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EVIDENCE REVIEW

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Efficacy of vaccination against equine herpesvirus type 1 (EHV-1) infection: Systematic review and meta-analysis of randomised controlled challenge trials

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Abstract

Background: Equid herpesvirus type 1 (EHV-1) infection can cause a range of disease syndromes of variable severity that can result in a lethal outcome and restriction of horse movements, especially in the case of outbreaks involving neurological disease. Vaccination is one of the tools used to control the infection. It is widely known that vaccination is not completely effective in ensuring protection against disease caused by this virus. In fact, the real efficacy of vaccination against EHV-1 related disease has not been measured and no systematic reviews exist on this topic.

Objectives: To perform a systematic review and meta-analysis on the efficacy of commercial or candidate vaccines against EHV-1 in randomised controlled trials (RCT) all of which involved experimental challenge of the test subjects.

Study design: Systematic review and meta-analysis.

Methods: RCTs were searched using the search algorithm (([equid herpesvirus* OR equine herpesvirus* OR EHV-1]) AND vaccin*) AND (trial OR experimental OR challenge) on PubMed, Science Citation Index Expanded, Scopus, and CAB Abstracts. Where appropriate, meta-analysis was performed using RevMan 5.4.

Results: Eight studies were selected and were analysed for their respective characteristics and possible shortcomings. The results of RCTs revealed that there was a general improvement in the clinical and virological outcomes of EHV-1 infection following vaccination, but that the effects were very slight. The reduced beneficial effect is probably amplified by the paucity of detailed data reported in the studies that did not allow for the comparison of parameters in many of the cases analysed.

Main limitations: The remarkable heterogeneity and the poor quality of reporting of the selected studies.

Conclusions: Meta-analysis has shown that EHV-1 vaccination generally results in a slight improvement in clinical and virological outcomes, although not to a significant extent. The cumulative results have probably been affected by the lack of information on some parameters not systematically reported in the studies. An improvement in the standard of reporting and better standardisation of the data collected would

likely have improved the quality of each study and enabled more effective comparison of the studies with each other.

KEYWORDS
effectiveness, EHV-1, horse, meta-analysis, systematic review, vaccine

1 | INTRODUCTION

Equid herpesvirus type 1 (EHV-1) is one of the most important equine viruses from a clinical, epidemiological and economic point of view.[1,2] Infection occurs worldwide, although with varying prevalence.[3–5] It generally involves foals within the first month of life, giving rise to infection that is localised to the respiratory tract. This is clinically self-limiting or inapparent in most cases, if not complicated by intercurrent infection with other microbial agents. After initial viral shedding by the respiratory route, a leukocyte-associated viraemia supervenes, resulting in spread of the virus to the peripheral tissues. In adult subjects, viraemia is a prerequisite to abortion and neurologic disease. Concomitantly, the virus becomes latent in specific sites of the body, as in nerve ganglia and T lymphocytes within the lymphoid tissues, where it can be reactivated under stressful circumstances for the animal and shed once again via the nasal secretions. Some horses can act as intermittent shedders; these represent a reservoir of the virus for susceptible in-contact individuals.[5,6]

The economic impact of EHV-1 related disease is especially important. It can result from any of four outcomes of infection with the virus: (1) abortion (epidemic or sporadic) and neonatal mortality[7–10]; (2) myeloencephalopathy (EHM), that can be associated with a high case-fatality rate or result in permanent neurological sequelae in an affected animal; (3) suspension of the training of 2–3 year-old racehorses that present with respiratory disease and an associated fever[5]; (4) restriction of movement of infected animals and implementation of restrictive health measures, with significant economic consequences where herpesvirus infection is a notifiable disease.[5] This was illustrated by the recent major occurrence of EHM in Europe.[11,12] Moreover, in situations where horses are considered companion animals or animals used in assisted therapies for humans, the emotional and social aspects of these various disease events must be borne in mind.

Some aspects of EHV-1 infection and related disease syndromes have still not been completely elucidated and this limits the effectiveness of measures to control the disease.[5,13–16]

Currently, prevention of EHV-1 infection and related disease is based on implementation of prophylactic measures. Early detection is critical, with the aim of isolating and segregating subjects potentially exposed to infection and thereby minimising the risk of introduction of EHV-1, both exogenous and endogenous. These measures are generally associated with vaccination that is not, however, considered the panacea to resolving the problem.[5]

Numerous vaccines have been developed over the years for prevention and control of infection and disease caused by EHV-1. The real impact of this intervention still remains in doubt, however, notwithstanding the fact that this has been the subject of numerous studies of various types over the years. Several have addressed this topic, but never in a systematic way.[1,13,17–21] For this reason, it was decided to undertake a systematic review to identify studies represented by randomised controlled clinical trials (RCT) that provided greater scientific evidence when assessing the efficacy of EHV-1 vaccines[22] following viral challenge.

The aims of this review are as follows: (1) evaluate, as a primary outcome, the efficacy of vaccination against EHV-1 in preventing the appearance of disease; (2) evaluate, as secondary outcomes, improvement in the virological and immunological parameters of infection.

2 | MATERIALS AND METHODS

2.1 | Inclusion criteria and search strategy

The review question, including inclusion criteria, was formulated using the acronym PICOS. P as population: the selected studies must be performed using horses or ponies, of any sex, age and physiological status; I as intervention: the selected studies must have evaluated the efficacy of vaccination against EHV-1 infection, using any type of vaccine or attenuated variant of EHV-1; C as a comparator: a control group subjected to administration of a placebo, a comparable vaccination, or no intervention, must have been included to act as a comparator for the intervention; O as outcome: efficacy of vaccination after experimental challenge with EHV-1 virus must be reported as the primary outcome; this is represented by a reduction in the incidence of EHV-1-related disease (respiratory, abortion or neurological); S as study design: the selected studies were RCT.

Abstracts, conference proceedings, editorials and letters to the editor were excluded from consideration when identifying appropriate studies for inclusion in the review.

The studies were selected following searches on Medline (since 1966), ISI's (Thomson) Science Citation Index Expanded (since 1950), Scopus (since 1975) and CAB Abstracts (since 1973) until 18 October 2021. The search algorithm used was the following: (([equid herpesvirus* OR equine herpesvirus* OR EHV-1]) AND vaccin*) AND (trial OR experimental OR challenge).

Google Scholar also was searched using the same keywords (carried out several times to implement the search algorithm) but introducing restrictions (display the first 15 pages).

References listed in the selected papers were further checked manually to identify possible additional useful citations. No language restrictions were employed in the search. If the data from the same trial was reported in several papers, the results of the most recently published work were used.

