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Review

Meta-analysis on the efficacy of foot-and-mouth disease emergency vaccination

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ABSTRACT

The objectives of this study were to provide a summary quantification of the efficacy of FMD emergency vaccination based on a systematic review and a meta-analysis of available literature, and to further discuss the suitability of this review and meta-analysis to summarize and further interpret the results. Peer-reviewed, symposium, and unpublished studies were considered in the analysis. Clinical protection and virological protection against FMD were used as parameters to assess the efficacy of emergency vaccination. The clinical protection was estimated based on the appearance of clinical signs including FMD lesions and fever, while the virological protection parameter was estimated based on the outcome of laboratory tests that were used to diagnose FMD infection. A meta-analysis relative risk was calculated per protection parameter. Results of the meta-analyses were examined using publication bias tests. In total, 31 studies were included in the analyses, of which 26 were peer-reviewed studies, 1 was a symposium study and 4 were unpublished studies. Cattle, swine and sheep were well protected against clinical disease and FMD infection following the use of emergency vaccine. Fortunately, no significant bias that would alter the conclusions was encountered in the analysis. Meta-analysis can be a useful tool to summarize literature results from a systematic review of the efficacy of FMD emergency vaccination.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting ruminants and pigs, which can cause huge economic damage (Cox and Barnett, 2009; Pendell et al., 2007). Although FMD remains as an endemic disease in several areas of the world, elsewhere FMD free status has been maintained or FMD was eradicated by imposing strict legislation and/or implementing a variety of control strategies including use of vaccination (Cox and Barnett, 2009). The eradication of the disease and the prohibition of vaccination in these countries have created a highly susceptible population of animals. This means that a potential outbreak could rapidly spread within and between the countries. Following the confirmation of FMD in any EU member state, slaughter and disinfection of infected herds, as well as movement restriction and surveillance of herds within zones around the infected herds must be applied to contain the outbreak as fast as possible (Council Directive 2003/85/EC). From January 1st 1992, prophylactic vaccination against FMD was prohibited in the EU countries. However, after public concerns regarding the mass killing of animals in the UK and the Netherlands during the FMD outbreak in 2001, the OIE and the EU have revised the policy to make use of emergency vaccination in case the risk of an extensive outbreak is suspected (Cox et al., 2005; Parida, 2009). Emergency vaccination may be defined as the use of vaccines to control an outbreak of FMD in a country free of the disease, in which routine prophylactic vaccination is not applied (Council Directive 2003/85/EC). Based on the literature, an ideal emergency vaccine should have the following characteristics: (1) contain no residues

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of a live virus and have minimal side effects to newborns and adults; (2) after a single application with the recommended dose, achieve a potency dose 50 (PD_{50}) of $\geq$6; (3) be compatible with serological tests that identify infection in vaccinated animals; (4) induce reasonably long lasting immunity and provide a broad spectrum of antigenic protection; and (5) be stable under storage once formulated; (6) provide a rapid protection after vaccination; and (7) reduce the reproduction ratio (R_0) to below 1 (Goris et al., 2007; Cox and Barnett, 2009).

The economic consequence of using emergency vaccination following an FMD outbreak is an essential consideration for livestock and livestock products exporting countries. Their export status is highly dependent on the freedom of FMD, which could be delayed because of vaccination (Mahul and Durand, 2000). Several experimental studies have quantified the efficacy of FMD emergency vaccines and these were recently reviewed by Cox and Barnett (2009). The authors observed a considerable variability between studies. This would make it difficult to conduct comprehensive economic assessment of the efficacy of emergency vaccination. It was, therefore, recommended to conduct a meta-analysis study to quantify the efficacy of FMD emergency vaccination. The outcome of this meta-analysis can be used for FMD modeling exercises and economic analysis to assist policy makers in national governments, the EU and worldwide on efficient FMD control strategies.

The objectives of this study were to provide a summary quantification of the efficacy of FMD emergency vaccination based on a systematic review and a meta-analysis of available literature, and to further discuss the suitability of this review and meta-analysis to summarize and further interpret the results.

2. Materials and methods

2.1. Selection of published and unpublished studies

A search was conducted on the literature related to FMD vaccine efficacy studies published between 1960 and the beginning of 2009. The search was carried out using the key words: foot-and-mouth disease; emergency; vaccine; efficacy; and protection in different combinations. Searches were conducted in PubMed (the National Library of Medicine, Bethesda, USA) and Google Scholar (Google Inc., CA, USA).

