Paratuberculosis control: a review with a focus on vaccination

Felix Bastida\(^1\) and Ramon A Juste\(^2*\)

**Abstract**

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection causes in ruminants a regional chronic enteritis that is increasingly being recognized as a significant problem affecting animal health, farming and the food industry due to the high prevalence of the disease and to recent research data strengthening the link between the pathogen and human inflammatory bowel disease (IBD). Control of the infection through hygiene-management measures and test and culling of positive animals has to date not produced the expected results and thus a new focus on vaccination against this pathogen is necessary. This review summarizes all vaccination studies of cattle, sheep or goats reporting production, epidemiological or pathogenetic effects of vaccination published before January 2010 and that provide data amenable to statistical analyses. The meta analysis run on the selected data, allowed us to conclude that most studies included in this review reported that vaccination against MAP is a valuable tool in reducing microbial contamination risks of this pathogen and reducing or delaying production losses and pathogenetic effects but also that it did not fully prevent infection. However, the majority of MAP vaccines were very similar and rudimentary and thus there is room for improvement in vaccine types and formulations.

**Keywords:** Mycobacteria, paratuberculosis, cattle, sheep, goats, vaccine, protection, production effects, epidemiological effects, pathogenetic effects

**Introduction**

Paratuberculosis poses a big challenge to Veterinary Medicine and in particular to ruminant production. Since the first description of the disease in 1895 in a cow from Oldenburg, Friesland, its etiological agent, *Mycobacterium avium* subsp. *paratuberculosis* (MAP), has been shown to cause the disease in the majority of wild and domestic ruminant species [1,2]. This microbe is also present in many other hosts as well as the environment [3,4]. Even though the most important mycobacterial infection in animals, bovine tuberculosis, has been successfully controlled in nearly all developed countries, the other important mycobacterial infection, paratuberculosis, remains an unsolved problem for the veterinary scientific community still incapable of reaching a consensus on the better way to deal with it. This is so despite large control efforts in different countries during the past three decades.

The mounting evidence showing that MAP is a factor in the pathogenesis of human inflammatory bowel disease (IBD) has increased the pressure to overcome this challenge. In spite of this, most of the undertakings are nevertheless based on the old principle that the only way to control an infectious disease is to eradicate its agent. This principle has worked well for some acute infections in times of survival struggle and profligate use of means but is increasingly difficult to apply because of demonstrated lack of efficacy and sustainability philosophy [5,6]. We are no longer faced with a live or death dilemma due to infectious diseases, but we have to deal with a need to increase productivity for the sake of improved and prolonged use of scarce resources. From this perspective, it is necessary to simultaneously exploit the three classical main approaches to eradicate or reduce the impact of paratuberculosis in herds or flocks. These are: 1) to introduce management changes to decrease the transmission of MAP, 2) to apply test and cull practices to eliminate the sources of infection, 3) to vaccinate replacers in order to increase their resistance to infection. The advantages and drawbacks of these strategies will be briefly examined.
Management measures to decrease transmission of MAP

Management changes to reduce the transmission rate are widely accepted strategies that are compatible with all other approaches [7]. Furthermore, these changes have other positive side effects on farm productivity. Management measures focus mainly on avoiding contact between infected and susceptible young animals [8]. These measures include separating offspring from dams immediately after birth, feeding calves paratuberculosis-free colostrum supplement and milk replacement, raising replacement heifers in separate locations, avoiding manure fertilization of fields where replacement heifers grace, improving general farm hygiene, and eliminating practices that can bring infected foods or materials in contact with susceptible animals. In practice, it implies duplication of facilities and equipment, and meticulous working procedures. Also, another very important factor in the spread of paratuberculosis, which complicates the control of this disease through management measures, is the ability of MAP to survive in the environment for around one year [9,10]. Given the different settings and economic constraints of each individual farm, control measures may greatly vary from farm to farm. In addition, control measures should not be neglected when new animals are brought into a herd. Microbiological and serological results of all new animals, as well as, the paratuberculosis status and history of the herd of origin should always be taken into account before introducing new animals into the farm.

Although these measures might be viable for large dairy farms, the required changes may not be economical for many small dairy farms and are probably impossible to implement in beef cattle and sheep operations due to costs and disrupting effects. Moreover, these measures usually yield no immediate results and are easily abandoned when other productive constraints become more pressing [11]. In summary, this type of strategy has low engaging force and has little chance of being widely and successfully implemented in a whole region.

Culling strategies to eliminate sources of infection

Three variants of the testing and culling strategy prevail depending on the diagnostic method used to detect infected animals: fecal culture, ELISA or Polymerase Chain Reaction (PCR). The slow turn around rate or the low sensitivity of some of these test are the major problems in the efforts to control the disease [12].

Fecal culture and culling

It is generally accepted that this method detects infected animals first and is the most sensitive method [13,14]. Since it is based on identifying the agent when it is shed into the environment, culling these animals has a direct effect in preventing new infections. Fecal positive animals will also become clinical cases, and, therefore, the most visible effect of culling them is that clinical cases quickly disappear. The main problem with this approach is that the laboratory test is expensive, requires specialization, and its results are not available for several weeks or even months. As a result, progress in control of the disease is slow and often rather disappointing since positive animals keep on appearing over the years even after periods of negative results and absence of clinical cases. Its use for sheep and goats is prohibitively expensive unless it is carried out in pools. Another problem with this approach in farms heavily contaminated with MAP or in farms with super-shedders (animals that excrete 10,000 to 10 million MAP bacteria per gram of manure)[15] is the elimination of uninfected animals that give positive MAP results just because they are passing MAP bacteria through their gastrointestinal tract. This problem also affects PCR and culling strategy.

ELISA and culling

The ELISA test for paratuberculosis is generally considered to be highly specific, but of low sensitivity [14]. ELISA’s simplicity, speed, low cost, and potential for automation makes it an ideal tool for laboratory diagnostic work [16]. The problems with ELISA test are that it has not yet been well studied how it will perform to control the disease and that the minimal sensitivity to reach eradication in a reasonable period of time is not guaranteed. In the best case scenario, inferring from the experience with fecal culture it can be assumed that ELISA testing and culling, if done often enough, will prevent the appearance of clinical cases, and slightly decrease the transmission risk. Additional problems with paratuberculosis ELISA are that sample handling appears to affect substantially the results of the test [17] and that the different commercially available diagnostic kits have very different efficacies [18,19], which therefore, can severely affect control programs. Given its costs are low and the results are obtained in less than a week, it is more easily accepted when positive results keep trailing along time since it is always possible to intensify control by testing more frequently. The regional ELISA specific strategies implemented up to now are rather complex and still not proven successful.

PCR and culling

The new type of strategy, albeit sparsely implemented, is the combination of PCR analysis of feces and culling of positive animals. In theory, this strategy should detect animals early in the infection process before antibodies are developed, and thus can quickly reduce the overall bacterial burden in the farm. However, the costs and the requirement of specialized personnel are major drawbacks of this technique. Until recently costs of PCR were extremely high for its use in animal health diagnostics. Dramatic reductions in reagent prices accompanied by improvements in
Vaccination

Vaccination, as a control measure for paratuberculosis, is probably the less accepted strategy although it is or has been used in all countries with substantial problems with this disease [26,27]. It is a highly cost-efficient strategy, which clearly prevents the appearance of clinical cases if done properly [27]. Vaccination strategies have been widely implemented for sheep in different countries with great success [27]. The main drawback to vaccination is that, since vaccines used in the field are not DIVA (differentiating infected from vaccinated), it can interfere with serological diagnosis of paratuberculosis and tuberculosis infections. Thus MAP vaccination might not allow eradication of the disease and it can interfere with national tuberculosis eradication programs. The latter is in fact the major hurdle affecting MAP vaccine approval for cattle by medical and agricultural authorities all over the world and the major deterrent for pharmaceutical companies to design new MAP vaccines for cattle. The most widely used tuberculosis diagnostic test in cattle is the single intradermal tuberculin test, and some cattle vaccinated with the currently available ovine or experimental MAP vaccines will become positive to this test. According to legislation in many countries, these animals are banned from international trade and should be slaughtered unless it can be proved that they are not infected with tuberculosis. New tuberculosis immunological diagnostic test, such as the gamma interferon release assay or the Enferplex™ TB assay, could help in the differentiation between MAP vaccinated and tuberculosis infected animals, but, improvements of these test might be required, since interference with tuberculosis diagnosis can still occasionally occur in MAP infected animals [28]. However, a modification of the single intradermal tuberculin test, the comparative intradermal tuberculin test, could solve the interference problem in the vast majority of cases. This test, which has been available for many years and is actually an official tuberculin test according to the OIE and EU legislation, consists of the simultaneous intradermal injection in two different sites of tuberculins from Mycobacterium bovis (PPDbov) and Mycobacterium avium subsp. avium (PPDav). Higher reactivity to the avian tuberculin indicates infection or vaccination with avian type mycobacteria and allows to rule out mammal tuberculosis infection according to standardized criteria.

