

REVIEW

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Paratuberculosis control: a review with a focus on vaccination

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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.

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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 graze, 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

technique sensitivity and especially in efficient high-throughput processing of samples and extraction of nucleic acids have made this approach a valuable strategy due to its high specificity, good sensitivity, and fast turnaround time [20,21]. The majority of paratuberculosis PCR detection tests are based on the detection of IS900 sequence, which has the benefit of multiple copies of target DNA per bacteria (higher sensitivity) but the disadvantage of a lower specificity since a few environmental mycobacteria also contain this insertion sequence. Other tests use MAP specific single copy genes (i.e. F57, 251) with theoretically lower sensitivity but higher specificity [22,23]. Multiplex PCRs, using combinations of target genes, have also been reported [24]. PCR has the additional benefit over the ELISA technique that, like fecal culture, it can provide quantitative bacterial content results, and thus high shedders and medium shedders can readily be identified and eliminated. Recently in the Netherlands, fecal culture has been replaced by a PCR based test in the Dutch paratuberculosis control program. As with the ELISA and culling strategy, PCR and culling is not yet proven in the field, however, a new study by Lu et al has shown that the use of faster detection tests such as PCR might be important in farms with poor management [25].

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* (PPD_{bov}) and *Mycobacterium avium* subsp. *avium* (PPD_{av}). 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.

An additional drawback to MAP vaccination, which at least in sheep appears not to be of economical relevance [29], is the granulomatous lesion at the injection site produced by most oil-based bacterin vaccines.

In summary, there are several strategies for paratuberculosis control, but there is no generalized consensus on which one or which combination of strategies should be the standard approach. In our opinion, this is in part due to the fact that paratuberculosis control programs emphasize too heavily MAP eradication.

