

Effects of an inactivated vaccine for bovine mycoplasmosis on calves naturally affected with *Mycoplasma bovis*

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Abstract

Three autogenous vaccine trials were carried out on farms where *Mycoplasma bovis* had been identified as a major pathogen. The first trial was carried out on a veal farm in the Lombardia region of northern Italy. Vaccine, prepared by saponising an *M bovis* strain taken from the farm some months before, was given as single inoculation to 24 calves on arrival while 19 were left unvaccinated. Six months later calves were sent to the abattoir where lungs were examined for gross pathological lesions. The mean weight of the vaccinated group was higher though not significantly so and mean lesion scores were similar; however the percentage of vaccinated calves with severe lung lesions and pleuritis was lower than in the non vaccinates. A second trial was carried out in northern England on a farm where monthly batches of male calves from a nearby dairy herd were reared under a feedlot system. One group of 27 calves were vaccinated with a saponised strain of *M bovis* isolated from the lungs of a pneumonic calf on the farm prior to the start of the trial. A second batch of 25 calves was left unvaccinated. The groups of calves were monitored for nine months prior to slaughter and records kept of antibiotic usage and mortality. The mortality rates in the vaccinated calves were about 15% compared to 28% in unvaccinated calves; however there was no difference in the number of times calves were treated for respiratory disease. A third batch of calves, treated with oxytetracycline on arrival because a number were already showing respiratory signs, had a lower mortality rate and fewer subsequent treatments. A third trial was carried out on a milk veal unit in the Veneto region of Italy. A group of 19 calves were vaccinated on arrival with a saponised isolate taken earlier from the farm; a similar number of calves of the same batch were left unvaccinated. After 6 months animals were routinely processed at the abattoir and lungs inspected. Results showed that vaccinated calves had higher mean body weights and although similar mean lung scores there was a smaller percentage of vaccinated calves with severe lung lesions and pleuritis.

Introduction

Mycoplasma bovis is a major cause of respiratory disease, mastitis and arthritis in cattle. Having first been isolated in 1961 in the USA from a case of severe mastitis in cattle [1], *M. bovis* has now spread via animal movements or products throughout the world including all European countries and most recently to Finland and New Zealand [2]. In Europe, *M. bovis* is responsible for at least a quarter of losses due to calf pneumonia although this is probably an underestimate as few laboratories routinely monitor for mycoplasmas [1].

The inability of antibiotics to control bovine mycoplasmosis caused by *M. bovis* has focused attention on vaccines as a more sustainable and cost effective solution and critically reducing threat of antimicrobial resistance [3]. However many experimental vaccine studies have proved ineffective or even damaging to affected calves so consequently it is unlikely that a commercial vaccine will appear in Europe in the next few years. In the USA a number of vaccines have been used in cattle but there is no published evidence that they are effective [3].

Under experimental conditions, an *M. bovis* vaccine inactivated by saponin was shown to be safe, immunogenic and protective to challenge by a heterologous strain [4]. Attempts to commercialise the vaccine are on-going but in the meantime it has been possible to evaluate autogenous vaccines, using saponin both as an inactivant and adjuvant, from isolates taken and used on individual premises.

Material and method

Vaccine production

Strains of *M. bovis* were isolated from lungs and grown in mycoplasma medium for 3 days at 38.5°C and then subcultured in fresh medium for a further 2 days [4]. The mycoplasmas were centrifuged at 10,000g for 30 minutes, resuspended and washed once in 0.1M phosphate buffered saline (pH 7.2). Cells were centrifuged again and resuspended in 1/50th of the original volume. To the washed cells was added 2mg/ml of filter sterilised saponin (Sigma, Poole) and incubated for one hour at 38.5°C. The saponised cells were then placed at 4°C. The titre of the washed cells was adjusted to 10⁸ colony forming units (CFU) /ml and protein content estimated at approximately 2mg/ml. The vaccine was plated onto blood agar to check for bacterial contamination and into mycoplasma medium to ensure inactivation of mycoplasmas.