2.2 | Selection of studies and data extraction

Pertinent studies were selected in accordance with the inclusion criteria and search strategies used in the study. Duplicates were removed and records obtained were independently screened by two of the authors (MLM, CDW). The selection was carried out in two phases; first, eligibility was assessed based on titles and abstracts; if they were suitable, an examination of the full text followed. Possible disagreements on eligibility among the reviewers during this process were resolved by discussion, reaching a consensus (MLM, CDW).

The data were independently extracted by two blinded researchers (MLM, CDW), using an Excel sheet previously prepared and shared at the time the protocol was developed. Disagreements were resolved by discussion among the authors. The sources for data extraction were the original articles. Data on study characteristics (authors and year, country), vaccine characteristics (type of vaccine, dose, type of adjuvant, association with EHV-4), host features (species, breed, sex, age, number of randomised animals, enrolment criteria, EHV-1 prevaccinal conditions), vaccine protocol (number of administrations, timing, challenge), characteristics of the follow-up (duration, number of clinical and diagnostic evaluations, test performed for diagnosis), and the presence of competing interest were extracted from each study. Data on the primary outcomes of efficacy (number of clinical cases of EHV-1 infection, either respiratory, abortion, or neurological after challenge of vaccinated compared with unvaccinated horses) and secondary outcomes (extent and duration of viral shedding and number of shedders after challenge; extent and duration of viraemia and number of viraemic subjects after challenge; levels of prechallenge antibodies; adverse vaccine reactions) were also extracted separately for vaccinated and unvaccinated horses.

2.2.1 | Quality assessment of the studies

The quality of the selected studies was assessed by applying the JADAD scale,[23] revised in accordance with the REFLECT Statement,[24,25] that considers the following: presence of randomisation; presence of masking of operators involved in the vaccination and outcome assessment; control of lost animals at follow-up; appropriateness of the random allocation, based on items 8, 9 and 10 of the REFLECT Statement; and appropriateness of blinding.

2.3 | Data analysis

The data were combined using the RevMan software in the presence of at least two relevant studies.[26] The results were expressed in terms of risk ratio (RR) and 95% confidence intervals (95% CI) in the case of dichotomous outcomes (if there were no events, zero was replaced by a value of 0.5 to allow for the calculations to be made); whereas for continuous variables, it was measured by using the mean difference (MD) with 95% CI. Analysis was performed on a subgroup of commercial vaccines. The heterogeneity among the selected studies was analysed based on contextual and statistical heterogeneity[26]; statistical heterogeneity was ascertained through I^2 and the Q statistics (with fixed effects in the presence of $I^2 < 50\%$ and $p \geq 0.10$ or at random effects with $I^2 \geq 50\%$ and $p < 0.10$). Based on the overall results, the most suitable model was chosen. When data from the studies was not comparable due to differences in reporting or measurement methods, it could not be combined (meta-analysis is not possible) and a descriptive analysis of the extracted data was resorted to. A funnel plot was used to analyse for publication bias.

3 | RESULTS

3.1 | Identification of relevant studies

Overall, the search identified 1278 citations. After removal of duplicates, 857 studies were screened for inclusion by title and abstract assessment, of which 97 were deemed acceptable. Among these, 8 studies containing 16 RCT met the inclusion criteria.[27–34] The other 89 were excluded due to lack of randomisation in the selection of subjects participating in a trial ($n = 29$ studies), nonimplementation of the challenge or challenge with viruses other than EHV-1 ($n = 26$), type of article that does not meet the inclusion criteria, that is, abstracts, editorials, etc. ($n = 31$), absence of a control group ($n = 3$) (Figure 1).

3.2 | Description of the characteristics of the studies

The eight papers, selected by the search strategy, described overall 16 randomised controlled clinical trials; in details, a single study[30] described 7 RCTs; two studies[31,32] reported 2 RCTs each; the other studies[27–29,33,34] reported respectively 1 RCT for each. On review of these 8 studies, one study[28] included four groups of animals which were used to perform three comparisons; only one of which was randomised. As randomisation was a requirement for study inclusion, the paper was included with respect to this single comparison but the other two comparisons were excluded from analysis. For the same reason, a study on EHV-1 in pregnant mares[29] was excluded because the animals were not stated to be allocated randomly; however, data from the same study was used and analysed purely with respect to a single RCT on foals.

The vaccines used in the included studies are very different from one another in terms of type (live attenuated, inactivated and DNA) and technology. In all cases, the comparator was a nonactive (negative) control (Table 1).

The study populations exhibited extreme variability in terms of type of animal used (pony or horse), sex, age and breed. None of the selected studies included pregnant mares as the study population.

The number of animals used in the studies was small, ranging from a maximum of 10 to a minimum of 2 subjects per group, for a total of 97 subjects in the vaccinated group and 87 in the control group. The inclusion criteria for the population generally were not