Studies included in the analysis had to: (1) be a research or symposium paper published in English; (2) be an experimental challenge study, which showed the efficacy of an FMD emergency vaccine in pigs, cattle, and/or sheep; (3) report the rate or the number of FMD infected or protected animals and the total number of animals in at least 2 groups (challenged vaccinated and non-vaccinated control group); (4) include vaccination with the full vaccine dose. Protocols with sub-preventive doses were not included so as not to under-estimate the efficacy of the tested vaccine; (5) include a challenge with a homologous virus to that used in the vaccine, similarly in both groups (vaccinated and non-vaccinated); (6) in case of research papers, report the outcome of a new experiment or protocol not previously reported in a symposium paper, to prevent data sets from being included twice in the analyses.

The quality of the studies was further assessed by insuring that a study: (1) met the above mentioned inclusion criteria; (2) provided sufficient information on animal handling, vaccine application and virus challenge procedure; (3) provided sufficient information regarding the measure of clinical disease and laboratory procedures to diagnose infection; (4) provided sufficient information about the duration of the experiment, and sampling frequency; (5) had an epidemiologic design that would meet the objectives of that study.

The primary check of the studies was carried out by reading the abstract of the studies. When a study was found to fit our inclusion criteria, the study was read to insure a good quality and thereafter a careful reading accompanied with data extraction was carried out. When a study lacked essential information, the authors were contacted and asked to provide the necessary information.

Unpublished data might be an important source of information and should be considered in meta-analysis studies (Dohoo et al., 2003). Therefore, several researchers and research groups that are involved in FMD vaccine studies were approached to provide unpublished data that fitted the above mentioned inclusion criteria from point 2 to 6. The researchers were asked to provide further information as needed to assess the quality of the data. This is important to correctly pool unpublished data with published studies in a meta-analysis (Dohoo et al., 2003).

Because different studies differ in the design, discrepancies were expected that could affect the validity of the meta-analysis. Data related to the study design, emergency vaccination and challenge are presented in the next sections and in supplementary file.

2.2. Emergency vaccination and challenge

The majority of the studies were performed in disease secure facilities. At the start of each study, the animals were FMD free. Frequently, animals were given a few days to adapt to the new facility before assignment to either of the vaccinated or control groups. In the majority of the studies, the emergency vaccine application was carried out using the intramuscular route of injection using a full dose (Supplementary file, column VA). Thereafter, a challenge with a live homologous FMD virus was carried out. Some studies examined the efficacy of emergency vaccination using short and long periods between vaccination and challenge, while other used only long intervals (Supplementary file, footnote j). In the majority of the studies conducted on cattle, a period of 21 days between emergency vaccination and challenge was used. In studies conducted on swine and sheep, a period of 14 days between emergency vaccination and challenge was often used. However, several protocols were frequently tested within these studies. Control animals were separated from the vaccinated animals. In the majority of the studies conducted on cattle, contact with infected cattle for several days or a pig for 1 h was used as the challenge method, while intra-dermal injection of the virus in the tongue was carried out in other studies (Supplementary file, column CR). In the majority of

the studies conducted on pigs, challenge was carried out by direct or less frequently indirect contact with infected pigs for a period from 1 h to several days. In few studies, intra-dermal injection of the virus in the heel bulb was used (Supplementary file, column CR). In the majority of the studies conducted on sheep, challenge with infected pigs for 2–9 h was used (Supplementary file, column CR).

2.3. Follow-up days post-challenge

After challenge animals were monitored for clinical infection over variable time periods (Supplementary file, column FC) and samples for laboratory analyses and diagnosis were obtained (Supplementary file, column FS). In most studies conducted on cattle, 7–14 days post-challenge was used as the follow-up period for clinical diagnosis. However, some studies recorded clinical disease up to the end of the trial. Follow up for laboratory diagnosis varied from 14 to 35 days post-challenge, but was mainly up to 28 days post-challenge.

In most studies conducted on pigs, the follow-up period for clinical diagnosis was also 7–14 days post-challenge. However, in one study (Eblé et al., 2009), clinical disease was recorded up to 42 days post-challenge. Follow up for laboratory diagnosis varied from 8 up to 42 days post-challenge, but was frequently up to 14 days post-challenge.

In studies conducted on sheep, follow up for clinical diagnosis varied from 10 up to 14 days post-challenge, while in one study (Barnett et al., 2004), FMD lesion recording was carried out up to 42 days post-challenge. Follow up for laboratory diagnosis varied from 14 up to 42 days post-challenge and was frequently up to 14 days post-challenge.