Pathogenic background

If we take a general view of our knowledge on paratuberculosis, we should point out that MAP is not a classical infectious agent fully complying with Koch’s postulates. Indeed, we know that many experimental infections fail to establish the infectious agent in the intestinal tissue and to cause the disease [30-33]. We also know that frequently the initial focal lesions do not progress to clinical stages. More recent evidence has revealed that it is not rare for herds with no clinical history of paratuberculosis and even with a history of negative fecal culture to occasionally show positive fecal culture results [34]. In addition, recent studies on paratuberculosis prevalence have revealed that as many as 60% of some national herds are actually infected [35]. Finally, Pickup and collaborators have shown that MAP is present in the environment at a previously unsuspected high frequency [4]. All this evidence indicates that MAP might be a necessary, but not a sufficient cause of paratuberculosis. Under these conditions, we should therefore ask ourselves: Is paratuberculosis eradication a realistic goal? Is it necessary? Is it profitable for the society in general? Answers to these questions are not readily available because we lack accurate information on the actual distribution of MAP and its potential impact on
human health. Reviewing aspects of the pathogenesis and epidemiology may lay the grounds on which control alternative(s) to choose.

**Forms of infection**

Multiple forms of infection can be observed in MAP infected animals. The form present in an animal will not only depend on the progression of the infection or stage of the disease, but also on many other factors including an individual’s genetic resistance or susceptibility to the pathogen, age at the time of infection, and previous exposure to other environmental mycobacteria. On Figure 1, we illustrate the balance between the infection and the animal’s immune system and their corresponding forms of infection. According to different studies, about 46% of cattle, 51% of sheep, and 50% of goats in a MAP-contaminated environment do not show any signs of infection [36-38]. Since these animals live in a heavily contaminated environment, they must continuously be exposed to MAP, and, therefore, they either prevent the infection or very quickly clear up the establishment of local infection foci.

Because it is not rare for such animals to carry MAP and plenty of experimental evidence has shown that administration of large amounts of MAP not always results in the development of a full blown infection, quite the opposite frequently produces very regressive lesions, the more likely explanation is that there is a balance between MAP and the host that in about half of the exposed individuals results in containing the infection (Figure 1). Beyond this balance point there are also different stages of infection. About 19% of cattle, 24% of sheep and 12% of goats carry an infection which is very focal and delimited. Around 17% and 9% of cattle and sheep, respectively, have multifocal forms. Of the animals presenting diffuse forms, approximately 19% of cattle, 16% of sheep and 38% of goats develop into diffuse forms which lead to animals showing clinical signs and to their death.

**Vaccine types**

Both live (non-attenuated and attenuated) and killed whole cell vaccines have been used against paratuberculosis. In a few cases, subunit vaccines consisting of sonicated...
bacteria, bacterial cell fractions or recombinant MAP antigens have been used but they have shown a much lower degree of protection [39,40]. More recently, DNA vaccines, consisting of the inoculation of mammalian expression vectors containing MAP genes have also been used in mice, humans and sheep but not in cattle [41-47]. Most MAP vaccine formulations have been based on mycobacteria and a water-in-oil emulsion (olive, mineral, liquid, paraffin, etc). Some have also an irritant like pumice powder in order to increase and stimulate the local inflammatory response, and therefore enhance the immuno- 
genicity of the vaccine. The goal of these vaccines is to establish a focus of inflammation where the antigens can permanently stimulate the host immune system. Under this principle, it would not be necessary to revaccinate animals because the slow liberation of antigens from the vaccination site keeps on stimulating the immune system, at least during the period before the age of initial clinical disease presentation.

**Vaccination age**

Paratuberculosis vaccines are recommended for exclusive use in very young animals on the grounds that this is necessary to prevent infection and to decrease interfering responses with the diagnosis of tuberculosis. Actually, the experience on animals older than 1 month is rather scarce, however recent studies on the pathogenesis as well as some field data suggest that vaccination of adult or subadult animals might have some management (no need for separate handling, vaccination of only replacers) and therapeutic (stronger humoral and cellular responses) advantages that need to be taken into account [48,49]. More recent evidence from Australian sheep vaccination trials indicate that there might be an age threshold for vaccine efficacy that can be drawn at around 8 months of age [50].

**Reassessment of vaccination results**

**Literature on vaccination**

There is an increasing number of vaccination studies in ruminant species focused on different aspects of the use of MAP vaccines including two recent reviews on the topic [51,52]. The most recent review by Rosseels et al. focused mainly on the immunological aspects of MAP vaccination [52]. For the purpose of the present review we have used only vaccinations studies of cattle, sheep or goats reporting production, epidemiological or pathogenetic effects and data that could be used to estimate the reduction rates of damage or contamination. Production effects relate to the losses measured as the frequency of clinical cases or mortality rates. We considered epidemiological effects as the microbiological contamination risks measured by the frequency or amount of MAP isolations in fecal or tissue cultures. And finally, pathogenetic effects pertain to the modification in the course of the disease as measured by the frequency of specific histopathological lesions. Searches of published material before January 2010 were run using three strategies: First, specific searches of combinations of the words vaccination, vaccine and paratuberculosis were run on Current Contents or Pubmed and the hits were screened for articles meeting the conditions stated above. Second, the same combinations of words were used in Google (http://www.google.com) to obtain studies from doctoral dissertations and other sources. Third, literature data on vaccination trials collected over a period of 25 years at NEIKER was also examined systematically. More than half the published studies included in this meta-analysis describe field reports, which actually might give a better view of the whole problem of vaccination, since highly controlled experimental trials might be misleading because of the lack of interferences from field conditions.

The very first report on paratuberculosis vaccination of cattle is that by Vallée and Rinjard in 1926 [53]. It is not until 1960 that a similar vaccine was reported to have been used in sheep [54]. As for goats, although it is known that vaccines have been used in Spain in the 70’s, the first written report on its efficacy dates back to 1985 in Norway [26].

**Paratuberculosis vaccination meta-analysis**

Taking worldwide published reports on paratuberculosis vaccination available to us but not restricted to peer-reviewed papers, we have classified the studies according to species (cattle, sheep or goats), and type of evaluation of vaccine efficacy (production, epidemiological or pathogenetic effects). We have kept only those studies were the authors reported either vaccinated versus control group or pre-vaccination versus post-vaccination cohorts in numerical terms. In all, except in one study where a scoring system was used for MAP isolation, results were presented as the frequency of positive/affected individuals over total animals in the study. We have not been overly critical on the criteria applied by authors, but instead we have assumed that they knew well the disease and that their study design was sound.

All data have been transformed into a reduction percent calculated as the frequencies difference divided by the frequency in the control group. For each category of species and type of evaluation, we have calculated a size-weighted reduction average for the whole set of studies in that category. The same size-weighting method has been previously used to calculate a standard deviation in order to define the 95% confidence limits of the estimate [55].

**Results**

A total of 118 experiments from 63 reports and 14 countries have been used for the meta-analysis in this review (Tables 1 and 2). The USA was the country with the highest number of studies included (26.3%), followed by New Zealand (14.4%) and then closely by Spain (13.6%).
### Table 1 Countries where the vaccination experiments* used in the meta-analysis were carried out

| Country       | Number of Experiments | Percent |
|---------------|-----------------------|---------|
| Australia     | 12                    | 10.2    |
| Denmark       | 1                     | 0.8     |
| France        | 5                     | 4.2     |
| Germany       | 1                     | 0.8     |
| Greece        | 6                     | 5.1     |
| Hungary       | 1                     | 0.8     |
| Iceland       | 2                     | 1.7     |
| India         | 4                     | 3.4     |
| Netherlands   | 12                    | 10.2    |
| New Zealand   | 17                    | 14.4    |
| Norway        | 1                     | 0.8     |
| Spain         | 16                    | 13.6    |
| United Kingdom| 9                     | 7.6     |
| United States | 31                    | 26.3    |
| Total         | 118                   |         |

* An experiment is defined as vaccine trial whose results are measured according to one of the three outcome variables: clinical signs, MAP isolation, gross or microscopic lesions.