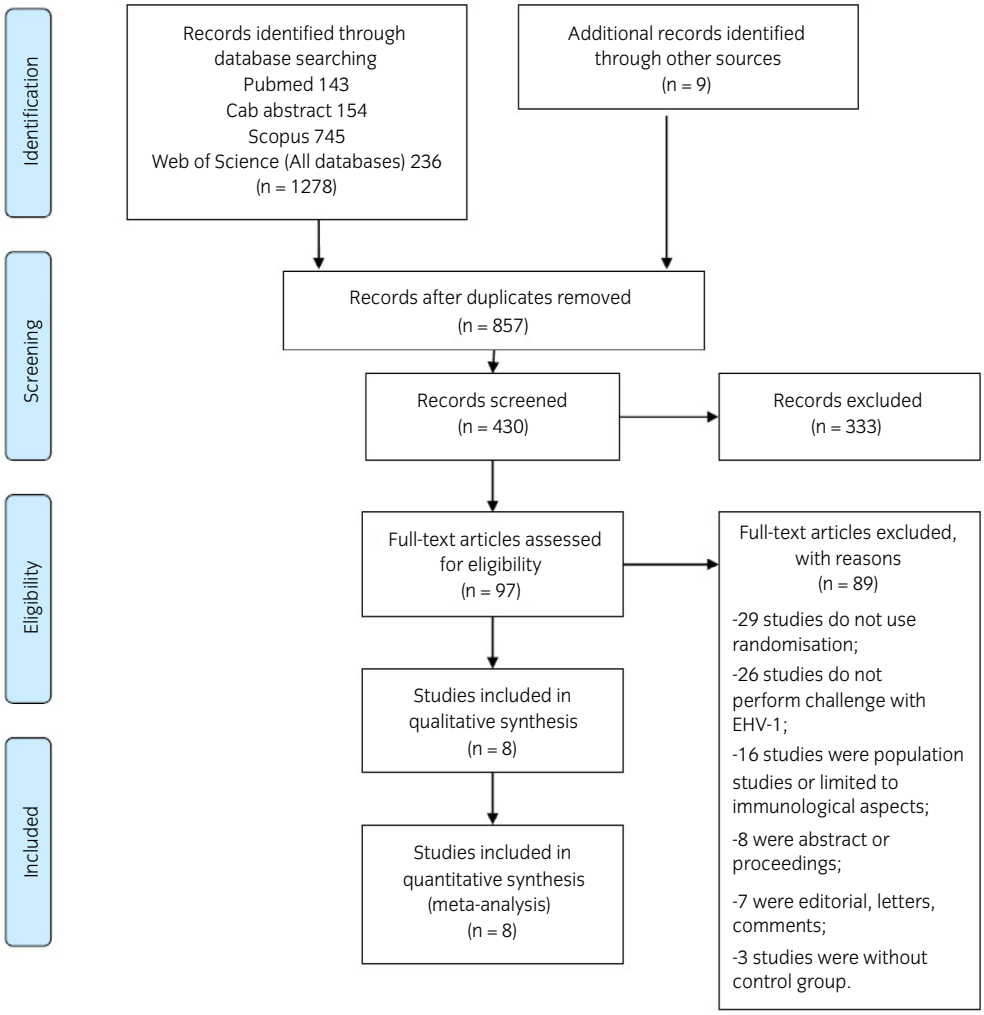

FIGURE 1 Flow chart describing the process of selecting, including and excluding of the studies

explicitly stated. In some cases, the general conditions of breeding management prior to the start of an experiment and the status of infection at the onset of an experiment are described (Table S1).[35,36]

The vaccination schedules, virus strain used for the challenge, and the time between the last vaccination and virus challenge are variable, which could result in differences in the effectiveness of the response to vaccination (Table S2). The viral strains used for the challenge were all isolated from outbreaks of natural disease that were characterised by severe clinical signs. These were generally not genetically typed at the time of a trial (however many of them were typed later in the years subsequent to the study). The viral dose and route of administration (via respiratory tract) were essentially homogeneous (Table S3).

The characteristics of the follow-up were similar in terms of duration of observation period (median 21 days, minimum 14 days and maximum 200 days) and frequency of tests undertaken. A relevant difference observed over time was variation in the methods of analysis. For direct viral investigation, tests ranged from viral isolation and titration, to PCR. The latter included both real-time and quantitative PCR for determination of the viral load in a nasal swab or blood. Molecular methods are generally more sensitive than traditional methods in being able to detect lower quantities of viral nucleic acid (including infectious virus/inactivated virus/fragments of viral nucleic acid) and for a longer time-frame. Furthermore, PCRs (real-time or quantitative) only identify DNA, regardless of whether what is detected is infectious virus or not. For virus-specific immune response investigations, conventional methods (virus neutralisation, complement fixation assay, ELISA) were replaced in the most recent studies[32–34] by more definitive methods describing the humoral immune response at the antibody isotype level (Table 2).

Finally, the outcomes were probably the most heterogeneous aspects of the selected studies and these were difficult to compare. Some studies used clinical scores,[29,31–34] which, however, were not defined in their composition and not standardised. Sometimes results were attributed to the group and not to the individual test subjects, so data on the number of animals with multiple clinical signs could not be extracted. Standardisation of some parameters was present in the most recent studies.[33,34] Different parameters were sometimes measured for viral nasal shedding and for viraemia. Viral load was evaluated in some studies, the duration of virus shedding in others, and in still some others, the number of shedders or viraemic animals were determined. In some studies involving duration, only the group means were reported, without any measure of dispersion within the group. This also applied in the case of standard deviation, making it

TABLE 1 Summary of the characteristics of the vaccines used in the clinical trials included in the analysis

First author and year of publication	Country	Type of vaccine	Commercial name (marketed by * at the time of the study)	Vaccine strain used in experimental challenge	Vaccinal viral titre per dose	Adjuvant type	Presence of EHV-4	Type of administration in the control group
Perkins 2019[33]	USA	MLV (candidate vaccine)	N/A	Ab4ΔORF1/71	1×10^7 PFU	N/A	No	Saline placebo
Schnabel 2019[34]	USA/Germany	MLV (candidate vaccine)	N/A	Ab4 ΔORF2	1×10^7 PFU	N/A	No	Not infected
Goehring 2010[32]-a	USA	MLV	Rhinomune, Boehringer	Rac-H strain	Not reported	N/A	No	Saline placebo
Goehring 2010[32]-b	USA	Killed vaccine	Pneumabort-K, Pfizer Animal Health	EHV-1 1P and 1B strains	Not reported	Oil	No	Saline placebo
Goodman 2006[31]-a	USA	MLV	RhinomuneT Pfizer	Rac-H strain	Not reported	N/A	No	Placebo (MEM)
Goodman 2006[31]-b	USA	Killed vaccine	Fluvac Innovator 6, Fort Dodge	Not reported	Not reported	MetaStim	Yes	Placebo (MEM)
Minke 2006[30]-1a	France/UK	Recombinant canarypoxvirus vector expressing gB/gC/gD	ALVAC-EHV	KY strain	10^8 TCID 50	Carbopol	No	Other vaccine (ALVAC-EIV)
Minke 2006[30]-1b	France/UK	Recombinant canarypoxvirus vector expressing gB/gC/gD	ALVAC-EHV	KY strain	10^8 TCID 50	Nothing	No	Other vaccine (ALVAC-EIV)
Minke 2006[30]-1c	France/UK	Killed vaccine	Not reported	Not reported	Not reported	Carbopol	Yes	Other vaccine (ALVAC-EIV)
Minke 2006[30]-2a	France/UK	Plasmid DNA (expressing gB/gC/gD)	N/A	Neurologic EHV-1 strain 2234/88-2	500 μg/plasmid	No	No	Nothing
Minke 2006[30]-2b	France/UK	Plasmid DNA (expressing gB/gC/gD)	N/A	Neurologic EHV-1 strain 2234/88-2	500 μg/plasmid	Aluminium phosphate	No	Nothing
Minke 2006[30]-2c	France/UK	Plasmid DNA (expressing gB/gC/gD)	N/A	Neurologic EHV-1 strain 2234/88-2	500 μg/plasmid	Carbopol	No	Nothing
Minke 2006[30]-2d	France/UK	Plasmid DNA (expressing gB/gC/gD)	N/A	Neurologic EHV-1 strain 2234/88-2	500 μg/plasmid + 200 μg GM-CSF	DMRIE-DOPE	No	Nothing
Breathnach 2001[28]	USA	MLV-killed	Rhinomune + Pneumabort K	Not reported	2.35×10^7 PFU/ml plus killed vaccine	No/oil	No	Nothing
Heldens 2001[29]	UK/Ireland/ Netherlands	Killed vaccine	Duvaxyn, Fort Dodge	EHV-1438/77	$>10^{7,3}$ TCID 50	Carbopol	Yes	Nothing
Hannant 1993[27]	UK/USA	Killed vaccine	N/A	V592	25 μg of whole virus	ISCOM	No	Nothing

Abbreviations: MEM, minimum essential medium (medium used for viral transport); MLV, modified live attenuated virus; N/A, not applied; PFU, plaque forming units.