2.4. Samples collection, storage and laboratory methods

Sample collection, storage and laboratory methods were generally similar among the different studies. We will only describe these procedures briefly, as detailed information can be found in the original publications.

Heparinised and unheparinised blood samples were stored at −70 °C, while serum samples were stored at −20 °C until processing. Nasal fluids were collected on cotton buds and stored at −70 °C. Oropharyngeal fluid was collected using cotton swabs. For ELISA, oropharyngeal fluid samples were stored at −20 °C, but they were stored at −70 °C for virus isolation (VI). Probang samples were collected using a probang sampler on sedated animals and then the samples were stored at −70 °C.

VI or titration was carried out by inoculation of the sample onto monolayers of mammalian cells in tubes or plates. Infection was determined either by staining and counting of plaques or by microscopic examination for the cytopathic effect with subsequent confirmation of serotype by ELISA. The presence of antibodies against the non-structural proteins (NSP) 3ABC was tested using commercial ELISA. The RT-PCR was used to confirm and identify infection from the samples, mostly using the MagNA Pure® LC kit (Roche) and a light cycler® RT-PCR system.

All studies that confirmed infection using laboratory tests used VI, PCR and NSP, except (Barnett et al., 2002; Cox et al., 1999; Donaldson and Kitching, 1989; Gibson et al., 1984; Salt et al., 1995, 1998) that used only VI to confirm infection from the samples. Orsel et al. (2005) used VI and PCR to confirm infection from the samples.

2.5. Evidence of FMD

2.5.1. Clinical disease

Clinical signs of FMD were diagnosed in all studies. However, there was variability between studies in the consideration of which clinical signs represent clinical disease. The majority of studies conducted on cattle and pigs considered the presence of FMD lesions and/or fever as a sign of disease (Supplementary file, column Fe). In the other studies only lesions on the feet were considered to diagnose clinical infection. In studies conducted on sheep, the presence of FMD lesions and/or fever was considered to diagnose clinical infection.

In the studies that used fever as a clinical sign, discrepancies between the studies in the consideration of fever were also observed. In only one study conducted using cattle, where fever was considered a clinical sign of the disease, fever was specified to be a rectal temperature of ≥40 °C (Aggarwal et al., 2002). In studies conducted using swine, 40 °C was used as a cut-off value, except in one study (Parida et al., 2007), which used 39.5 °C as a cut-off value. Two studies conducted with swine included fever to diagnose FMD, but did not specify a cut-off value (Doel et al., 1994; Orsel et al., 2007a). In studies conducted using sheep, fever was defined as ≥40 °C, except one study (Parida et al., 2008), which used ≥39.5 °C.

2.5.2. Virological infection

In the majority of studies, FMD infection was confirmed by laboratory diagnosis (Supplementary file, column FS). In the current meta-analysis we defined infection based on the results of: (1) VI from the blood, oral, nasal and/or esophageal–pharyngeal fluids samples; (2) presence of antibodies against NSP; or (3) presence of viral RNA in oral, nasal and/or esophageal–pharyngeal fluids samples diagnosed using RT-PCR.

2.6. Meta-analysis procedure

2.6.1. Outcome parameters

In the current meta-analysis we defined two main parameters to determine the efficacy of FMD emergency vaccination: clinical protection and virological protection.

The relative risk (RR) of clinical disease was considered to represent clinical protection. For each study, the RR was calculated as the incidence risk of clinically FMD diseased animals in the vaccinated group divided by the incidence risk in the unvaccinated control group. The incidence risk of clinical FMD was calculated for each group as the number of clinically diseased animals divided by the total number of animals. The length of the follow-up period post-challenge was assumed not to affect the outcome, given that animals were followed up at least 7 days. Seven days after challenge was assumed to be a sufficient period for clinical signs to appear.

The virological protection was estimated based on the definition of infection, which considered an animal to have FMD infection when it was found positive to VI, NSP or RT-PCR tests from the original studies. The RR of infection was used to represent virological protection. For each study, a RR of infection was calculated as the incidence risk of infection in the vaccinated group divided by the incidence risk in the unvaccinated control group. The incidence risk of infection was calculated for each group (vaccinated and unvaccinated) as the number of animals that were positive to any of the 3 tests divided by the total number of animals. As for clinical disease, we assumed that the length of the follow-up period post-challenge to diagnose infection did not affect the outcome, given that animals were followed for at least 7 days. Seven days after challenge was assumed to be a sufficient period for virus shedding to occur and 14 days for NSP antibodies to develop.