Some countries, such as the USA, have studies throughout the years, however, interest in MAP vaccination studies change among countries. For example, early large studies in the UK and France, gave way to studies in The Netherlands, New Zealand, Australia and Spain. This pattern might reflect MAP prevalence levels and research funding priorities in the different countries, but most likely it is also biased by administrative regulations limiting the availability of a successful commercial vaccine for sheep and goats (Gudair™), which is being widely used in countries with large sheep populations. 45 experiments were conducted in cattle, 49 in sheep, and 24 in goats (Table 2). Apart from the studies where small ruminants were used either because they were the target species of the commercial vaccine or because they are an easier to handle and a less costly animal model, there is a relation between the type of animal used in the study and the main livestock in the country.

Half of the studies are field trials where animals were naturally exposed to MAP. In these studies, results were assessed either by comparison between initial prevalence before vaccination, and final prevalence some time post-vaccination, or by following up a matched group within the same herd or flock. The later type of studies, when the control group is housed with vaccinated animals, frequently underestimates the positive effects of vaccination, because as herd immunity increases, bacterial shedding into the environment is reduced and thus the probability of a natural infection in the control group is also reduced. In three experiments the assessment was done using control unvaccinated herds, and one consisted of a questionnaire on clinical incidence in farms before and after using vaccination.

Tables 3, 4 and 5 summarize the results of all vaccination experiments used for the meta-analysis. Less than a third of them are not standard peer review journal publications (Doctoral Dissertations, non-peer review magazines, conference proceedings, bulletin reports, memoranda, or other types of documents). Some appear to be advances of results that have been published later. Since the information is different, we have treated them as individual experiments, although we were aware that they might introduce a bias to underestimate vaccination positive effects, particularly regarding culture results because of their lack of time span for the vaccine to make its mid- to long-term effects.

The vast majority of studies on all species showed positive reductions in all examined variables (Figure 2), that in cattle resulted in average reductions of 96.0%, 72.6% and 57.5% for production, epidemiological or pathogenetic effects, respectively. In sheep these reductions were of 67.5%, 76.4% and 89.7% and in goats of 45.1%, 79.3% and 94.8%, clearly demonstrating that MAP vaccination works well in all three species. The widest spread in reduction percentages, including several negative reduction rates, was observed with the epidemiological effects variable, which represents culture data. These differences are probably due to inherent aspects of each variable, since frequently the same study that gave negative reduction rates with the epidemiological variable, showed much better reduction results with the other variables, specially for the production effects variable. Most studies reported culture data as positive or negative result and did not include data on quantification of bacterial load in the sample. Thus, vaccinated animals with clinical signs reduction were still infected and excreted bacteria. This would imply that even though the amount of bacterial shedding might have been reduced, the proportion of shedding animals might have not. As a consequence, this would be in agreement with the widely accepted concept that, in general, current MAP vaccines can contain the infection and dramatically decrease clinical signs in a herd, but do not completely clear the infection.

Except for a few cases, vaccination in cattle was applied at early ages, in the first weeks of life, while in sheep more studies included adult sheep. The largest sample size studies, up to 150,000 animals, were done in cattle and preferentially recorded production effects in terms of

### Table 2 Experiments and reports used for the meta-analysis

| Species | Experiments | Reports* |
|---------|-------------|----------|
| Cattle  | 45          | 33       |
| Sheep   | 49          | 21       |
| Goats   | 24          | 9        |

* A report is a publication or communication that might contain results of one or more experiments.
| Name/Laboratory | Type    | Strain/Antigen | Adjuvant | Country and reference | Year | Number of animals | Age at vaccination | Reduction (%) | Type of trial |
|-----------------|---------|----------------|----------|-----------------------|------|-------------------|-------------------|---------------|---------------|
| NCV Live        | 6 strains | Oil | U.S.A. [65] | 1935 | 20 | 1 m | 100.00 | E, MC, CC |
| Weybridge Live  | 316F | P/O/P | U.K. [66] | 1959 | 63401 | 1 m | 93.45 | F, IF, CC |
| Weybridge Live  | 316F | P/O/P | U.K. [67] | 1964 | 2440 | 1 m | 98.36 | F, IF, CC |
| Weybridge Live  | 316F | P/O/P | U.K. [68] | 1965 | 84 | 1 w | 46.67 | E, MC, CC |
| Weybridge Live  | 316F | P/O/P | U.K. [69] | 1982 | 150000 | 1 m | 99.06 | F, IF, CC |
| Fromm Killed    | M. a. a strain 18 | Oil | U.S.A. [70] | 1983 | 48 | 1 m | 35.29 | F, MC, CC |
| - Live          | 316F | P/O/P | France [61] | 1988 | 902 | 1 m | 87.34 | F, IF, CC |
| - Live          | 316F | P/O/P | France [61] | 1988 | 1037 | 1 m | 97.22 | F, IF, CC |
| Lelystad Killed | - | Oil | Netherlands [59] | 1988 | 851 | 1-24 m | 87.05 | F, IF, CC |
| Lelystad Killed | - | Oil | Netherlands [71] | 1992 | 61050 | 1 m | 91.82 | F, MC, CC |
| NCV Killed      | - | Oil | Netherlands [72] | 1994 | 337 | 1 m | 79.01 | F, IF, CC |
| NCV Killed      | - | Oil | Netherlands [37] | 1996 | 573 | 1 m | 68.14 | F, CC |

**Average** 96.02 ± 0.01

| Sheep |
|-----------------|---------|----------------|----------|-----------------------|------|-------------------|-------------------|---------------|---------------|
| NCV Live        | 316F | Oil Paraffin | Greece [73] | 1988 | 1448 | 1 m | 76.14 | F, MC, TM |
| NCV Live        | 316F | Oil Paraffin | Greece [73] | 1988 | 5526 | Adults | 28.74 | F, MC, TM |
| Lio-Johne Live  | 316F | Oil | Spain [74] | 1993 | 1201 | Adults | 78.29 | F, MC, CC |
| Lio-Johne Live  | 316F | Oil | Spain [75] | 1995 | 570 | 1 m | 52.55 | F, MC, TM |
| Weybridge Live  | 316F | P/O/P | U.K. [76] | 1993 | 830 | Adults | 89.86 | F, IF, CC |
| Neoparasec & NCV Live & Killed | 316F | Oil | Spain [77] | 1995 | 857 | Adults | 54.55 | F, IF, CC |
| Neoparasec Live| 316F | Oil | New Zealand [78] | 2000 | 28 | 1-1.5 m | 71.43 | E, MC, CC |
| Gudair Killed   | 316F | Oil | Australia [79] | 2003 | 8000 | 3, 8, 2 y | 87.50 | F, IF, mortality rate |
| Gudair Killed   | 316F | Oil | Australia [80] | 2004 | 1200 | 1-4 m | 90.00 | F, MC, mortality reduction |
| Gudair Killed   | 316F | Oil | Australia [34] | 2006 | 400 | 1-3 m | 91.25 | F, MC, TM |
| Gudair Killed   | 316F | Oil | New Zealand [81] | 2009 | 65 | 4 m | 78.57 | E, MC, CA |
| NCV Killed      | 316F | Lipid-K formulation | New Zealand [81] | 2009 | 65 | 4 m | 57.14 | E, MC, CA |
| NCV Live        | 316F | Lipid-K formulation | New Zealand [81] | 2009 | 65 | 4 m | 14.29 | E, MC, CA |
| NCV Live        | 316F | Lipid-K formulation | New Zealand [81] | 2009 | 65 | 4 m | 35.71 | E, MC, CA |

**Average** 67.57% ± 0.35

| Goats |
|-----------------|---------|----------------|----------|-----------------------|------|-------------------|-------------------|---------------|---------------|
| NCV Live        | 316F | Oil Paraffin | Greece [73] | 1988 | 2178 | 1 m | 82.78 | F, MC, TM |
| NCV Live        | 316F | Oil Paraffin | Greece [73] | 1988 | 7773 | Adults | 34.52 | F, MC, TM |