Pathogenic background

MAP distribution

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

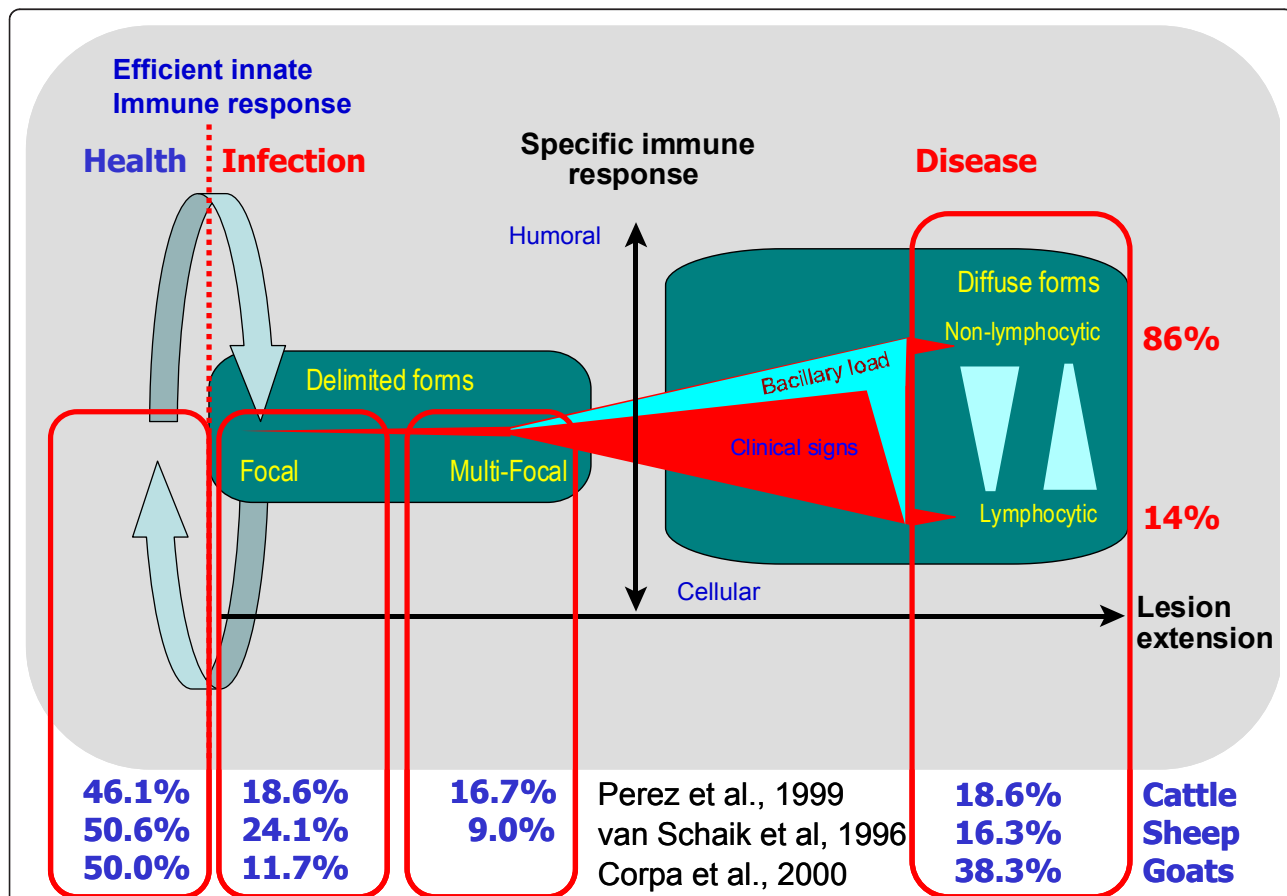


Figure 1 Immunopathological model of paratuberculosis. Continuous exposure of animals to MAP results in a dynamic balance where infection never gets established or is controlled by an efficient innate immune response in about half of the farm population, while in the other half it progresses to subclinical delimited focal or multifocal forms and, in a smaller fraction, to diffuse lymphocytic (cellular or Th1 type) or non-lymphocytic (humoral or Th2 type) forms that will result in open clinical disease.

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 immunogenicity 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 where 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

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.

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 3 Production effects (Paratuberculosis clinical cases or mortality rates).

Vaccine				Country and reference	Year	Number of animals	Age at vaccination	Reduction (%)	Type of trial
Name/Laboratory	Type	Strain/Antigen	Adjuvant						
Cattle									
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	<i>M.a.a</i> 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
		-	Oil						
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 m, 2 y	87.50	F, IF, mort rate
Gudair	Killed	316F	Oil	Australia [80]	2004	1200	1-4 m	90.00	F,MC, mort 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.

Table 4 Epidemiological effects (*Mycobacterium avium* subsp. *paratuberculosis* isolation from faeces or tissues).

Name/Laboratory	Vaccine		Country and reference	Year	Number of animals	Age at vaccination	Reduction (%)	Type of trial	
	Type	Strain/Antigen							Adjuvant
Cattle									
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	<i>M.a.a</i> strain 18	Oil	U.S.A.[70]	1983	158	1 m	79.28	F, MC, FC
Fromm	Killed	<i>M.a.a</i> 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	<i>M.a.a</i> 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
Neoparasec	Live	316F	Oil	Germany [89]	2002	521	1 m	86.87	F, MC, FC
Mycopar	Killed	<i>M.a.a</i> strain 18	Oil	U.S.A. [58]	2003	10	7 d	-28.00	E, MC, FC, TC
Mycopar IL-12	Killed	<i>M.a.a</i> strain 18	Oil	U.S.A. [58]	2003	10	7 d	32.00	E, MC, FC, TC
Mycopar	Killed	<i>M.a.a</i> strain 18	Oil	U.S.A. [58]	2003	14	8 d	40.00	E, MC, FC, TC
Mycopar IL-12	Killed	<i>M.a.a</i> strain 18	Oil	U.S.A. [58]	2003	14	8 d	23.60	E, MC, FC, TC
Silirum	Killed	316F	Oil	Spain [90]	2005	14	2 m	62.50	E, MC, TC
NCV	Rec	Hsp70	DDA	Netherlands [39]	2006	20	1 m boost 11 m	37.50	E, MC, FC
Mycopar	Killed	<i>M.a.a</i> 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	U.S.A. [93]	2009	12	14 d	84.61	E,MC,TC
Silirum	Killed	316F	Oil	Spain [49]	2009	371	all ages	68.20	F, IF, FC, FP
Average							72.55 ± 0.29		
Sheep									
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
Neoparasec	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)