From 4 studies (Eblé et al., 2004, 2007; Orsel et al., 2005, 2007b) only results of experiments using vaccinated and non-vaccinated animals challenged by direct injection of the virus were included in the current meta-analysis. Only results of experiments using vaccinated and non-vaccinated animals that had been challenged using the airborne route with unvaccinated infected seeders were included in the analysis from Orsel et al. (2007a). Results of other experiments from these studies were not included.

2.6.2. Statistical procedure

The RRs were pooled over the studies; separately per protection parameter, and animal species, using a commercial analytical package (Comprehensive meta-analysis 2.0, 2009). When the pooled RRs were calculated, classification on virus serotype was carried out to correct for potential differences between the different virus serotypes in reaction to vaccination. In studies that included more than one protocol, a combined effect was calculated per study. When one study reported the outcome of separate challenge trials with different virus serotypes, we treated each challenge trail as a separate study in the meta-analysis. Because studies are conducted by different people, in different areas and times, which could create a heterogeneous population of studies, a random effect model was used to estimate the pooled RR (Halasa et al., 2009). A meta-analysis was conducted when at least 4 studies were available (Halasa et al., 2009). Forest plots were used to illustrate the calculated RR per study and virus serotype and the overall pooled effect of all studies in the last line of the plot.

2.6.3. Meta-regression

As recommended by Lean et al. (2009), a weighted meta-regression was conducted in an attempt to explain the heterogeneity between studies. Explanatory variables of the heterogeneity were selected based on the literature and expert assessment of likely factors. The variables used are presented in the supplementary file. The country of origin and animal species were not included in the meta-regression, while the number of tests conducted to confirm infection per study was included. These variables were regressed against the RR results of each study and weighted by the inverse variance (Dohoo et al., 2003). The variables were first tested in univariable models and then combined in a multivariable model using a backward stepwise regression method. A liberal P-value <0.3 was chosen for the variables to be included in a combined multivariable model. In case of a significant association between the explanatory variable and the dependent variable (RR per study) with a P-value <0.05 in the multivariable model, the variable was believed to explain the heterogeneity significantly.

2.6.4. Publication bias

Because studies that result in large and interesting effects are more likely to be published than studies that show relatively small or no effects, the outcome could be a biased body of research (Halasa et al., 2009). The publication bias was assessed using funnel plots. A funnel plot is a plot of a measure of study size (standard error) on the vertical axis as a function of the effect size on the horizontal axis. Large studies appear toward the top of the graph, and tend to cluster near the mean effect value. Smaller studies appear toward the bottom of the graph and tend to disperse across a range of values. Publication bias methods included Duval and Tweedie's fill and trim method (Duval and Tweedie, 2000), Begg and Mazumdar correlation test (Begg and Mazumdar, 1994), and Egger's regression test (Egger et al., 1997). A significant publication bias was deemed to exist when adjustment for the bias altered our conclusion or when the confidence limits of the unadjusted and the adjusted RR did not overlap. When significant publication bias and change on the estimated pooled RR were detected, the number of studies necessary to reverse the pooled effect was calculated using Orwin's fail-safe N method (Orwin, 1983). The study influence was examined using the one study removed method (Dohoo et al., 2003). When significant publication bias was deemed to exist, the pooled RR was presented based on the Duval and Tweedie's fill and trim estimation after correcting for the publication bias. The interpretation of each of the above mentioned methods is presented in Sections 3 and 4.

It is important to mention that these methods are based on statistical theory. They do not necessarily prove existence of bias, but they do suggest it, and the confirmation of the bias should be based on biological and rational reasoning (Halasa et al., 2009).

3. Results

3.1. Descriptive results

The primary search identified 862 published studies of relevance. Of these, 69 studies examined FMD vaccines in cases and control groups in cattle, swine and/or sheep. However, only 29 published studies described a vaccine that would meet our criteria for an emergency vaccine. The authors of 4 studies were contacted to identify the PD_{50} of the vaccine used in their studies. Finally, 28 studies were identified to fit our criteria and 27 of them were included in the analysis. One study (Orsel et al., 2007c) was excluded to correct for age as a natural confounder, because the study only included older cattle. Of these 27 studies, 10 examined the efficacy of emergency vaccination using cattle; 9 studies using swine; 5 studies using sheep, and 3 studies

examined emergency vaccination in more than 1 species (Supplementary file). One of the 5 studies (Cox et al., 1999) conducted in sheep included 3 different virus strains, and hence each experiment was treated in the analysis and referred to as a separate study (Supplementary file). All studies were peer-reviewed research papers except one (Salt et al., 1995), which was a symposium paper.