**Average** 45.08 ± 0.39

NCV: non-commercial vaccine; Weybridge: Central Veterinary Laboratory, Weybridge, UK; Fromm: Fromm Laboratories, Grafton, Wisconsin USA; Lelystad: Central Veterinary Institute, Lelystad, The Netherlands; Lio-Johne, Ovejero, Spain; Neoparasec: Neoparasec®; Merial; Gudair: Gudair®; CZ Veterinaria/Pfizer; P/O/P: Paraffin, Olive Oil, Pumice Stone Powder; y: year(s); m: month(s); w: week(s); d: day(s); F: Field trial; E: Experimental infection; MC: Comparison to matched controls; IF: Comparison of initial versus final prevalence; TM: Total mortality; CC: clinical cases; NVH: Comparison to non-vaccinating herds.
| Name/Laboratory | Type | Strain/Antigen | Adjuvant | Country and reference | Year | Number of animals | Age at vaccination | Reduction (%) | Type of trial |
|----------------|------|----------------|----------|-----------------------|------|-------------------|-------------------|--------------|---------------|
| NCV            | Live | 6 strains     | Oil      | U.S.A. [65]           | 1935 | 20                | 1 m               | -14.29       | E, TC         |
| Weybridge      | Live | 316F          | P/O/P    | U.K. [68]             | 1965 | 84                | 1 w               | 11.54        | E, MC, TC     |
| Weybridge      | Live | 316F          | P/O/P    | Australia [82]        | 1971 | 82                | 1 m               | 24.18        | F, IF, MC, TC |
| NCV            | Live | avirulent     | P/O/P    | U.S.A. [83]           | 1974 | 16                | 16 d              | 81.47        | E, MC, FC     |
| NCV            | Live | avirulent     | P/O/P    | U.S.A. [83]           | 1974 | 16                | 16 d              | 0.00         | E, MC, TC     |
| Fromm          | Killed | M. a. strain 18 | Oil    | U.S.A. [70]           | 1983 | 158               | 1 m               | 79.28        | F, MC, FC     |
| Fromm          | Killed | M. a. strain 18 | Oil    | U.S.A. [70]           | 1983 | 3060              | 1 m               | 99.11        | F, IF, FC     |
| NCV            | Live | 316F          | Oil      | Denmark [84]          | 1983 | 5446              | 1 m               | 92.90        | F, MC, FC     |
| Lelystad       | Killed | -            | Oil      | Netherlands [71]      | 1992 | 2065              | 1 m               | -21.25       | F, IF, FC     |
| NCV            | Live | 316F          | P/O/P    | France [85]           | 1992 | 22988            | 1 m               | 81.68        | F, IF, MC, FC |
| Phylaxia       | Killed | 5889 Bergey  | Oil      | Hungary [86]          | 1994 | 2738              | 1 m               | 94.70        | F, IF, FC     |
| NCV            | Killed | -            | Oil      | Netherlands [72]      | 1994 | 499               | 1 m               | -36.72       | F, IF, TC     |
| NCV            | Killed | -            | Oil      | Netherlands [37]      | 1996 | 573               | 1 m               | 13.34        | F, IF, TC     |
| Mycopar        | Killed | M. a. strain 18 | Oil    | U.S.A.[87]            | 2000 | 372               | < 35 d            | 71.43        | F, MC, FC     |
| NCV            | Killed | -            | Oil      | Netherlands [88]      | 2001 | 4452              | 1 m               | 33.83        | F, NVH, FC    |
| Neoparasac     | Live  | 316F          | Oil      | Germany [89]          | 2002 | 521               | 1 m               | 86.87        | F, MC, FC     |
| Mycopar        | Killed | M. a. strain 18 | Oil    | U.S.A. [58]           | 2003 | 10                | 7 d               | -28.00       | E, MC, FC, TC |
| Mycopar        | Killed | M. a. strain 18 | Oil    | U.S.A. [58]           | 2003 | 10                | 7 d               | 32.00        | E, MC, FC, TC |
| Neoparasac     | Live  | 316F          | Oil      | Spain [90]            | 2005 | 14                | 2 m               | 62.50        | E, MC, TC     |
| NCV            | Rec   | Hsp70         | DDA      | Netherlands [39]      | 2006 | 20                | 1 m               | 37.50        | E, MC, FC     |
| Mycopar        | Killed | M. a. strain 18 | Oil    | U.S.A. [91]           | 2006 | 213               | < 35 d            | 77.12        | F, MC, FC     |
| NCV            | Rec   | MAP (85A, 85B, 85C, SOD) | MPLA +/- IL12 RIBI | U.S.A. [92] | 2008 | 24               | 5-10 d            | 41.67        | E, MC, FC, TC |
| Silirum        | Killed | 316F          | Oil      | Spain [49]            | 2009 | 371               | all ages          | 68.20        | F, IF, FC, FP |
| Average        |       |               |          |                       |      |                   |                   | 72.55 ± 0.29 |              |

**Sheep**

| Name/Laboratory | Type | Strain/Antigen | Adjuvant | Country and reference | Year | Number of animals | Age at vaccination | Reduction (%) | Type of trial |
|-----------------|------|----------------|----------|-----------------------|------|-------------------|-------------------|--------------|---------------|
| NCV             | Killed | 101 sheep & VB/4 cattle | Oil    | U.K. [94]             | 1961 | 44                | 1 m               | 52.63        | E, MC, TC     |
| NCV             | Killed | -              | Oil      | U.K. [95]             | 1962 | 126               | 1 m               | 29.05        | E, MC, TC     |
| Lio-Johne       | Live  | 316F          | Oil      | Spain [74]            | 1993 | 1201              | Adults            | 80.01        | F, MC, TC     |
| Neoparasac      | Live  | 316F          | Oil      | Spain [96]            | 1994 | 13                | 2 m               | 38.89        | E, MC, TC     |
Table 4 Epidemiological effects (*Mycobacterium avium* subsp. *paratuberculosis* isolation from faeces or tissues). (Continued)

| Vaccine                  | Type          | Oil/Paraffin   | Country | Year | Month(s) | Prevalence |
|-------------------------|---------------|----------------|---------|------|----------|------------|
| Neoparasec & NCV        | Live          | Oil            | Spain [77] | 1995 | 97       | Adults -10.95 |
| NCV                     | Killed        | Oil Paraffin   | Greece [97] | 1997 | 226      | 1 m 93.27 F, IF, TC |
| Neoparasec              | Live          | Oil            | New Zealand [78] | 2000 | 28       | 1-1.5 m 66.67 E, MC, TP |
| Gudair                  | Killed        | Oil            | Australia [80] | 2004 | 1200     | 1-4 m 90.00 F, MC, FC |
| Gudair                  | Killed        | Oil            | Australia [98] | 2005 | -        | 16 w 52.21 F, IF, FC |
| Gudair                  | Killed        | Oil            | Australia [34] | 2006 | 400      | 1 m 76.14 F, MC, FC |
| Gudair                  | Killed        | Oil            | Australia [34] | 2006 | 400      | 1 m 84.15 F, MC, FC |
| Gudair                  | Killed        | Oil            | Australia [99] | 2007 | 998      | 2-3 m 76.14 F, MC, FC |
| Gudair                  | Killed        | Oil            | New Zealand [81] | 2009 | 62       | 4 m 25.30 E, MC, FC |
| NCV                     | Killed        | Oil            | New Zealand [81] | 2009 | 62       | 4 m 36.03 E, MC, FC |
| NCV                     | Live          | Lipid-K formulation | New Zealand [81] | 2009 | 62       | 4 m 36.03 E, MC, FC |
| NCV                     | Live          | Lipid-K formulation | New Zealand [81] | 2009 | 62       | 4 m 34.09 E, MC, FC |