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Experimental design

Three autogenous vaccine trials were conducted on farms experiencing bovine mycoplasmosis caused by *M. bovis*. Routine vaccination for other respiratory pathogens was also carried out. Evaluation of the effects of the vaccine on the different farms varied. In trials 1 and 3 it was based on a comparison of lung lesions between vaccinated and unvaccinated calves at post mortem examination; and in trial 2 it was based on mortality and antibiotic treatment records between groups of vaccinated and unvaccinated calves. No attempt was made to alter the management of the farms during the trials

Trial 1

Mycoplasma bovis was isolated and identified from the lungs of a 3-month-old calf which had died of pneumonia on a farm of veal calves in Lombardy, northern Italy with a history of respiratory disease. The affected lung consisted of congested apical lobe which contained caseous necrotic lesions which is pathognomic for *M. bovis*. An inactivated vaccine was produced by growing the isolate in mycoplasma medium and inactivating the washed antigen in saponin as described above. Alternate calves of 2-4 weeks of age were vaccinated with single 1ml dose of vaccine on arrival. In total 19 were vaccinated and 24 were unvaccinated. Serum samples were taken 4 weeks after vaccination. After 6-7 months all calves were weighed and sent to slaughter where lungs were inspected and scored as previously described where 0=non pneumonic; 1= mild pneumonic lesion; 2=moderate pneumonic lesion; and 3=severe pneumonic lesion [5].

There was poor serum conversion in vaccinated calves with only 60% obtaining satisfactory antibody levels 4 weeks after vaccination. The results from the experiment showed that vaccinated calves had slightly lower lung scores than unvaccinated calves with a lower percentage of vaccinated calves having significant lesions (Table 1); the number of calves with pleuritis was also significantly lower in the vaccinated group than the non vaccinates. The mean body weights were slightly, though not significantly higher, in the vaccinated groups. There was no mortality in calves from either the vaccinated or vaccinated groups.

While all calves had seroconverted to *M bovis* antibody by the end of the experiment, it was interesting that the caseous necrotic lesions associated with severe mycoplasmosis were not seen in the calves at post-mortem examination. Indeed, respiratory disease during this winter was unusually mild. Antibody to RSV and PI3 were detected in some cattle but were more likely associated with vaccine antibody for which the calves had been vaccinated.

Trial 2

A farm in northern England which fattened male calves obtained from a nearby dairy herd was identified as a site for a vaccination trial. The farm had previously used an inactivated vaccine made by AHVLA, UK which had resulted in lower mortality rates and treatment costs compared to previous years [1]. However, the owner stopped using the vaccine and mortality rates increased: typically, 25% of each monthly batch of approximately 20-25 calves. It was clear from observing successive batches of male calves that a number arriving from the dairy farm were already affected with respiratory disease from which *M bovis* could be detected.

In this trial, an isolate from the lung of a calf with severe caseous necrotic pneumonia on the farm was obtained and an inactivated vaccine was produced as described above: batch 1 consisting of 25 calves were left unvaccinated and acted as controls; batch 2 of 27 calves were vaccinated on arrival with 1ml of vaccine. The calves were bled 4 weeks

after vaccination to check antibody levels. Treatments (which consisted of single injections of enrofloxacin or tildipirosin administered by the private veterinary surgeon) were recorded for individual calves following signs of respiratory disease; deaths too were recorded over the next 9 months; finally batch 3 comprising of a small group of 10 calves, 3 of which showed signs of respiratory disease on arrival, were treated with oxytetracycline over two successive days following the manufacturer's recommendation. The calves were then given a single dose of autogenous vaccine

Antibody levels were satisfactory in all vaccinated calves tested 4 weeks later indicating high antigen content in the inactivated vaccine. Mortality rates were very high in the unvaccinated group of calves at 28% (Table 2). The effect of a single vaccination was to significantly ($p<0.01$) reduce mortality by 50% but there was little difference in individual calf treatments with antibiotics. However, the group that were treated with oxytetracycline before vaccination showed fewer subsequent treatments and lower mortality rates, but the group was too small to provide any statistical significance.

Trial 3

A veal calf unit in Veneto region of northern Italy was identified as consistently having respiratory disease from which *M bovis* was shown to be the most persistent pathogen. In all, 19 calves were vaccinated on arrival with 1ml of a saponised vaccine prepared at the IZS Sicily (who are licensed to make autogenous vaccine) from an isolate taken from a calf dying of respiratory disease with characteristic lesions of *M. bovis*; 17 calves from the same batch acted as unvaccinated controls. All calves were housed together and bled at four weeks and the vaccinated calves boosted with 1ml of vaccine. After 6 months calves were sent for slaughter; lungs were obtained from all calves and examined and scored at the IZS Venetie as for trial 1.