Four unpublished experiments conducted on swine were included in the analysis; all of good quality and with experimental design similar to other included studies (Supplementary file).

3.2. Protection in cattle

Studies included in investigating the clinical protection provided by emergency vaccination against FMD in cattle were homogeneous (Q-value = 16.3 with 12 degrees of freedom and a P-value = 0.178). The studies included virus serotypes O, A and Asia 1. In general, emergency vaccination protected cattle well against clinical disease (Table 1). There was no significant difference in clinical protection between the different vaccine serotypes (Fig. 1). Removing any of the studies did not alter the pooled effect significantly. Further investigation of the publication bias showed signs of bias. The Begg and Mazumdar test suggested a significant correlation between study size and study effect ($\tau = -0.45$ and a P-value = 0.03). Egger's regression test suggested a significant association between study size and study effect with an intercept = −1.46, a standard error = 0.45 and a P-value = 0.01. Correcting for the bias using the Duval and Tweedie's trim and fill method, resulted in an insignificant change to the pooled RR.

In the meta-analysis to examine virological protection against FMD, the studies were heterogeneous, and none of the variables in the meta-regression explained the heterogeneity significantly. Vaccinated cattle had 0.71 (0.59–0.85) times lower risk of FMD infection compared to non-vaccinated cattle (Table 1). Publication bias tests indicated no significant publication bias.

3.3. Protection in swine

Studies investigating the clinical protection provided by emergency vaccination against FMD in swine were heterogeneous. None of the tested variables in the meta-regression explained the heterogeneity significantly. The studies included virus serotypes O, A, C and Asia. In general, emergency vaccination provided good protection against clinical disease in swine (Table 1). There was no significant difference in clinical protection between the different vaccine serotypes. Publication bias tests did not indicate a significant bias that would alter the results.

Emergency vaccination protected swine against FMD infection (Fig. 2). Vaccinated swine had 0.67 (0.51–0.87) times lower risk of FMD infection compared to non-vaccinated swine (Table 1). The studies were heterogeneous, and none of the variables in the meta-regression explained the heterogeneity significantly. Only Egger's regression test suggested an association between study size and study effect. All other publication bias test indicated no publication bias.

Table 1
Pooled relative risk (RR) together with the 95% confidence interval as estimated based on the corresponding studies in the meta-analysis to quantify the clinical and virological protection against foot-and-mouth disease using emergency vaccination.

Animal species and measure of protection	Studies included in the meta-analysis	Pooled RR and 95% confidence limits
Cattle		
Clinical	(Aggarwal et al., 2002; Brehm et al., 2008; Cox et al., 2005; Cox et al., 2006; Cox et al., 2007; Doel et al., 1994; Donaldson and Kitching 1989; Goris et al., 2007, 2008; Graves et al., 1968; Mattion et al., 2004; Orsel et al., 2005; Salt et al., 1995)	0.13 (0.09–0.18)
Virological	(Cox et al., 2005, 2006, 2007; Donaldson and Kitching 1989; Orsel et al., 2005; Salt et al., 1995)	0.71 (0.59–0.85)
Swine		
Clinical	(Aggarwal et al., 2002; Barnett et al., 2002; Buonavoglia et al., 1998; Doel et al., 1994; Eblé et al., 2004, 2007, 2009; Graves et al., 1968; Orsel et al., 2007a,b; Parida et al., 2007; Sellers and Herniman, 1974; Salt et al., 1998; Unpublished data).	0.48 (0.36–0.65)
Virological	(Barnett et al., 2002; Eblé et al., 2004, 2007, 2009; Orsel et al., 2007a,b; Parida et al., 2007; Salt et al., 1998)	0.67 (0.51–0.87)
Sheep		
Clinical	(Aggarwal et al., 2002; Barnett et al., 2004; Cox et al., 1999; Gibson et al., 1984; Orsel et al., 2007a,b; Parida et al., 2008)	0.31 (0.18–0.53)
Virological	(Barnett et al., 2004; Cox et al., 1999; Gibson et al., 1984; Orsel et al., 2007a,b; Parida et al., 2008)	0.59 (0.44–0.80)

3.4. Protection in sheep

Studies investigating the clinical protection provided by emergency vaccination against FMD in sheep were heterogeneous. In the meta-regression, none of the tested variables explained the heterogeneity significantly. The included studies examined virus serotypes O, C and Asia 1. In general, emergency vaccination protected sheep well against clinical disease (Table 1) and no significant publication bias was found.