**Average**: 76.42 ± 0.54

| Vaccine                  | Type          | Oil/Paraffin   | Country | Year | Month(s) | Prevalence |
|-------------------------|---------------|----------------|---------|------|----------|------------|
| Neoparasec              | Live          | Oil            | France [100] | 1988 | 27       | 1 m 73.08 E, MC, FC |
| Neoparasec              | Live          | Oil            | France [100] | 1988 | 26       | 1 m 51.01 E, MC, TC |
| Fromm                   | Killed        | Freund's Complete | U.S.A. [101] | 1988 | 1075     | 1 m 80.23 F, MC, FC |
| NCV                     | Killed        | Oil Paraffin   | Greece [97] | 1997 | 297      | 1 m 95.57 F, NVH, FC |
| NCV                     | Killed        | Goat isolate (CWD) | QS21 | U.S.A. [102] | 2007 | 20 | 1-4 w 61.69 E, MC, FC, TC |
| NCV                     | Killed        | Goat isolate (CWC) | QS21 | U.S.A. [102] | 2007 | 20 | 1-4 w 85.19 E, MC, FC, TC |
| NCV                     | Killed        | Goat isolate (CWC) | Alum | U.S.A. [102] | 2007 | 20 | 1-4 w 79.31 E, MC, FC, TC |
| NCV                     | Killed        | Goat isolate (CWD) | Alum | U.S.A. [102] | 2007 | 20 | 1-4 w -57.68 E, MC, FC, TC |
| NCV                     | Killed        | Virulent Field Strain | Alum | India [48] | 2007 | 55 | 4-6 m 82.14 E, MC, FC |
| Gudair                  | Killed        | MAP (85A, 85B, SOD, 74F) | India [48] | 2007 | 55 | 4-6 m 52.38 E, MC, FC |
| NCV                     | Rec           | MAP (85A, 85B, SOD, 74F) | DDA | U.S.A. [40] | 2009 | 17 | 5-10 d 87.50 E, MC, TC |
| NCV                     | Rec           | MAP (85A, 85B, SOD, 74F) | none | U.S.A. [40] | 2009 | 17 | 5-10 d 37.50 E, MC, TC |

**Average**: 79.34 ± 0.89

NCV: non-commercial vaccine; Weybridge: Central Veterinary Laboratory, Weybridge, UK; Fromm: Fromm Laboratories, Grafton, Wisconsin USA; Lelystad: Central Veterinary Institute, Lelystad, The Netherlands; Phylaxia: Phylaxia Veterinary Biologicals Company, Budapest; Mycopar®: Mycopar Fort Doge/Solvay, USA; Neoparasec: Neoparasec®, Merial; Silirum: Silirum®, CZ Veterinaria/Pfizer; Lio-Johne, Ovejero, Spain; Gudair: Gudair®, CZ Veterinaria/Pfizer; Rec: recombinant; CWD Cell Wall Deficient MAP; CWC Cell Wall Competent MAP; P/O/P Paraffin, Olive Oil, Pumice Stone Powder; y: year(s); m: month(s); w: week(s); d: day(s); F: Field trial; E: Experimental infection; MC: Comparison to matched controls; IF: Comparison of initial versus final prevalence; NVH: Comparison to non-vaccinating herds; TC: Tissue culture; FC: Fecal culture.
| Name/Laboratory          | Type       | Strain/Antigen | Adjuvant | Country and reference     | Year | Number of animals | Age at vaccination | Reduction (%) | Type of trial |
|-------------------------|------------|----------------|----------|---------------------------|------|-------------------|-------------------|---------------|---------------|
| Cattle                  |            |                |          |                           |      |                   |                   |               |               |
| NCV Live 6 strains      | Oil        | U.S.A. [65]    | 1935     | calves                    | 20   | 42.86             | E, HP             |               |               |
| NCV Live avirulent P/O/P| Oil        | U.S.A. [83]    | 1974     | 16 d                      | 16   | 17.24             | E, HP             |               |               |
| Lelystad Killed         | None       | Netherlands [71]| 1992     | 1 m                       | 3209 | 58.34             | F, IF, HP         |               |               |
| NCV Killed              | Oil        | Netherlands [72]| 1994     | 1 m                       | 499  | 57.23             | F, IF, HP         |               |               |
| NCV Killed              | Oil        | Netherlands [37]| 1996     | 1 m                       | 573  | 58.09             | F, IF, HP         |               |               |
| Silirum Killed 316F     | Oil        | Spain [103]    | 2005     | all ages                  | 79   | 38.68             | F, MC, HP         |               |               |
| Silirum Killed 316F     | Oil        | Spain [90]     | 2005     | 2 m                       | 14   | 37.50             | E, MC, HP         |               |               |
| **Average**             |            |                |          |                           |      | 57.54 ± 0.11      |                   |               |               |
| Sheep                   |            |                |          |                           |      |                   |                   |               |               |
| NCV Killed              | -          | Oil            | Iceland [54]| 1960          | 419  | 83.58             | F, MC, PM         |               |               |
| NCV Killed              | -          | Oil            | Iceland [54]| 1960          | 2432 | 93.55             | F, MC, PM         |               |               |
| NCV Killed              | Oil        | U.K. [95]      | 1962     | 1 m                       | 126  | 52.22             | E, MC, HP         |               |               |
| Lio-Johne Live 316F     | Oil        | Spain [74]     | 1993     | 1 m                       | 570  | 100.00            | F, MC, HP         |               |               |
| Lio-Johne Live 316F     | Oil        | Spain [74]     | 1993     | Adults                    | 1201 | 53.36             | F, MC, HP         |               |               |
| Neoparasec Live 316F    | Oil        | Spain [96]     | 1994     | 2 m                       | 13   | 64.52             | E, MC, HP         |               |               |
| Neoparasec Live 316F    | Oil        | Australia [104]| 1995     | 3 m                       | 475  | 82.27             | F, MC, HP         |               |               |
| Neoparasec & Gudair     | 316F Oil   | Spain [77]     | 1995     | Adults                    | 135  | -3.03             | F, IF, HP,       |               |               |
| Neoparasec Live 316F    | Oil        | New Zealand [78]| 2000   | 1-1.5 m                   | 28   | 77.78             | E, MC, HP         |               |               |
| Gudair Killed 316F      | Oil        | Spain [105]    | 2002     | 1 m                       | 12   | 100.00            | E, MC, HP         |               |               |
| Mycopar Killed          | Oil        | U.S.A. [106]   | 2005     | 60-164 d                  | 178  | 75.31             | F, MC, HP         |               |               |
| Neoparasec Live 316F    | Oil        | New Zealand [57]| 2005   | 2-4 w                     | 59   | 68.52             | E, MC, HP         |               |               |
| AquaVax Live 316F       | saline     | New Zealand [57]| 2005   | 2-4 w                     | 58   | -2.48             | E, MC, HP         |               |               |
| Gudair Killed 316F      | Oil        | Australia [34] | 2006   | 1-3 m                     | 88   | 72.70             | F, MC, GL, HP     |               |               |
| Gudair Killed 316F      | Oil        | Australia [34] | 2006   | 1-3 m                     | 307  | 48.29             | F, MC, GL, HP     |               |               |
| Gudair Killed 316F      | Oil        | New Zealand [81]| 2009   | 4 m                       | 62   | 75.57             | E, MC, HP         |               |               |
| NCV Killed 316F Lipid-K formulation | New Zealand [81]| 2009 | 4 m                     | 63   | 37.17             | E, MC, HP         |               |               |
| NCV Live 316F Lipid-K formulation | New Zealand [81]| 2009 | 4 m                     | 63   | 51.32             | E, MC, HP         |               |               |
| NCV Live 316F Lipid-K formulation | New Zealand [81]| 2009 | 4 m                     | 62   | 57.56             | E, MC, HP         |               |               |
| **Average**             |            |                |          |                           |      | 89.70 ± 0.15      |                   |               |               |
| Goats                   |            |                |          |                           |      |                   |                   |               |               |
| NCV Live 2E/316F        | P/O/P      | Norway [26]    | 1985     | 1 m                       | 5535 | 97.18             | F, IF, PM         |               |               |
| Gudair Killed 316F      | Oil        | Spain [38]     | 2000     | Adults                    | 189  | 65.88             | F, MC, HP         |               |               |
| NCV Killed Goat isolate (CWD) | QS21 | U.S.A. [102] | 2007 | 1 w                      | 20   | 34.38             | E, MC, HP         |               |               |
| NCV Killed Goat isolate (CWD) | QS21 | U.S.A. [102] | 2007 | 1 w                      | 20   | 32.03             | E, MC, HP         |               |               |
| Pathogenetic effects (histopathological lesions). (Continued) |
|----------------------------------|
| NCV | Killed | Goat isolate (CWC) | Alum | U.S.A. [102] | 2007 | 20 | 1 w | 44.53 | E, MC, HP |
| NCV | Killed | Goat isolate (CWD) | Alum | U.S.A. [102] | 2007 | 20 | 1 w | -17.19 | E, MC, HP |
| NCV | Killed | Virulent Field Strain | Alum | India [48] | 2007 | 8 | 4-6 m | 75.00 | E, MC, HP |
| Gudair | Killed | 316F | Oil | India [48] | 2007 | 8 | 4-6 m | 50.00 | E, MC, HP |
| NCV | Rec | MAP(85A, 85B, SOD, 74F) | DDA | U.S.A. [40] | 2009 | 17 | 5-10 d | 66.67 | E, MC, HP |
| NCV | Rec | MAP(85A, 85B, SOD, 74F) | none | U.S.A. [40] | 2009 | 17 | 5-10 d | 33.33 | E, MC, HP |