Neoparasec & NCV	Live & Killed	316F	Oil	Spain [77]	1995	97	Adults	-10.95	F, IF, TC
		-							
NCV	Killed	-	Oil Paraffin	Greece [97]	1997	226	1 m	93.27	F, MC, FC
Neoparasec	Live	316F	Oil	New Zealand [78]	2000	28	1-1.5 m	66.67	E, MC, TP
Gudair	Killed	316F	Oil	Australia [80]	2004	1200	1-4 m	90.00	F, MC, FC
Gudair	Killed	316F	Oil	Australia [98]	2005	-	16 w	52.21	F, IF, FC
Gudair	Killed	316F	Oil	Australia [34]	2006	400	1 m	76.14	F, MC, FC
Gudair	Killed	316F	Oil	Australia [34]	2006	400	1 m	84.15	F, MC, FC
Gudair	Killed	316F	Oil	Australia [99]	2007	998	2-3 m	76.14	F, MC, FC
Gudair	Killed	316F	Oil	New Zealand [81]	2009	62	4 m	25.30	E, MC, FC
NCV	Killed	316F	Lipid-K formulation	New Zealand [81]	2009	63	4 m	36.03	E, MC, FC
NCV	Live	316F	Lipid-K formulation	New Zealand [81]	2009	63	4 m	36.03	E, MC, FC
NCV	Live	316F	Lipid-K formulation	New Zealand [81]	2009	62	4 m	34.09	E, MC, FC
Average								76.42 ± 0.54	
Goats									
Neoparasec	Live	316F	Oil	France [100]	1988	27	1 m	73.08	E, MC, FC
Neoparasec	Live	316F	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	316F	Oil	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.

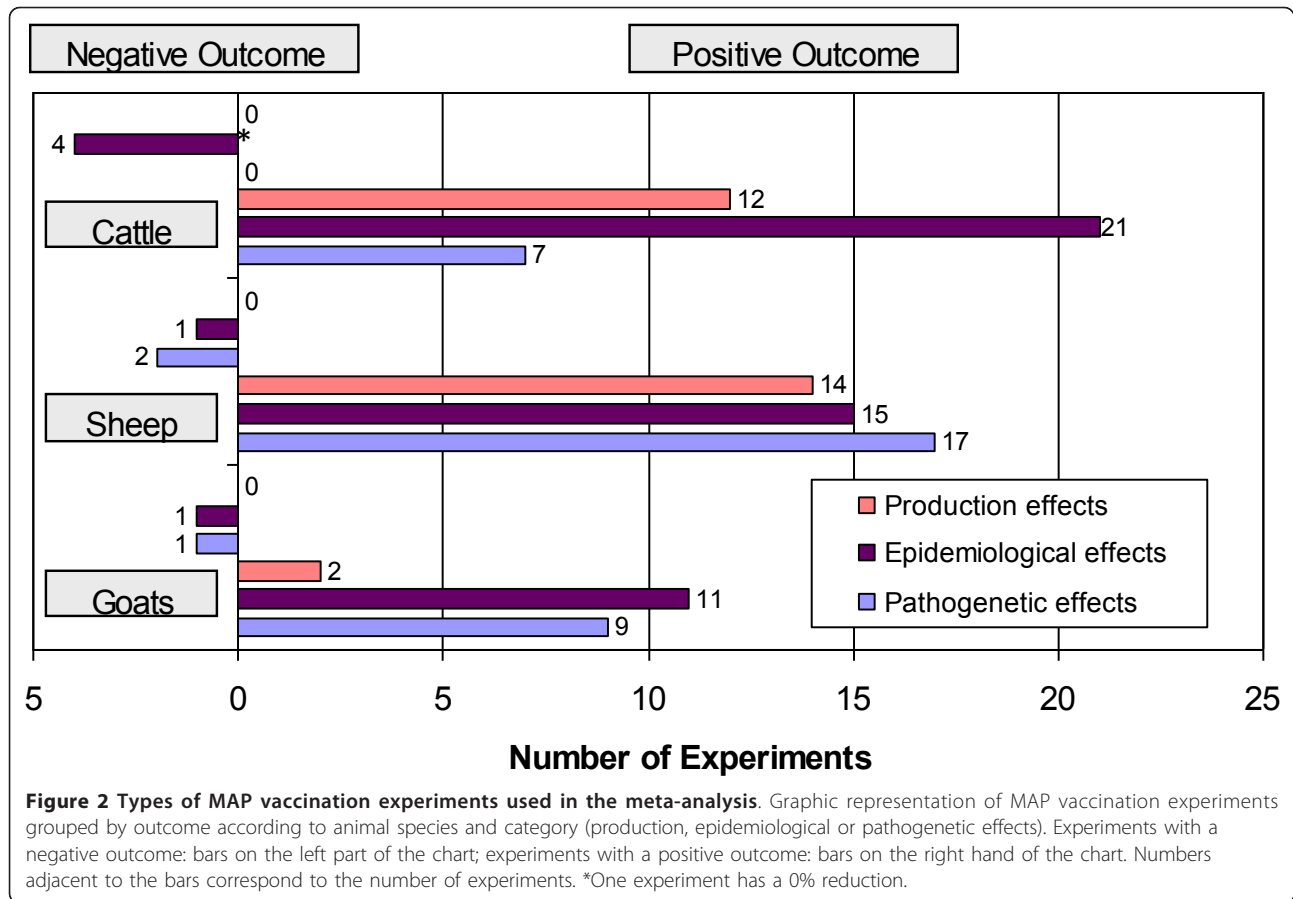
Table 5 Pathogenetic effects (histopathological lesions).