Emergency vaccination provided virological protection against FMD infection in sheep (Table 1). The studies were heterogeneous and none of the variables in the meta-regression explained the heterogeneity significantly. There was no significant difference in protection between the different vaccine serotypes. Removing any of the studies did not alter the effect. Begg and Mazumdar correlation test

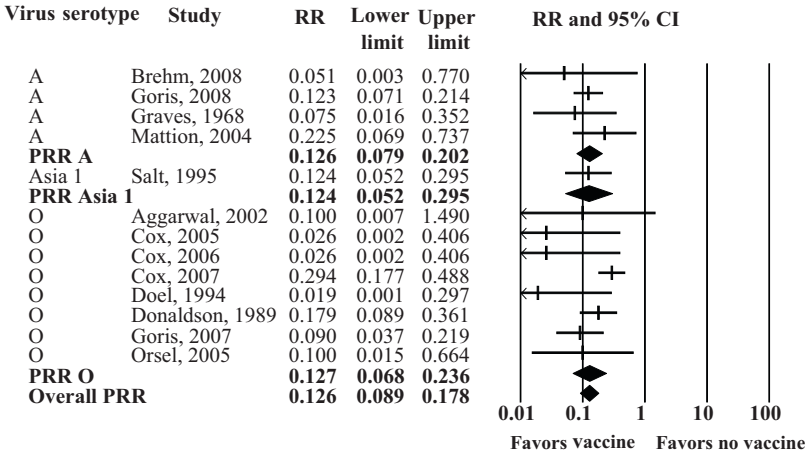

Fig. 1. Forest plot of the relative risk (RR) of the clinical disease in vaccinated *cattle* for each of the 13 included studies, the pooled RR (PRR) per virus serotype and the overall PRR together with the 95% confidence interval (CI).

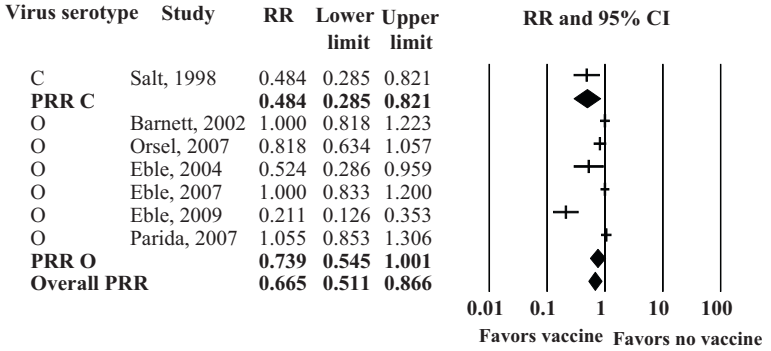

Fig. 2. Forest plot of the relative risk (RR) of the FMD infection of *swine* for each of the 7 included studies, the pooled RR (PRR) per virus serotype and the overall PRR together with the 95% confidence interval (CI).

suggested an absence of correlation between study size and study effect. This result was contradicted by Egger's regression test that suggested a strong association between study size and study effect with an intercept = −2.84, a standard error = 0.54 and a *P*-value = 0.003. Duval and Tweedie's trim and fill method suggested adding 3 studies to the right side of the funnel plot (Fig. 3), which would shift the effect from 0.59 (0.4–0.8) (the white diamond under the *X*-axis), to 0.68 (0.5–0.99) (the black diamond under the *X*-axis). Nonetheless, this adjustment would not alter the result that emergency vaccination protected sheep against FMD infection significantly than non-vaccinated animals.