Average 94.79% ± 0.29

NCV: non-commercial vaccine; Lelystad: Central Veterinary Institute, Lelystad, The Netherlands; Silirum: Silirum®; CZ Veterinaria/Pfizer; Lio-Johne, Ovejero, Spain; Neoparasec: Neoparasec®; Merial; Gudair: Gudair®; CZ Veterinaria/Pfizer; Mycopar®: Mycopar Fort Doge/Solvay, USA; AquaVax; Rec: recombinant; CWD Cell Wall Deficient MAP; CWC Cell Wall Competent MAP; P/O/P Paraffin, Olive Oil, Pumice Stone Powder; y: year(s); m: month(s); w: week(s); d: day(s); F: Field trial; E: Experimental infection; MC: Comparison to matched controls; IF: Comparison of initial versus final prevalence; GL: Gross lesions; HL: Histological lesions.
paratuberculosis culling rates, since measurement of the other variables is much more time-consuming and costly. This is also reflected in the median sample size for the studies that looked at the production variable, 876, 700, and 4975 animals for cattle, sheep and goat studies, respectively, while studies analyzing the epidemiological or pathogenetic variables had median sizes around 100 or less.

The range of study length was between a few months and 16 years covering a period of 74 years. A large increase in sheep studies in the last decade coincided with the availability of the successful small ruminant commercial vaccine Gudair™ and its extended application in Australia and New Zealand. In the majority of the studies (68 experiments) killed vaccines were used. Most experiments used MAP strain 316F from Weybridge, nine used Strain 18 (now known to be *M. avium* subsp. *avium* rather than MAP [56]), and the rest used local isolates or subunit vaccines consisting of recombinant proteins. Not surprisingly, 316F is the most frequently used strain in sheep studies, since the above mentioned commercial vaccine for sheep and goats is based on this strain.

Bacterial content varied widely, from 1000 CFU to $3 \times 10^9$ CFU, and from 2.5 mg to 100 mg. The large majority of studies used some type of oily adjuvant (mineral oil, olive oil, liquid paraffin etc.) and in very few cases alum. In one study [57], AquaVax experimental vaccine was used, which contains no adjuvant but saline instead. More recent studies have started using other newer adjuvants such as MPLA, RIBI, cytokines, DDA, QS21, and lipid formulations, some of them with good results.

**Discussion**

A wide variation in the efficacy of vaccines was observed, especially in cattle and sheep, where negative reductions are described in some studies. However, the overall results are pretty homogeneous, with very small error ranges due to the large numbers of observations included. In general, vaccine strain or administration route differences do not seem to substantially alter the outcome of vaccination, however, type of antigen formulation or adjuvant appears to have been important in a few experimental studies where different formulations were compared side by side [58].

The goal of this review was to evaluate vaccination as a whole, summarizing the results into a single table for each type of measure used to determine vaccine success. This analysis has revealed that, in average, vaccination...
has a positive effect. However, since the efficacy figures are rather poor in comparison to vaccines for other microorganisms, it is relevant to at least try to discuss possible reasons for some of the low success rates. In order to simplify, one possible approach is to find an explanation for studies where vaccination performed below the average. Production effect studies where the measurement was total mortality may be considered flawed because in most cases mortality did not differentiate between paratuberculosis and other pathologies, possibly diluting the “vaccine effect”. This is evident in the case of sheep and goat trials were adults were considered. Since the more sensitive part of the population might have already died of paratuberculosis before vaccination the remaining animals could be considered more resistant to paratuberculosis, and therefore, less likely to show any effect of vaccine protection. Because young animal studies showed larger effects, this becomes a very likely explanation for low reduction rates. An additional explanation for the poor results could be the fact that, frequently, vaccination programs coincide with the initiation of other control measures making it difficult to assess the real effect of the vaccine on paratuberculosis control.

Under the epidemiological effects analyzed, reduction in the proportion of fecal shedders appears to be one of the measurements showing the widest variability. This happens mostly in small studies or in studies carried out in the Netherlands. Besides the qualitative effects in terms of protection conferred, vaccination should be assessed from another, perhaps even more important standpoint, such as is the reduction of amounts of bacteria shed by vaccinated and non-vaccinated animals.

When considering reduction in pathogenetic effects, it should be pointed out that some of the studies had a very short follow up and that the presence of focal lesions of paratuberculosis are weighted the same as the presence of large areas of affected intestine.

Since the vast majority of the studies show a positive effect, the question as to why vaccination has not been given more opportunities comes out with force. Especially, because for years MAP eradication efforts have only shown very moderate success or straight failure due to their enormous costs and frequent relapses of infected animals. Already in the eighties [59] and nineties [37,60] several studies showed the profitability of vaccination. Over a period of a few years, the economic advantages of vaccination may be up 20 times higher than any testing and culling strategy which, in addition to yielding uncertain results, it results in a much higher economic cost. Other strategies based on certification are compatible with vaccination, and moreover, vaccination might allow a spectrum of other approaches to paratuberculosis control dependant on the financial resources of the farm, region or farmers association, and the actual economic losses sustained by the enterprise. It has been estimated that only a 5% annual clinical incidence of paratuberculosis will justify entering a mixed vaccination and testing and culling strategy [61].

In our opinion, there is a mixture of vested interests on control programs based in testing and culling, simplistic thinking comparing tuberculosis and paratuberculosis, fear of cross-reactions, academic detachment and confusion between ideal objectives and practical needs for the livestock industry. It is clear that the main reason for the opposition to MAP vaccination in cattle has been the problem of expected interference with the diagnosis of tuberculosis and its consequences in trade and national TB programs, however, the availability of an OIE official test -the comparative intradermal tuberculin test- that can very easily solve this problem in the majority of cases, should eliminate this concern on MAP vaccination in cattle. Recent field vaccination trials in cattle with an experimental MAP vaccine (Silirim™, CZV), have shown that less than 0.5% of vaccinated animals will give interference problems when the comparative intradermal tuberculin test is used even if the most restrictive interpretation of results proposed by the OIE is applied (Joseba Garrido, personal communications). The benefits obtained from production increases and reduction in clinical cases of MAP, will largely outweigh the small loss due to culling of these tuberculosis cross-reactive animals. In addition, new plans for the introduction of improved tuberculosis vaccines for cattle [62], will also affect the prospects of MAP vaccination in cattle, since the accompanying DIVA diagnostic test will probably allow for the identification of MAP infected or vaccinated animals.

MAP vaccination concerns in cattle have been further aggravated by the fear of the dairy industry to a crisis of confidence in their products, particularly, if a potential zoonotic link between paratuberculosis and a human disease (IBD/Crohn’s disease) is confirmed [63] or if too much discussion and research efforts are focused on this subject. At this moment in which the paratuberculosis scientific community has finally accepted that the key to the paratuberculosis problem might not be eradication, but just control, vaccination offers the solution to this problem, since it not only allows to confine the paratuberculosis problem within the limits of a livestock production issue, while downright calming the worries of farmers, but also provides the perfect cover for doing something against paratuberculosis from a Public Health point of view, without incurring in massive costs. Vaccination might be the beginning of the end of the huge worldwide paratuberculosis problem and might mark the difference between doing nothing and advancing towards global control [64].
Conclusions

Paratuberculosis control poses a though challenge for farmers and veterinarians. Test and cull strategies can be useful in some settings but do not seem to have reached extensive success. Control by vaccination is an alternative that has been longtime in use in some regions and species. A substantial number of vaccination studies where objective information is amenable to meta-analysis treatment have been published in peer reviewed journals or in conference proceedings or other media. The high heterogeneity among reports makes it difficult to accept that the narrow statistical confidence interval obtained in these meta-analyses actually represents the true range of reduction in the whole set of trials. However, the results analyzed here clearly show a general positive effect from vaccination, negative effects only in a few trials, and a positive average balance according to all three variables considered (production, epidemiological or pathogenetic effects).

In terms of quantitative reduction, the minimum is an 11% reduction in MAP isolation, which could be considered the worst case average, but with a common outcome at over 50% which is highly profitable from a production point of view. This strategy thus has high chances of have a effect on the overall environmental contamination with MAP, which would mean a significant advance in the fight against paratuberculosis, both in the animal and in the (potential) human public health fields.