Seroconversion to the *M bovis* vaccine was not strong in the vaccinated calves with only 55% animals showing satisfactory levels after the first vaccination. However, all seroconverted after the booster dose. Of 17 non vaccinated calves, 6 had significant lesions (35%) and of the 19 vaccinated, 5 had significant lesions (26%). While mean lung scores were similar, there were significantly fewer cattle ($p < 0.01$) with pleuritis in the vaccinated group. The mean weight of the vaccinated calves was also significantly higher than the non-vaccinates (Table 3).

Table 1. Trial 1: Summary of results of autogenous vaccine trial in veal calf unit (Lombardy, Italy)

Group	No	Mean lung score*	No of calves with significant lesions (>1)* / (%)	No with pleuritis/ (%)	Mean weight
Non vaccinated	19	0.8	8 (42)	16 (84)**	154.9
Vaccinated	24	0.6	8 (33)	12 (50)	155.2

* Radaelli et al., 2008

** $p < 0.01$

Table 2. Trial 2: Summary of results of autogenous vaccine trial in calf rearing unit (Northern England)

Group	Batch	No	No calves treated/ (%)	No of treatments	Deaths/%
Non vaccinated	B1	25	14 (56)	28	7 (28)
Vaccinated	B2	27	15 (55.6)	30	4 (14.8)*
Treated/ vaccinated	B3	10	4 (40%)	7	1 (10%)

* total antibiotic treatments for respiratory disease (often including the same calf) during trial

** $p < 0.05$

Table 3. Trial 3: Summary of results of autogenous vaccine trial in veal calf unit (Veneto, Italy)

Group	No	Mean lung score*	No of calves with significant lesions (>1)* (%)	No with pleuritis (%)	Mean weight
Non vaccinated	17	0.65	6 (35)	9 (53)**	130.5
Vaccinated	19	0.63	5 (26)	5 (26)	136.1**

* Radaelli *et al.*

** P<0.01

Discussion

The aim of these trials was to see the effect of an autogenous vaccine for *M. bovis* in real farm settings. There were a number of factors which were not possible to control e.g. the weather and the degree and nature of infection to which the calves were exposed. In fact, the weather during trial 1 was very mild and infection was not severe although all calves were exposed to infection as measured by serological test. There was however significant evidence to show that the vaccine had some beneficial effects in terms of a reduction in the number of calves with severe lesions despite little difference in mean finishing weights.

Trial 3 which took place on another Italian veal farm had similar results in terms of a reduction in number of calves showing severe lesions and pleuritis. Although it is difficult to compare results on the two different farms, giving a second dose of vaccine did not bring about any obvious improvements. It could however have compensated for the reduced antigen content of the vaccine prepared in Italy as only just over half of the vaccinated group seroconverted after primary vaccination.

Trial 2 took place on a male calf rearing unit with annual mortality rates regularly reported up to 30%. It was apparent that the nearby dairy farm from which the calves were sourced was affected with *M bovis* as clinical disease was evident in 10-30% of animals arriving on the feed lot in the monthly batches. However, the effect of a single vaccination on the calves on arrival was to reduce the mortality rate to nearly half compared to the non vaccinates clearly showing that *M bovis* was the major pathogen here. A small sub trial in which all calves (some of which were showing early signs of pneumonia) were treated with

oxytetracycline on arrival before vaccination gave some encouraging results in terms of reduced subsequent treatments and lower mortality rates. However, it should be noted that some *M. bovis* strains have shown *in vitro* resistance to this and other antibiotics, so susceptibility testing is recommended before treatment [6]. Further reduction in the mortality and treatment rates on this farm can only be brought about by tackling the health issues on the dairy herd from which the calves originated as it is unlikely that a vaccine against *M. bovis* can fully be fully protective in the face of this persistent challenge.

In conclusion it is important to recognise that this autogenous vaccine works optimally where *M bovis* is the sole or major pathogen causing respiratory disease in a herd, so accurate laboratory diagnosis is necessary prior to vaccine use. In addition, these results show that this vaccine works optimally when given to young calves on arrival at the farm reducing mortality rates and treatment costs. Antibiotic treatment of calves on arrival (which is normal practice on many farms to offset the stress of animal movements) should also be considered as many calves may already be showing disease signs. Finally, we recommend that the *M. bovis* vaccine should eventually be included in the multivalent vaccines that are currently used to combat respiratory infections.

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