4. Discussion

The results indicate that emergency vaccination protect cattle, swine and sheep against FMD clinical disease compared to unvaccinated animals (Table 1). More importantly, emergency vaccination provided protection against virological infection with FMD in these species. The purpose of an emergency vaccine is to limit the spread of the disease or in other words, decrease the reproduction ratio to <1. Therefore, virological protection is more important than the clinical protection. Still, reducing the clinical signs is also important, because the virus can be excreted through the lesions. Preventing the appearance of lesions might, therefore, decrease virus shedding. However, if vaccination induces protection against clinical signs without virological protection, clinical disease can be concealed

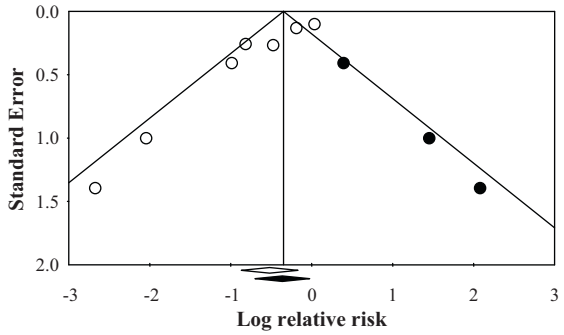

Fig. 3. Funnel plot of the logarithm pooled relative risk (RR) of 7 studies (empty circles) quantifying the effect of FMD vaccination against FMD infection in *sheep*. The dark spots are the potential missing studies according to Duval and Tweedie's trim and fill method (if they had existed, the pooled effect would have shifted from 0.59 (0.4–0.8); the white diamond under the *X*-axis, to be 0.68 (0.50–0.99); the black diamond under the *X*-axis).

making early disease detection of vaccinated farms more difficult.

Despite the finding that emergency vaccination protects cattle against FMD infection, it was observed that several cattle persisted as carriers. Some cattle were found to excrete virus up to 553 days post-inoculation (Moonen et al., 2004). This will pose the risk of harboring and transmitting the virus for a long period, which could delay regaining FMD free status, and hence increase the economic damage due to the outbreak. This problem can be solved by culling all vaccinated animals after the epidemic. However, this might not be an option, because mass culling has, in previous outbreaks, triggered public concerns (Cox et al., 2005; Parida, 2009), which lead to the introduction of the so-called "vaccine-to-live policy". Consequently, the OIE and EU regulations were adjusted, implying that if a vaccine-to-live policy is used, countries can now regain the FMD free status 6 months instead of one year after the last case has been detected (Council Directive 2003/85/EC). Given the long period of potential virus excretion from the carrier animals, a strict post-vaccination screening program is essential to find carrier animals and cull the infected herds to prevent subsequent outbreaks.

First, we observed that sheep that had emergency vaccination had 0.59 (0.4–0.8) lower risk of FMD infection compared to non-vaccinated sheep. However, after thorough examination of publication bias, we noticed an existing trend in the studies (Fig. 3). Small studies showed large effect, while large studies showed small effect. This could be an indication of publication bias. This finding was supported by other publication bias tests such as Egger's regression test, which has been reported to be powerful in detecting publication bias, especially when the data is heterogeneous, such as ours (Peters et al., 2006). Correcting for publication bias by including the "missing" studies would move the RR toward the null effect. The "missing" studies could have been truly missing, conducted but not published, due to several reasons discussed thoroughly by Thornton and Lee (2000), Bornstein et al. (2009) and Halasa et al. (2009). More important than accepting or rejecting this bias was that the correction for the bias did not alter the conclusion that there was significant protective effect of vaccinating sheep against FMD. Moreover, the confidence limits of the corrected and uncorrected pooled RR overlap, making the difference between them statistically insignificant.

In the current study, we defined infection to be a positive result based on VI, NSP or RT-PCR tests. This could have included bias, because some studies conducted more tests than other studies. When more tests are applied, the chance of positive diagnosis (true or false) might become higher. Nonetheless, the majority of studies applied the 3 tests (VI, RT-PCR and NSP), and all studies conducted VI, which is considered the gold standard test to diagnose FMD infection (Paixão et al., 2008). In an outbreak situation, one positive test result will most likely be interpreted as an FMD infection and further measures will be taken according to the EU Council Directive 2003/85/EC. Therefore, the tests do not replace each other; likewise, they supplement each other.

In some of the studies included in the analysis, several periods of time between vaccination and virus challenge were tested. A longer period between vaccination and challenge gives the immune system more time to develop neutralizing antibody titers, resulting in better protection (Doel et al., 1994; Salt et al., 1998). This could have created bias in our study, because some studies used longer periods between vaccination and challenge compared to other studies. Nevertheless, an emergency vaccine should provide rapid protection after vaccination (Cox and Barnett, 2009) and hence, the use of long or short duration between vaccination and challenge should not affect the efficacy of the vaccine. Because the pooled RRs of clinical and virological protection from the current study were calculated from studies that included different periods between vaccination and challenge, and because it is actually unknown when a virus could infect a herd after emergency vaccination, the pooled RRs of the virological protection are useful in simulation exercises to represent the efficacy of the emergency vaccine. Simulation exercises are important to estimate the cost-effectiveness of FMD emergency vaccination during an outbreak and its effects on the national economy. The results from such exercise may assist policy makers to decide whether and how to use emergency vaccines during an outbreak.