TABLE 2 Summary of the primary and secondary outcomes obtained during the clinical trials included in the analysis

First author and year of publication	Number of vaccinated animals with at least 1 clinical sign (%)	Number of control animals with at least 1 clinical sign (%)	Mean days of fever in vaccinated animals	Mean days of fever in control animals	Number of vaccinated animals with >1 clinical sign (%)	Number of control animals with >1 clinical sign (%)	Mean viral titre in ns of vaccinated animals	Mean viral titre in ns of control animals	Days of viral shedding in vaccinated animals	Days of viral shedding in control animals	Number of viral shedder in vaccinated animals (%)	Number of viral shedder in control animals (%)
Perkins 2019[33]	0 (0)	5 (100)	0	5 (biphasic)	Reported a clinical score < 2.2	Reported a clinical score 5	0 PFU/ml	30 000 PFU/ml	0 (VI)	4–5 (VI)	2 (40)	5 (100)
Schnabel 2019[34]	3 (37.5)	8 (100)	1–3	5	Reported a clinical score < 4	Reported a clinical score < 6	0 PFU/ml	10^5 PFU/ml	8 (VI)	8 (VI)	1 (12.5)	8 (100)
Goehring 2010[32]-a	8 (100)	8 (100)	3 (biphasic)	6 (biphasic)	Reported a clinical score < 1	Reported a clinical score < 5	4.8 viral copies $\log_{10}$ (qPCR)	6 viral copies $\log_{10}$ (qPCR)	10 (qPCR)	17 (q PCR)	Not reported	Not reported
Goehring 2010[32]-b	8 (100)	8 (100)	7	6 (biphasic)	Reported a clinical score < 3	Reported a clinical score < 5	4.2 viral copies $\log_{10}$ (qPCR)	6 viral copies $\log_{10}$ (qPCR)	6 (qPCR)	16 (qPCR)	Not reported	Not reported
Goodman 2006[31]-a	Not reported	Not reported	1.2 (SD 0.45)	3.4 (SD 1.52)	Not reported	Not reported	10 PFU/ml	~9000 PFU/ml	0.2	3.8	1 (20)	5 (100)
Goodman 2006[31]-b	Not reported	Not reported	2.25 (SD 0.5)	3.4 (SD 1.52)	Not reported	Not reported	~800 PFU/ml	~9000 PFU/ml	3.2	3.8	5 (100)	5 (100)
Minke 2006[30]-1a	5 (100)	3 (60)	2.2 (range 1–4)	0.8 (range 0–2)	3 (60)	2 (40)	1.37 $\log_{10}$	4.6 $\log_{10}$	1 (range 0–3)	4.6 (range 4–5)	2 (40)	5 (100)
Minke 2006[30]-1b	3 (60)	3 (60)	0.6 (range 0–1)	0.8 (range 0–2)	1 (20)	2 (40)	4.5 $\log_{10}$	4.6 $\log_{10}$	4.8 (range 4–6)	4.6 (range 4–5)	5 (100)	5 (100)
Minke 2006[30]-1c	4 (80)	3 (60)	1.4 (range 0–4)	0.8 (range 0–2)	1 (20)	2 (40)	2.6 $\log_{10}$	4.6 $\log_{10}$	3.2 (range 1–5)	4.6 (range 4–5)	5 (100)	5 (100)
Minke 2006[30]-2a	5 (100)	5 (100)	3.2	3	2 (40)	4 (80)	1.16 $\log_{10}$	1.68 $\log_{10}$	4	5.4	4 (80)	5 (100)
Minke 2006[30]-2b	5 (100)	5 (100)	2.8	3	3 (60)	4 (80)	0.38 $\log_{10}$	1.68 $\log_{10}$	1.4	5.4	4 (80)	5 (100)
Minke 2006[30]-2c	5 (100)	5 (100)	3.6	3	3 (60)	4 (80)	0.85 $\log_{10}$	1.68 $\log_{10}$	3.2	5.4	5 (100)	5 (100)
Minke 2006[30]-2d	4 (80)	5 (100)	2.2	3	3 (60)	4 (80)	0.66 $\log_{10}$	1.68 $\log_{10}$	3	5.4	4 (80)	5 (100)
Breathnach 2001[28]	Not reported	Not reported	6.7	14	Not reported	Not reported	Not reported	Not reported	3.3 (by PCR)	9 (by PCR)	Not reported	Not reported
Heldens 2001[29]	10 (100)	5 (100)	6.3	7.2			0.7 ± 0.92 $\log_{10}$	1.83 ± 0.84 $\log_{10}$	5.2 ± 1.6	10 ± 3.2	10 (100)	5 (100)

TABLE 2 (Continued)

First author and year of publication	Number of vaccinated animals with at least 1 clinical sign (%)	Number of control animals with at least 1 clinical sign (%)	Mean days of fever in vaccinated animals	Mean days of fever in control animals	Number of vaccinated animals with >1 clinical sign (%)	Number of control animals with >1 clinical sign (%)	Mean viral titre in ns of vaccinated animals	Mean viral titre in ns of control animals	Days of viral shedding in vaccinated animals	Days of viral shedding in control animals	Number of viral shedder in vaccinated animals (%)	Number of viral shedder in control animals (%)
					Mean clinical score 51.4 ± 17.8	Mean clinical score 68.9 ± 16.3						
Hannant 1993[27]	7 (77.8)	6 (100)	1.6 ± 0.5	2.6 ± 0.8	5 (55.5)	4 (66.7)	13.6 $TCID_{50}$	28.6 $TCID_{50}$	4.8	7.8	9 (100)	6 (100)

Abbreviations: ns, nasal swab; PCR, polymerase chain reaction; PFU, plaque forming units; qPCR, quantitative PCR; VI, viral isolation.

impossible to perform a comparison and quantitative analysis (Tables 2 and 3). Even where the same methods were used to evaluate the outcomes, differences in the time of final reading of the tests used to measure the antibody response and/or viral titres made it difficult to compare the results.

Animals lost in the follow-up were generally not reported, probably because the studies were limited in the number of individuals and period of observation (Table 4).