Name/Laboratory	Vaccine			Country and reference	Year	Number of animals	Age at vaccination	Reduction (%)	Type of trial	
	Type	Strain/Antigen	Adjuvant							
Cattle										
NCV	Live	6 strains	Oil	U.S.A. [65]	1935	20	calves	42.86	E, HP	
NCV	Live	avirulent	P/O/P	U.S.A. [83]	1974	16	16 d	17.24	E, HP	
Lelystad	Killed	-	None	Netherlands [71]	1992	3209	1 m	58.34	F, IF, HP	
NCV	Killed	-	Oil	Netherlands [72]	1994	499	1 m	57.23	F, IF, HP	
NCV	Killed	-	Oil	Netherlands [37]	1996	573	1 m	58.09	F, IF, HP	
Silirum	Killed	316F	Oil	Spain [103]	2005	79	all ages	38.68	F, MC, HP	
Silirum	Killed	316F	Oil	Spain [90]	2005	14	2 m	37.50	E, MC, HP	
Average								57.54 ± 0.11		
Sheep										
NCV	Killed	-	Oil	Iceland [54]	1960	419	3 m	83.58	F, MC, PM	
NCV	Killed	-	Oil	Iceland [54]	1960	24323	3 m	93.55	F, MC, PM	
NCV	Killed	-	Oil	U.K. [95]	1962	126	1 m	52.22	E, MC, HP	
Lio-Johne	Live	316F	Oil	Spain [74]	1993	570	1 m	100.00	F, MC, HP	
Lio-Johne	Live	316F	Oil	Spain [74]	1993	1201	Adults	53.36	F, MC, HP	
Neoparassec	Live	316F	Oil	Spain [96]	1994	13	2 m	64.52	E, MC, HP	
Neoparassec	Live	316F	Oil	Australia [104]	1995	475	3 m	82.27	F, MC, HP	
Neoparassec & Gudair	Live and Killed	316F	Oil	Spain [77]	1995	135	Adults	-3.03	F, IF, HP,	
Neoparassec	Live	316F	Oil	New Zealand [78]	2000	28	1-1.5 m	77.78	E, MC, HP	
Gudair	Killed	316F	Oil	Spain [105]	2002	12	1 m	100.00	E, MC, HP	
Mycopar	Killed	<i>M.a.a.</i> Strain 18	Oil	U.S.A. [106]	2005	178	60-164 d	75.31	F, MC, HP	
Neoparassec	Live	316F	Oil	New Zealand [57]	2005	59	2-4 w	68.52	E, MC, HP	
AquaVax	Live	316F	saline	New Zealand [57]	2005	58	2-4 w	-2.48	E, MC, HP	
Gudair	Killed	316F	Oil	Australia [34]	2006	88	1-3 m	72.70	F, MC, GL, HP	
Gudair	Killed	316F	Oil	Australia [34]	2006	307	1-3 m	48.29	F, MC, GL, HP	
Gudair	Killed	316F	Oil	New Zealand [81]	2009	62	4 m	75.57	E, MC, HP	
NCV	Killed	316F	Lipid-K formulation	New Zealand [81]	2009	63	4 m	37.17	E, MC, HP	
NCV	Live	316F	Lipid-K formulation	New Zealand [81]	2009	63	4 m	51.32	E, MC, HP	
NCV	Live	316F	Lipid-K formulation	New Zealand [81]	2009	62	4 m	57.56	E, MC, HP	
Average								89.70 ± 0.15		
Goats										
NCV	Live	2E/316F	P/O/P	Norway [26]	1985	5535	1 m	97.18	F, IF, PM	
Gudair	Killed	316F	Oil	Spain [38]	2000	189	Adults	65.88	F, MC, HP	
NCV	Killed	Goat isolate (CWD)	QS21	U.S.A. [102]	2007	20	1 w	34.38	E, MC, HP	
NCV	Killed	Goat isolate (CWC)	QS21	U.S.A. [102]	2007	20	1 w	32.03	E, MC, HP	

Table 5 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; Neoprasec: Neoprasec[®], 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 3×10^9 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 where 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 (Silirum™, 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 tough 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; NCV: Non-commercial vaccine; NVH: 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/O/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.

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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 Gudair®, 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.

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