Abbreviations

CC: Clinical Cases; CWC: Cell Wall Competent; CWD: Cell Wall Deficient; E: Experimental infection; F: Field trial; FC: Fecal culture; GL: Gross lesions; HL: Histological lesions; IBD: Inflammatory Bowel Disease; IF: Comparison of initial versus final prevalence; MAP: Mycobacterium avium subsp. paratuberculosis; MC: Comparison to matched controls; NOV: Non-commercial vaccine; NNV: Comparison to non-vaccinating herds; PCR: Polymerase Chain Reaction; PPDbov: Purified Protein Derivative from Mycobacterium bovis; PPDav: Purified Protein Derivative from Mycobacterium avium subsp. avium; P/D/P: Paraffin, Olive Oil, Pumice Stone Powder; Rec: Recombinant; TC: Tissue culture; TM: Total mortality; PCR: Polymerase Chain Reaction; MAP: Mycobacterium avium subsp. paratuberculosis; IBD: Inflammatory Bowel Disease.

Author details

Vacunek, Bizkaiko Teknologia Parkea, Ibaizabal Bidea 800, Debro 48160, Bizkaia, Spain. *NEIKER-Tecnalia, Department of Animal Health, Berreaga 1, 48160 Debro, Bizkaia, Spain.

Authors’ contributions

RAJ conceived of the study and performed the statistical analysis. Both authors (FB and RAJ) participated in the design of the study, acquisition of data and helped to draft the manuscript. Both read and approved the final manuscript.

Competing interests

Felix Bastida works for Vacunek, a small animal health biotechnology company. He is currently working on the development of a new paratuberculosis vaccine for cattle in collaboration with NEIKER and CZ Veterinaria, the producer of Guadär®, a commercial paratuberculosis vaccine for use in sheep and goats.

Ramon A. Juste works for a Regional Government funded Agricultural Research Institute that receives funding for research projects from local, regional, national and European Governments, as well as from, companies such as CZ Veterinaria and Vacunek.

Received: 18 May 2011 Accepted: 31 October 2011
Published: 31 October 2011

References

1. Chioldini RJ, Van Kruiningen HJ, Merkal RS: Ruminant paratuberculosis (Johnes disease): the current status and future prospects. Cornell Vet 1984, 74:218-262.
2. Chioldini RJ, Van Kruiningen HJ: Eastern white-tailed deer as a reservoir of ruminant paratuberculosis. J Am Vet Med Assoc 1983, 182:168-169.
3. Manning EJ: Mycobacterium avium subspecies paratuberculosis: a review of current knowledge. J Zoo Wild Med 2001, 32:293-304.
4. Pickup RW, Rhodes G, Bull TJ, Amott S, Sidi-Boumedine K, Hurley M, Henman-Taylor J: Mycobacterium avium subsp. paratuberculosis in lake catchments, in river water abstracted for domestic use, and in effluent from domestic sewage treatment works: diverse opportunities for environmental cycling and human exposure. Appl Environ Microbiol 2006, 72:4067-4077.
5. Stop the cull. Animal vaccines prevent disease but founder because of political motivations. Nat Biotechnol 2001, 19:1239.
6. Torgerson P, Torgerson D: Benefits of stemming bovine TB need to be demonstrated. Nature 2009, 457:657.
7. Rossiter CA, Burhans WS: Farm-specific approach to paratuberculosis (Johnes disease) control. Vet Clin North Am Food Anim Pract 1996, 12:383-415.
8. Goodger WJ, Collins MT, Nordlund KV, Eisele C, Pelletier J, Thomas CB, Sackett DC: Epidemiologic study of on-farm management practices associated with prevalence of Mycobacterium paratuberculosis infections in dairy cattle. J Am Vet Med Assoc 1996, 208:1877-1881.
9. Whittington RJ, Marshall DI, Nichols PJ, Marsh IB, Reddaff LA: Survival and dormancy of Mycobacterium avium subsp. paratuberculosis in the environment. Appl Environ Microbiol 2004, 70:2989-3004.
10. Whittington RJ, Marsh IB, Reddaff LA: Survival of Mycobacterium avium subsp. paratuberculosis in dam water and sediment. Appl Environ Microbiol 2005, 71:5304-5308.
11. Muskens J, Elbers AR, van Weerding HJ, Noordhuizen JP: Herd management practices associated with paratuberculosis seroprevalence in Dutch dairy herds. J Vet Med B Infect Dis Vet Public Health 2003, 50:372-377.
12. Kudahl AB, Sorensen JT, Nielsen SS, Ostergaard S: Comparison of different detection methods for detection of MAP super-shedder cows in a large dairy herd. Veterinary Microbiology 2007, 121:118-129.
13. Zimmer K, Drager KG, Klawonn W, Hess RG: Contribution to the diagnosis of Johnes’ disease in cattle. Comparative studies on the validity of Ziehl-Neelsen staining, faecal culture and a commercially available DNA-Probe test in detecting Mycobacterium paratuberculosis in faeces from cattle. Zentralbl Veterinärmed B 1999, 46:137-140.
14. Whitlock RH, Wells SJ, Sweeney RW, Van Tiem J: ELISA and fecal culture for paratuberculosis (Johnes’ disease): sensitivity and specificity of each method. Vet Microbiol 2000, 77:387-398.
15. Ayl S, Anderson R, Gardiner I, Whitlock R, Fryack T, Adaika J: Comparison of methods for detection of MAP super-shedder cows in a large dairy herd. Johnes Disease Integrated Program (JDIP) Annual Meeting, Michigan 2008.
16. Collins MT, Gardiner IA, Garry FB, Roussel AJ, Wells SJ: Consensus recommendations on diagnostic testing for the detection of paratuberculosis in cattle in the United States. J Am Vet Med Assoc 2006, 229:1912-1919.
17. Almovri CA, Ward MP, Lin TL, Wu CC: Sample handling substantially affects Johnes’ ELISA, Prev Vet Med 2009, 90:278-283.
18. Garrido JM, Aduriz G, Geijo MV, Sevilla I, Juste RA: Comparison of different indirect ELISA methods for reference cattle. In Proceedings of 7th International Colloquium on Paratuberculosis, June 11-14, 2002, Bilbao, Spain. Edited by: Juste RA. International Association for Paratuberculosis, 2002:52-53.
19. Dieguez FJ, Gonzalez AM, Menendez S, Vilar MU, Sanjuan ML, Yus E, Amaz I: Evaluation of four commercial serum ELISAs for detection of Mycobacterium avium subsp. paratuberculosis infection in dairy cows. Vet J 2009, 180:231-235.
mycobacterial antigens as fusion proteins with green fluorescent protein. Infect Immun 1999, 67:4243-4250.

42. Sechi LA, Mara L, Cappi P, Frothingham R, Ortu S, Leonc A, Ahmed N, Zanetti S: Immunization with DNA vaccines encoding different mycobacterial antigens elicits a Th1 type immune response in lambs and protects against Mycobacterium avium subspecies paratuberculosis infection. Vaccine 2006, 24:229-235.

43. Kadam M, Shurl D, Bhagat J, Tiwari V, Prasad N, Goswami P: Coexpression of 16.8 kDa antigen of Mycobacterium avium avium subspecies paratuberculosis and murine gamma interferon in a bacilinostic vector and studies on its potential as DNA vaccine. Vet Res Commun 2000, 23:597-610.

44. Rouppe V, Leroy B, Rosseels V, Piersoel V, Noel-Georis P, Romano M, Govaerts M, Letesson JJ, Watte R, Huygen K: Immunogenenicty and protective efficacy of DNA vaccines encoding MAP5086C and MAP4308C of Mycobacterium avium subspecies paratuberculosis secretome. Vaccine 2008, 26:4783-4794.

45. Park SJ, Karhaperumal K, McDonough S, Akey B, Huntley J, Bannantine J, Chang Y: Immunization with a DNA vaccine cocktail induces a Th1 response and protects mice against Mycobacterium avium subspecies paratuberculosis challenge. Vaccine 2008, 26:4329-4337.

46. Bull TJ, Gilbert SC, Sirtud S, Linedale R, Dierkes N, Sidi-Boumedine K, Hermann-Taylor J: A novel multi-antigen virally vectored vaccine against Mycobacterium avium subspecies paratuberculosis. PLoS ONE 2007, 2:e1229.

47. Huntley JF, Bannentine J, Paustian ML, Reinhardt TA, Bannantine J: Expression library immunization confers protection against Mycobacterium avium subspecies paratuberculosis infection. Infect Immun 2005, 73:6877-6884.