In summary, the included studies had statistical and contextual heterogeneity due to the type of population involved (breed, age and sex), country in which studies were conducted, type of vaccines, viral challenge, different methodologies applied, and so on. For this reason, these data are extracted and reported in a qualitative way.

3.3 | Quality assessment

Except for a single study,[32] that obtained a score of 4, the quality of the included works was poor. One study[31] had a score of 2, four[27,29,33,34] had a score of 1 and two[28,30] with a score of zero. Randomisation is described but appears appropriate only in one study (12.5% of the studies), while in two studies (25%), it was not addressed. Masking was considered only in two studies (25%), while it was not considered in others. Probably due to the small number of animals used in the studies, it is rare that they were lost in the follow-up; however, these data were never explicitly reported. The detail of the quality score is shown in Table 4.

3.4 | Meta-analysis

Meta-analysis was carried out for the primary outcomes; this was based on the number of subjects with at least one or more than one clinical sign suggestive of EHV-1 related disease (fever, depression, decrease in appetite, coughing, ocular discharge, nasal discharge, lymph node enlargement, dyspnoea, abortion, neurological signs, ataxia), the number of viraemic subjects and the number of viral shedders. Counts are simple measures that have the advantage of being comparable, whereas outcomes collected and reported by different scores or different tests and units for reporting are very often not comparable, as individual data or appropriate measures of dispersion of data for a group are often not reported.

Although the statistical analysis generally did not show heterogeneity between the included studies (Figures 2–4, Figure S1), a random effects model was used to investigate and analyse the level of contextual heterogeneity.

The pooled estimate in reduction of the number of animals with at least one clinical sign (pooled RR 0.97, 95% CI 0.86–1.10, $p = 0.62$) did not reflect significant vaccine efficacy among the selected studies (Figure 2). This is even more striking if only the subgroup of commercial vaccines is considered (pooled RR 1.02, 95% CI 0.90–1.15, $p = 0.76$, Figure 3). In contrast however, the efficacy of the vaccines improves if analysis considers a reduction of more than one clinical

TABLE 3 Summary of the secondary outcome obtained during the clinical trials included in the analysis

First author and year of publication	Mean titre of viraemia in vaccinated animals	Mean titre of viraemia in control animals	Viraemia in days in vaccinated animals	Viraemia in days in control animals	Number of viraemic vaccinated animals	Number of viraemic control animals	Mean peak antibody titre in vaccinated animals	Mean peak antibody titre in control animals	Adverse reactions to vaccine in vaccinated animals	Adverse reactions to vaccine in control animals	Presence of the declaration of interest	Presence of competing interest
Perkins 2019[33]	>38 Ct (real time PCR)	32 Ct (real time PCR)	9 (with days with undetectable virus)	15	1 (20)	5 (100)	15 000 MFI	20 000 MFI	Not reported	Not reported	Yes	No
Schnabel 2019[34]	>39 Ct (real time PCR); >5 PFU/1 × 10^7 PBMC	35 Ct (real time PCR); 22 PFU/1 × 10^7 PBMC	8 with VI; 2 with real time PCR	8 with VI; 9 with real time PCR	1 (12.5)	8 (100)	<9000 MFI	13 000 MFI	Not reported	Not reported	No	Not reported
Goehring 2010[32]-a	1.5 viral copies log_{10} (real time PCR)	2.8 viral copies log_{10} (real time PCR)	3 (real time PCR)	5 (real time PCR)	4 (50)	6 (75)	7680 ± 1435 SE	2016 ± 518 SE	1	0	Yes	Yes
Goehring 2010[32]-b	1.6 viral copies log_{10} (real time PCR)	2.8 viral copies log_{10} (real time PCR)	2 (real time PCR)	5 (real time PCR)	2 (25)	6 (75)	7680 ± 1435 SE	2016 ± 518 SE	13	0	Yes	Yes
Goodman 2006[31]-a	5.5 viral copies log_{10}	4 viral copies log_{10}	7 (real time PCR)	8 (real time PCR)	5 (100)	5 (100)	165 $TCID_{50}$	120 $TCID_{50}$	0	0	Yes	Yes
Goodman 2006[31]-b	4.8 viral copies log_{10}	4 viral copies log_{10}	8 (real time PCR)	8 (real time PCR)	5 (100)	5 (100)	240 $TCID_{50}$	120 $TCID_{50}$	3	0	Yes	Yes
Minke 2006[30]-1a	Not reported	Not reported	Not reported	Not reported	5 (100)	5 (100)	3.1 log_{10}	3.1 log_{10}	Not reported	Not reported	Yes	Yes
Minke 2006[30]-1b	Not reported	Not reported	Not reported	Not reported	5 (100)	5 (100)	3.2 log_{10}	3.1 log_{10}	Not reported	Not reported	Yes	Yes
Minke 2006[30]-1c	Not reported	Not reported	Not reported	Not reported	4 (80)	5 (100)	3 log_{10}	3.1 log_{10}	Not reported	Not reported	Yes	Yes
Minke 2006[30]-2a	Not reported	Not reported	Not reported	19	5 (100)	5 (100)	2.6 log_{10}	2.6 log_{10}	Not reported	Not reported	Yes	Yes
Minke 2006[30]-2b	Not reported	Not reported	Not reported	19	5 (100)	5 (100)	2.6 log_{10}	2.6 log_{10}	Not reported	Not reported	Yes	Yes
Minke 2006[30]-2c	Not reported	Not reported	13.6	19	5 (100)	5 (100)	2.8 log_{10}	2.6 log_{10}	Not reported	Not reported	Yes	Yes
Minke 2006[30]-2d	Not reported	Not reported	Not reported	19	4 (80)	5 (100)	2.6 log_{10}	2.6 log_{10}	Not reported	Not reported	Yes	Yes
Breathnach 2001[28]	Not reported	Not reported	0 (by PCR)	7.5 (by PCR)	0 (0)	Not reported	Not reported	Not reported	Not reported	Not reported	No	No
Heldens 2001[29]	Not reported	Not reported	0.3 ± 0.5	2.2 ± 1.5	3 (30)	4 (80)	1675 $TCID_{50}$ with SN 608 with CF	235 $TCID_{50}$ with SN 116 with CF	No	No	Yes	Yes
Hannant 1993[27]	6 $TCID_{50}$	121.8 $TCID_{50}$	6	11.5	9 (100)	6 (100)	Seroconversion reported for 6/9 vaccinated ponies by CF and 5/9 by SN	Seroconversion reported for all controls by CF and SN	Not reported	Not reported	No	No

Abbreviations: C, control; CF, complement fixation test; Ct, cycle threshold value obtained by real-time PCR; MFI, median fluorescence intensities; V, vaccinated.

