methods

Animal selection and vaccination procedure

A dairy goat herd located in Western France consisting of 220 adults with a history of clinical paraTB was selected for vaccination. Prior to vaccination in 2006, clinical paraTB and/or early culling due to weight loss had been estimated at 14% of the herd. In 2006 and 2007, half of the young replacement does were vaccinated with a heat-killed paraTB vaccine (Gudair) before they reached 1 month of age: the remaining animals served as controls. All replacement animals born in 2008 and 2009 were vaccinated between 20 and 28 days-old. The herd was considered free of TB given there were no clinical signs of the disease, no epidemiological links with cattle and no lesions had been found in any animals at slaughter over the previous 30 years. The vaccine used was a commercial, heat-killed strain 316F of MAP (Gudair). Each dose contained approximately 2.5 mg of dry micro-organisms/mL and three adjuvants (mineral oil, mannide mono-oleate, polysorbate 80). The goats were vaccinated subcutaneously high on the neck caudal to the ear.

Study design

Animals born in 2006 (34 unvaccinated, 36 vaccinated) and those born in 2008 and 2009 (40 and 38, respectively [all vaccinated]) were included in the survey of November 2009. The mean ages of the groups were 3.5 years, 20 months and 8 months, respectively. Intradermal skin testing was performed on all animals and an interferon (IFN)-γ assay was only carried out on the 2006 cohort. The 2007 group was excluded from the survey because of another ongoing study.

Intradermal skin testing for TB

Intradermal skin testing involved the injection of 0.1 mL of bovine (Bovituber, Synbiotics) and 0.1 mL of avian (Avituber, Synbiotics) purified protein derivative (PPD) into a clean clipped area on the left and right sides of the lower part of the neck, respectively. The immunological response was assessed by measuring the skin-fold thickness at this site with callipers prior to injection and 72 h later.

According to the Organisation Mondiale de la Santé Animale (OIE) protocol for the tuberculin testing of cattle, the SID test was considered positive when the increase in skin-fold thickness at the bovine PPD injection site was ⩾4 mm, and the CID test was considered positive when the increase in skin-fold thickness at the bovine PPD injection site was >2 mm and >4 mm greater than the reaction at the site of the avian PPD injection (OIE, 2008). However, as no specific criteria are set for the diagnosis of TB in goats (Gutiérrez et al., 1998; Alvarez et al., 2008), we also applied a more stringent interpretation of the skin test by considering goats inconclusive on the CID test as positives. These animals had an increase in skin-fold thickness of between 2 and 4 mm on the SID test, and a larger response to bovine than to avian PPD on the CID test.

Interferon-γ testing for TB

The IFN-γ assay has been used with varying success to detect TB and paraTB in goats (Liebana et al., 1998; Storset et al., 2001, 2005; Alvarez et al., 2008; Bezos et al., 2010). In the current study, blood samples containing lithium heparin were taken before the intradermal skin test and were processed within 4 h of collection. This very short assay turn-around time mitigated against the testing of blood samples for T cell reactivity.

Three, 1.5 mL aliquots of each sample were dispensed into microplate wells along with either 100 μL of: sterile PBS; avian PPD (300 μg/mL) or bovine PPD (300 μg/mL). Blood cultures were incubated for 20 h at 37 °C in a humidified atmosphere (5% CO_2). After centrifugation, 500 μL of plasma was pipetted off the sedimented red blood cells and stored at −20 °C. The plasma samples were then assayed in duplicate to determine the IFN-γ concentration using a two-step enzyme immunometric assay (Easia commercial kit, BioSource Europe SA). Results were calculated as Δ optical density (OD) = [(mean of PPD stimulated wells − mean of control wells)/(test positive control − test negative control)] for bovine (ODb) and avian (ODa) PPD. The interpretation of assay positivity, including values for both PPDb and PPDa, was tested at three different cut-off points based on previously published data: (cut off point 1) ΔODb > 0.2 and ΔODb ⩾ ΔODa (Storset et al., 2005); (cut off point 2) mean ODb > mean OD PBS + 0.1 and ⩾mean ODa; (cut off point 3) mean ODb > mean OD PBS + 0.05 and ⩾ODa (Alvarez et al., 2008; Bezos et al., 2010).

Statistical analysis

The chi-square and Student's *t* tests were used to compare percentages and mean skin-fold increases, respectively (considered significant where $P < 0.05$). The ΔOD values between groups were compared using a non-parametric, one-way ANOVA (Kruskal–Wallis test). These analyses were performed using Systat 1.9 (1999).

Results

Intradermal skin testing for TB

Following intradermal skin testing of the 2006 cohort the skin-fold thickness was significantly greater in vaccinated vs. unvaccinated animals at the site of avian PPD injection but no difference in thickness was observed at the 'bovine' site (Table 1). All controls were negative on both SID and CID tests, irrespective of selection criteria. Using the OIE definition of test positivity in the vaccinated group, all animals were negative on both SID and CID tests. However, using more stringent criteria, with a 2 mm cut-off, 2.7% of vaccinated goats were found positive at the site of injection of bo-

Table 1
Comparison of intradermal skin test results in 3.5 year-old goats given an inactivated paratuberculosis vaccine, with unvaccinated controls. The animals were vaccinated at 1 month-old.

	Skinfold increase (mm) mean ± SD (range)		Positive animals (%) ± confidence interval (95%)			
	Avian PPD	Bovine PPD	SID test		CID test	
			Strict (>2 mm)	OIE (⩾4 mm)	Strict[A]	OIE[B]
Unvaccinated controls ($n = 34$)	0.73 ± 0.85[a] (0.1–3.8)	0.27 ± 0.29 (−0.4–1.5)	0 ± 1.47	0 ± 1.47	0 ± 1.47	0 ± 1.5
Vaccinated ($n = 36$)	3.16 ± 3.88[b] (0.1–15.0)	0.40 ± 0.41 (−0.2–2.2)	2.7 ± 6.7	0 ± 1.38	0 ± 1.38	0 ± 1.4

[a,b]Different letters in the same column indicate significant difference ($P < 0.05$).
[A] Response to bovine PPD > 2 mm and response to bovine > response to avian PPD.
[B] Response to bovine PPD > 2 mm and response to bovine PPD 4 mm greater than to avian PPD.

Table 2
Comparison of intradermal skin test results in 8 and 20 month-old goats given an inactivated paratuberculosis vaccine. The animals were vaccinated at 1 month-old.

Age	Skinfold increase (mm) mean ± SD (range)		Positive animals (%) ± confidence interval (95%)			
	Avian PPD	Bovine PPD	SID test		CID test	
			Strict (>2 mm)	OIE (⩾4 mm)	Strict[A]	OIE[B]
8 months ($n = 38$)	8.47 ± 5.18[a] (1.3–21.6)	3.00 ± 2.62[a] (0.3–10.0)	50.0 ± 17.2[a]	23.7 ± 14.8[a]	2.6 ± 6.4	0 ± 1.3
20 months-old ($n = 40$)	4.38 ± 4.00[b] (−0.1–16)	1.31 ± 0.98[b] (−0.1–5)	15.0 ± 12.3[b]	2.5 ± 6.1[b]	0 ± 1.2	0 ± 1.2

[a,b]Different letters in the same column indicate significant difference ($P < 0.05$).
[A] Response to bovine PPD > 2 mm and response to bovine > response to avian PPD.
[B] Response to bovine PPD > 2 mm and response to bovine PPD 4 mm greater than to avian PPD.