We included only studies with homologous virus challenge. Three published studies and two of the unpublished experiments involved semi-homologous virus strain challenge to the vaccine (Supplementary file, footnote l), but were included, because the challenge virus strain and the vaccine virus antigen are categorized within the same topotype on the basis of their VP1 gene and are antigenically closely related (Barnett et al., 2001; Cox et al., 2006).

A major target of emergency vaccination is to reduce the reproduction ratio to <1. This means that an infected case will produce <1 secondary case during its entire infectious period, which will cause the epidemic to fade out. Recent advancements in statistics make it possible to measure the reduction of the reproduction ratio of FMD virus in vaccinated groups (Eblé et al., 2008). It is important to quantify the effect of emergency vaccination on the reproduction ratio of FMD virus for example by use of meta-analysis. Such a meta-analysis has been published, but was restricted to outcomes of 10 experiments on swine from one project (Eblé et al., 2008). Therefore, it would be interesting to estimate the reproduction ratio with and without emergency vaccination from all relevant studies and for the different species. Such a parameter would be useful for modeling within herd spread in simulation models.

In the current meta-analysis, experimental data from (Eblé et al., 2004, 2007; Orsel et al., 2005, 2007b) were included only for vaccinated and non-vaccinated animals challenged by direct injection of the virus. Moreover, only experimental data using vaccinated and unvaccinated challenged animals with the airborne route with non-vaccinated infected seeders were included in the analysis from Orsel et al. (2007a). This was carried out, because the excluded experiments would represent the dynamics of infection within a population rather than the efficacy of the vaccine. In the excluded experiments, the seeders were vaccinated; this means that their infectiousness could be

lower compared to non-vaccinated seeders and hence different than other studies used in the current meta-analysis. Moreover, infection of the contact animals in the excluded experiments is a stochastic process, because the number of infected animals to which a given contact animal is exposed is not predetermined by the experiment setup, as the challenged seeders might be protected following vaccination. These are important differences between these experiments and the included studies in the current meta-analysis, and therefore it is necessary to exclude the results of such experiments to avoid bias.

Several attempts were made to restrict the heterogeneity of studies, such as: Only including challenge studies after emergency vaccination, controlled studies, challenge studies with animals of the same age category, challenge studies with a homologous virus, and inclusion of symposium studies and unpublished data. Nevertheless, the heterogeneity existed in most of the analyses. Including only studies published in English language could have been a source of heterogeneity. Language could be a source of heterogeneity and bias, because non-English speaking researchers might publish their positive results in English language to have more publicity, they would on the other hand publish their negative results in their native language (Gregoire et al., 1995). Although attempts were made to explain the heterogeneity using meta-regression with several potential variables, none of them was able to explain it significantly. Another source of heterogeneity could have been the virus challenge dose. Studies that have challenged animals by direct injection of the virus showed variability in the challenge dose (Cox and Barnett, 2009). Studies that challenged animals using the airborne route showed even more variability in the challenge procedure and challenge intensity (Cox and Barnett, 2009). Unfortunately, because these variables varied largely between studies and given the small number of studies included in some analyses, it was not possible to examine the effect of these variables on the heterogeneity in the meta-regression. Nevertheless, the use of random effect model to calculate the pooled RR took into account the existing heterogeneity to provide the pooled RR by assuming a true RR per study instead of assuming only one true RR for all studies (Bornstein et al., 2009).

5. Conclusions

Emergency vaccination against FMD provided protection against clinical disease and against FMD infection in cattle, swine and sheep. For clinical protection, the pooled RRs with the 95% confidence intervals were 0.13 (0.09–0.18), 0.48 (0.36–0.65) and 0.31 (0.18–0.53), respectively. For virological protection, the pooled RRs with the 95% confidence intervals were 0.71 (0.59–0.85), 0.67 (0.51–0.87) and 0.59 (0.44–0.80), respectively. Fortunately, no significant publication bias was identified in the different meta-analyses.

The current systematic review provided valid outcomes that can be used in simulation models to examine the economic consequences of applying emergency vaccination during an FMD outbreak situation.

Conflict of interest statement

The authors declare that no financial or personal relationships with other people or organizations could have inappropriately influenced our work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.prevetmed.2010.08.005.

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