TABLE 4 Summary of the study quality based on the Jadad scale, revised according to the REFLECT statement

First author and year of publication	Presence of randomisation	Presence of blinding	Lost to follow-up analysis	Appropriate randomisation	Appropriate blinding	Total
Perkins 2019[33]	1	0	0	0	0	1
Schnabel 2019[34]	1	0	0	0	0	1
Goehring 2010[32]	1	1	0	1	1	4
Goodman 2006[31]	1	1	0	−1	1	2
Minke 2006[30]	1	0	0	−1	0	0
Heldens 2001[29]	1	0	0	0	0	1
Breathnach 2001[28]	1	0	0	−1	0	0
Hannant 1993[27]	1	0	0	0	0	1

sign (pooled RR 0.76, 95% CI 0.53–1.09, $p = 0.13$), even if the selected studies had fewer animals (Figure 4). Much information was lost in the different trials due to lack of reporting of these parameters[28,31] or for use of a cumulative and nonstandardised clinical score.[29,32–34]

Vaccines did not significantly reduce the number of virus shedders via the nasal route (pooled RR = 0.86, 95% CI 0.71–1.05, $p = 0.14$, Figure 5) and the effect is less favourable if only commercial vaccines are considered (pooled RR = 0.94, 95% CI 0.77–1.16, $p = 0.59$, Figure S1). Although not significant, the number of viraemic animals was reduced in vaccinated animals (pooled RR = 0.88, 95% CI 0.73–1.05, $p = 0.16$) compared with the control groups (Figure 6). This effect is less evident in the case of commercial vaccines (pooled RR = 0.91, 95% CI 0.75–1.10, $p = 0.34$, Figure S2).

A funnel plot (Figure 7) demonstrated that studies with a positive (reducing frequency of clinical signs in vaccinated animals) or a null effect are more frequent. Studies comprising a larger population of test subjects were lacking.

4 | DISCUSSION

In the absence of any previous systematic review of the efficacy of vaccination against EHV-1 infection, we undertook a systematic review and meta-analysis of RCT involving viral challenge to assess EHV-1 vaccine efficacy. In general, modest vaccination efficacy was evident (most marked in the case of a reduction of more than one clinical sign: pooled RR 0.76, 95% CI 0.53–1.09, $p = 0.13$, as showed in Figure 4, and least apparent for the subgroup of commercial vaccines: pooled RR 1.02, 95% CI 0.90–1.15, $p = 0.76$, Figure 3), even though the results were not significant when evaluated by quantitative methods (meta-analysis). Trials were heavily penalised by the heterogeneity of the reported data and by not reporting some data at an individual level or degree of variability if measured in groups. In this respect, dispersion measures (i.e., standard deviation) would need to have been reported to allow comparison of the different parameters.

Despite the heterogeneity that hindered combination of the studies, a quantitative analysis was attempted on the most solid and comparable outcomes of vaccine efficacy. Overall, the results of the quantitative analysis show a slight but nonsignificant efficacy of the EHV-1 vaccines in reducing clinical signs of the disease (pooled RR 0.97, 95% CI 0.86–1.10, $p = 0.62$ considering animals with at least one clinical sign, Figure 2, and pooled RR 0.76, 95% CI 0.53–1.09, $p = 0.13$ considering animals with more than one clinical sign, Figure 4). A greater, albeit still nonsignificant efficacy, was evident with regard to the number of nasal shedders and viraemic animals (pooled RR = 0.86, 95% CI 0.71–1.05, $p = 0.14$, Figure 5, and pooled RR = 0.88, 95% CI 0.73–1.05, $p = 0.16$, Figure 6, respectively). All the estimates of EHV-1 vaccine efficacy were reduced when only commercial vaccines were considered (pooled RR 1.02, 95% CI 0.90–1.15, $p = 0.76$ considering animals with at least one clinical sign, Figure 3; pooled RR = 0.94, 95% CI 0.77–1.16, $p = 0.59$ considering nasal shedders, Figure S1; and pooled RR = 0.91, 95% CI 0.75–1.10, $p = 0.34$ considering viraemic animals, Figure S2).

A limitation in the assessment of these outcomes is that much relevant information was missing due to incomplete or nonstandardised reporting, as discussed below in respect of qualitative analysis. Some studies did not describe clinical parameters[28,31] or state cumulative and nonstandardised clinical scores.[29,32–34] Guidelines provided by the REFLECT Statement,[24,25] as well as some systematic reviews already published on the topic,[37,38] should help to eliminate these reporting defects by providing useful indications of how they should be addressed in future studies. The use of different, nonstandardised, clinical scores prevents comparison of observations between different study groups. It would be desirable to create a single, standardised score, such as a body condition score in veterinary medicine or the Visual Analogue Scale (VAS) of pain used in human medicine to manage and compare these data more easily. The most recent studies[33,34] seem to be more standardised in terms of methods and clinical scores. Otherwise, if specific clinical scores are used, it would be worth reporting the composition of these scores at least as a supplementary item to allow comparative data to be extracted from the study. Additionally, recommending that all raw data is archived in repository would assist future meta-analysis by enabling the same outcome measures to be calculated for all included studies.

Results on the number of virus shedders via the nasal route and viraemic animals provided moderate but not significant evidence of efficacy of vaccination. These analyses only referred to the number of