48. Singh SV, Singh PK, Singh AV, Sahal JS, Gupta VK, Vihan VS: Comparative efficacy of an indigenous 'inactivated vaccine' using highly pathogenic field strain of Mycobacterium avium subspecies paratuberculosis 'Bison type' with a commercial vaccine for the control of Capri-paratuberculosis in India. Vaccine 2007, 25:7102-7110.

49. Juste RA, Alonso-Hearn M, Molina E, Geijo M, Vazquez P, Sevilla IA, Garrido JM: Significant reduction in bacterial shedding and improvement in milk production in dairy farms after the use of a new inactivated paratuberculosis vaccine in a field trial. BMC Res Notes 2009, 2:233.

50. Windsor P: Research into vaccination against ovine Johne's disease in Australia. Small Ruminant Research 2006, 62:139-142.

51. Emery DL, Whittington RJ: An evaluation of myophage therapy, chemotheraphy and vaccination for control of Mycobacterium avium subspecies paratuberculosis infection. Vet Microbiol 2004, 104:143-155.

52. Rosseels V, Huygen K: Vaccination against paratuberculosis. Expert Rev Vaccines 2008, 7:817-832.

53. Vallée H, P: Études sur l'enterite paratuberculeuse des bovins (note préliminaire). Rev Gen Med Vet 1926, 35:1-9.

54. Sigurdsson Ó: A killed vaccine against paratuberculosis (Johne's disease) in sheep. Am J Vet Res 1960, 21:54-62.

55. Domenach JM: Bioestadistica Métodos Estadísticos Para Investigadores. In Temas Fundamentales de Psicología. 4 edition. Edited by: Massons I. Barcelona, 1982.

56. Chiodini R: Abolish Mycobacterium paratuberculosis strain 18. J Clin Microbiol 1999, 31:1956-1959.

57. Begg DJ, Griffin J: Vaccination of sheep against M. paratuberculosis: immune parameters and protective efficacy. Vaccine 2005, 23:4999-5008.

58. Uzonna JE, Chilton P, Whittlock RH, Habecker PL, Scott P, Sweeney RW: Efficacy of commercial and field-strain Mycobacterium paratuberculosis vaccinations with recombinant IL-12 in a bovine experimental infection model. Vaccine 2003, 21:3101-3105.

59. Benedictus G, Dinkla ETB, Wermink GH: Preliminary results of vaccination against paratuberculosis in adult dairy cattle. In Proceedings International Colloquium on Paratuberculosis, I; Laboratoire Central de Recherches Veterinaires, Maisons-Affort, France. Edited by: Thorel MF, Meralk RS. International Association for Paratuberculosis, 1988:136-140.

60. Juste RA, Casal J: An economic and epidemiologic situation of different control strategies for ovine paratuberculosis. Prev Vet Med 1993, 15:101-115.

61. Argenté G: Utilisation de la culture fécale dans un plan de prevention de la paratuberculeose dans 500 boeufs; Justifications techniques et économiques. In Proceedings of the International Colloqium on Paratuberculosis, I; Laboratoire Central de Recherches Veterinaires. Maisons-
81. Griffin JF, Hughes AD, Liggett S, Farquhar PA, Mackintosh CG, Bakker D: Infection of dairy cattle with
80. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S:
79. Windsor PA, Eppleston J, Sergeant E: Vaccination against Johne
77. Pérez V, García Marín JF, Bru R, Moreno B, JJ B:
76. Cranwell MP: Vaccine efficacy of a killed vaccine
72. Wentink GH, Bongers JH, Zeeuwen AA, Jaartsveld FH:
68. Stuart P: Vaccination against Johne
66. Doyle TM: Vaccination against Johne
62. Vordermeier M, Gordon SV, Hewinson RG: Mycobacterium bovis antigens
57. Bastida and Juste & Journal of Immune Based Therapies and Vaccines 2011, 9:8
http://www.jibtherapies.com/content/9/1/8
Page 16 of 17

Johne's disease. Cornell Vet 1935,
25:344-351.

66. Doyle TM: Johne's disease. In Infectious Diseases of Animals. Volume 1. Edited by: I.A. SAWaG. London: Butterworth's Scientific Publications; 1959:319-345.

65. Hagan WA. Vaccination against Johne's disease. Cornell Vet 1935,
25:344-351.

64. Juste RA, Gepp MV, Sevilla I, Adurzú G, Gamito JM: Control of paratuberculosis by vaccination. In Proceedings of the 7th International Colloquium on Paratuberculosis, Bilbao, Spain. Edited by: Juste RA. International Association for Paratuberculosis; 2002:331.

63. Possible links between Crohn's disease and Paratuberculosis. Book Possible links between Crohn's disease and Paratuberculosis 2000, SANCO/B3/ R16/2000. EUROPEAN COMMISSION DIRECTORATE-GENERAL HEALTH & CONSUMER PROTECTION Directorate B - Scientific Health Opinions Unit B3 - Management of scientific committees II.

62. Vordermeier M, Gordon SV, Hewinson RG: Mycobacterium bovis antigens
61. Vordermeier M, Gordon SV, Hewinson RG: Mycobacterium avium subsp paratuberculosis proteins induces differential immune responses and protects calves against infection by oral challenge. Vaccine 2008, 26:1652-1663.

61. Vordermeier M, Gordon SV, Hewinson RG: Mycobacterium avium subsp paratuberculosis proteins induces differential immune responses and protects calves against infection by oral challenge. Vaccine 2008, 26:1652-1663.

60. Vordermeier M, Gordon SV, Hewinson RG: Mycobacterium avium subsp paratuberculosis proteins induces differential immune responses and protects calves against infection by oral challenge. Vaccine 2008, 26:1652-1663.

59. Vordermeier M, Gordon SV, Hewinson RG: Mycobacterium avium subsp paratuberculosis proteins induces differential immune responses and protects calves against infection by oral challenge. Vaccine 2008, 26:1652-1663.

58. Vordermeier M, Gordon SV, Hewinson RG: Mycobacterium avium subsp paratuberculosis proteins induces differential immune responses and protects calves against infection by oral challenge. Vaccine 2008, 26:1652-1663.

57. Bastida and Juste & Journal of Immune Based Therapies and Vaccines 2011, 9:8
http://www.jibtherapies.com/content/9/1/8
Page 16 of 17

47 - Management of scientific committees II.

46. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

45. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

44. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

43. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

42. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

41. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

40. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

39. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

38. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

37. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

36. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

35. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

34. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

33. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

32. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

31. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

30. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.

29. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jonbes S: The effect of vaccination on the excretion of Mycobacterium paratuberculosis in large dairy herds. Vet Microbiol 1994, 41:117-125.
102. Hines ME, Stiver S, Giri D, Whittington L, Watson C, Johnson J, Musgrove J, Pence M, Hurley D, Baldwin C, et al: Efficacy of spheroplast and cell-wall competent vaccines for Mycobacterium avium subsp. paratuberculosis in experimentally-challenged baby goats. *Vet Microbiol* 2007, 120:261-283.

103. García-Pariente C, Pérez V, Geijo M, Moreno O, Muñoz M, Fuertes M, Puentes E, Doce J, Ferreras MC, García Marin JF: The efficacy of a killed vaccine against paratuberculosis (SILIRUM®) in cattle. A field study. In *Proceedings of the 8th International Colloquium on Paratuberculosis*; Copenhagen, Denmark. Edited by: Manning EJB, Nielsen SS. International Association for Paratuberculosis; 2005:52.

104. Sommerville EM, Wakelin RL, Hutton JB: Vaccination of lambs at 3 to 4 months of age protects against Johne’s disease. *PTBC Newsletter* 1995, 7:25-29.

105. Reyes LE, González J, Benavides J: Nuevos adyuvantes en la vacunación frente a la paratuberculosis ovina. In *XXVII Jornadas Científicas y VI Jornadas Internacionales de la Sociedad Española de Ovinotecnia y Caprinocecnia, SEOC; Spain*. Edited by: Peris Palau B, PMP, LA M, GM A. SEOC; 2002:758-761.

106. Thonney ML SS, Smith MC: Control of Johne’s disease in sheep by vaccination Preliminary Report. *Control of Johne’s disease in sheep by vaccination Preliminary Report Cornell University; 2005.*

Cite this article as: Bastida and Juste: Paratuberculosis control: a review with a focus on vaccination. *Journal of Immune Based Therapies and Vaccines* 2011 9:8.

doi:10.1186/1476-8518-9-8