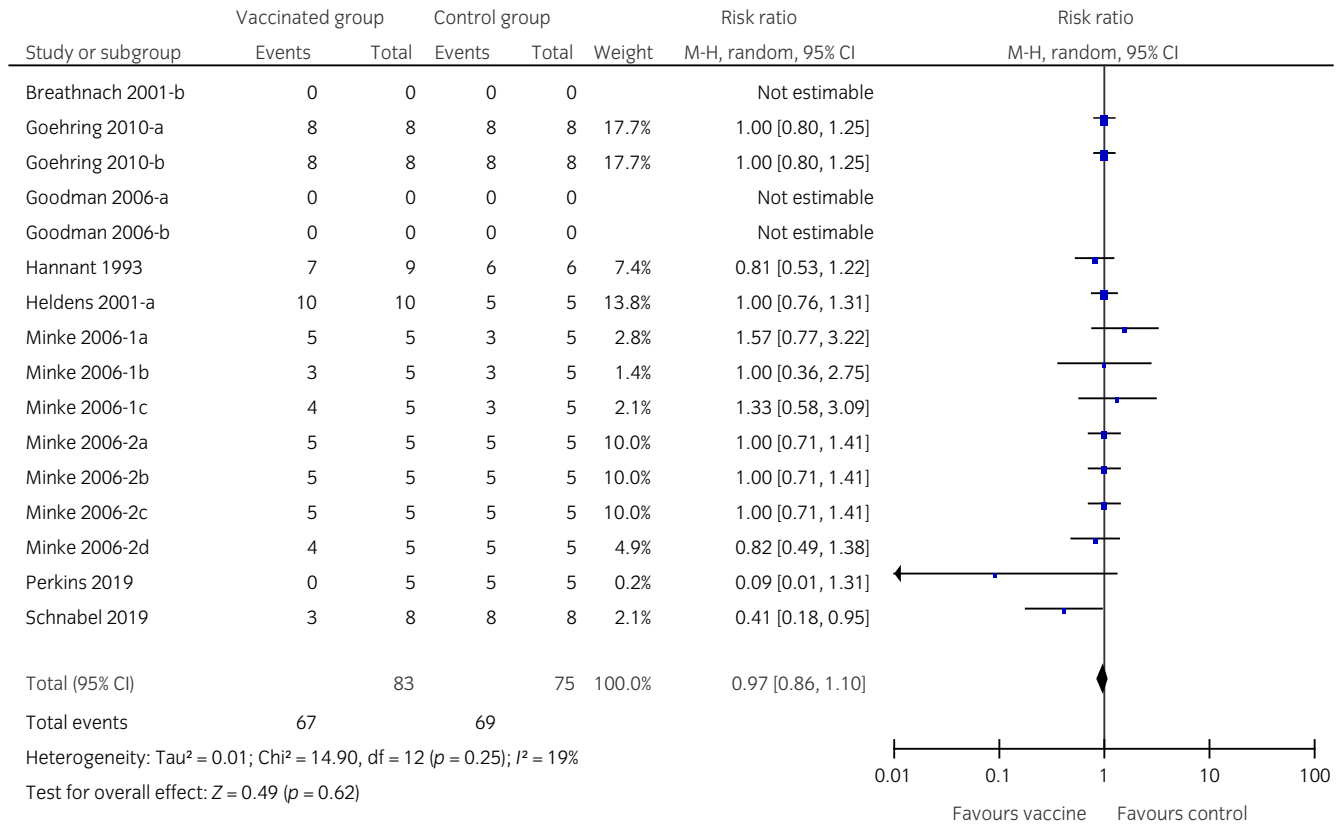

FIGURE 2 Efficacy of vaccination in reducing clinical signs, based on the presence of at least one clinical sign, after EHV-1 challenge

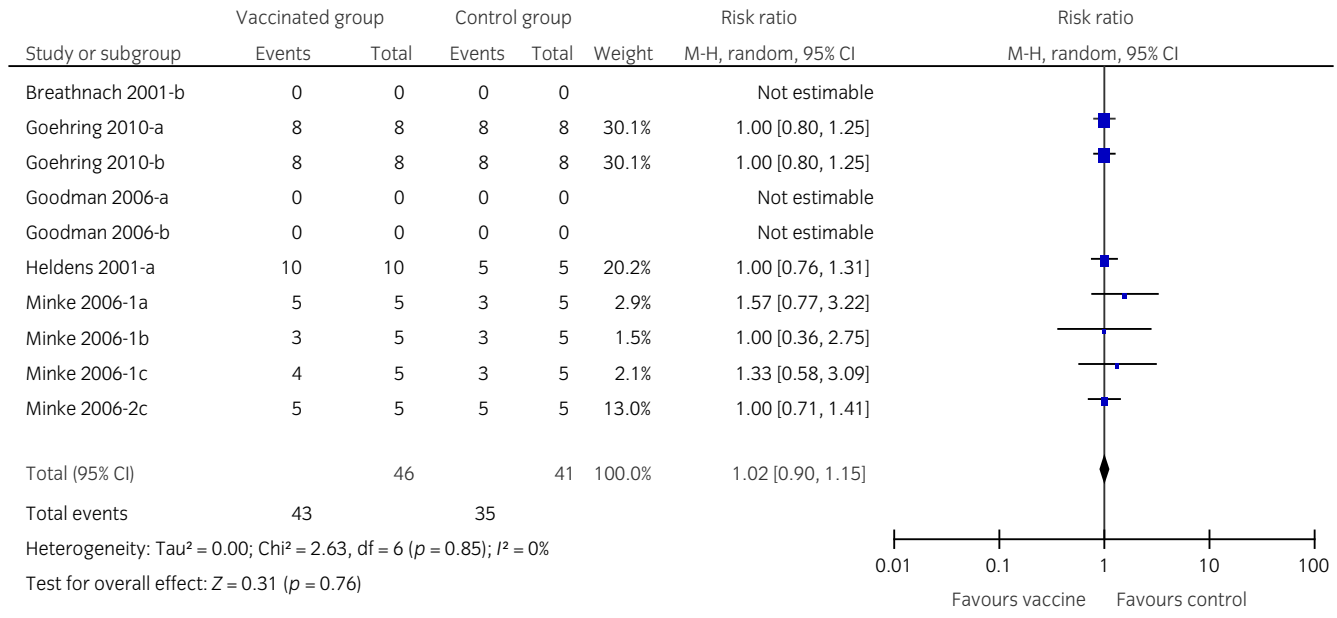

FIGURE 3 Efficacy of commercially available vaccines in reducing clinical signs, based on the presence of at least one clinical sign, after EHV-1 challenge

animals involved and could only be completed by information about the mean titre of the virus shed by the nasal route, duration of the viral shedding, mean values of viraemia, and mean duration of viraemia. Nevertheless, the heterogeneity and lack of standardisation in measuring these outcomes, together with their poor reporting, did not permit meta-analysis of these studies; had they been addressed, the studies would probably have confirmed evidence of more consistent vaccine efficacy. In fact, on evaluation of the raw data, this is the only

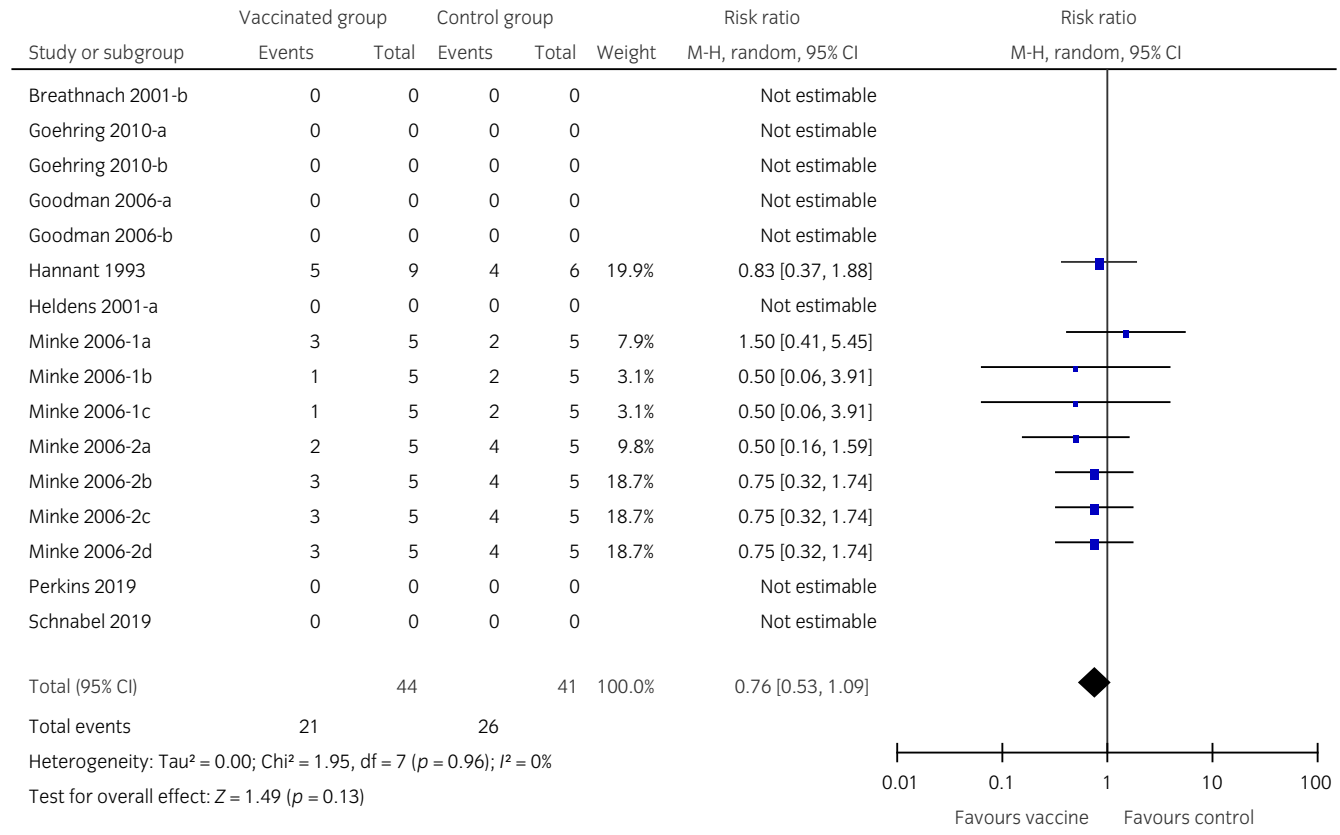

FIGURE 4 Efficacy of vaccination in reducing clinical signs (more than one clinical sign) after EHV-1 challenge

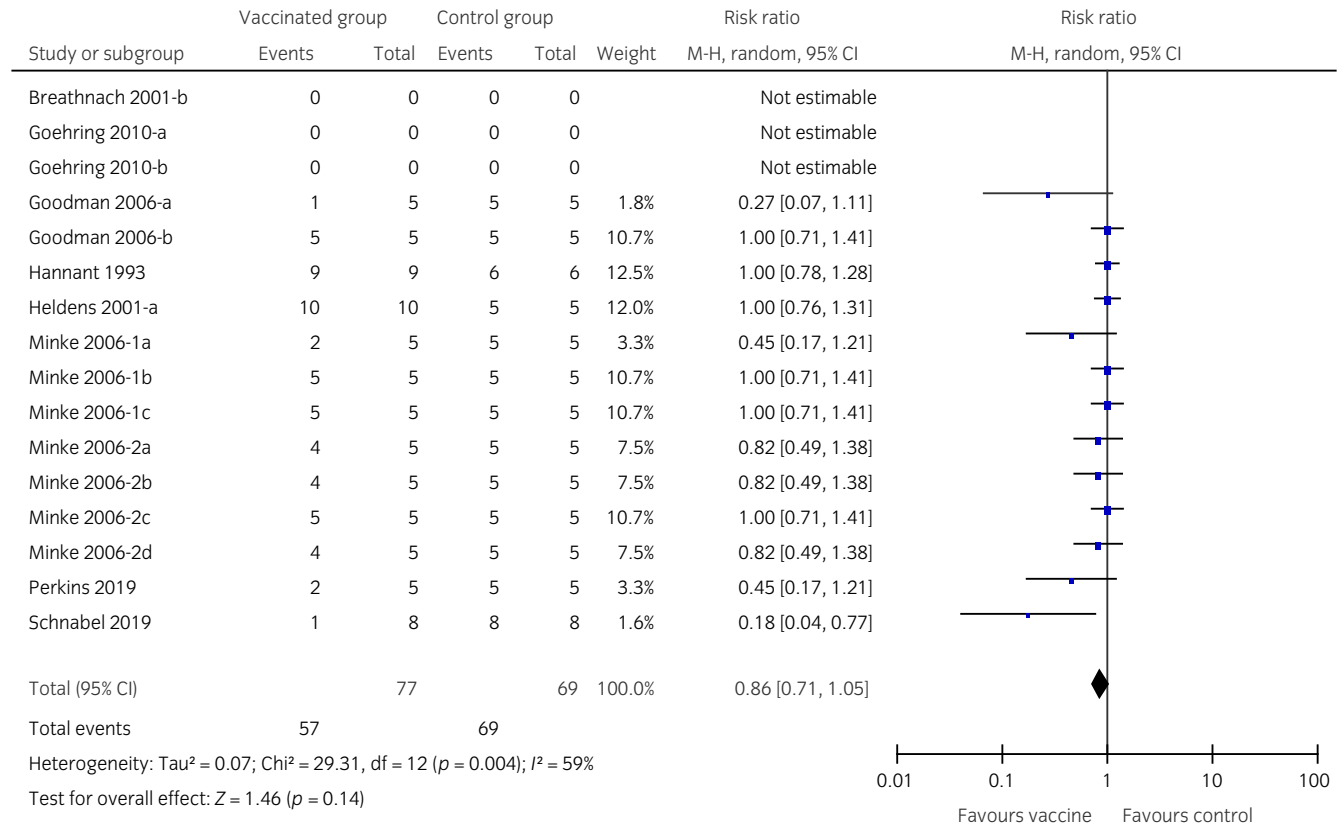

FIGURE 5 Efficacy of vaccination in reducing EHV-1 animal shedders after EHV-1 challenge

Study or subgroup	Vaccinated group Events	Total	Control group Events	Total	Weight	Risk ratio M-H, random, 95% CI
Breathnach 2001-b	0	0	0	0		Not estimable
Goehring 2010-a	4	8	6	8	3.8%	0.67 [0.30, 1.48]
Goehring 2010-b	2	8	6	8	1.9%	0.33 [0.09, 1.18]
Goodman 2006-a	5	5	5	5	9.3%	1.00 [0.71, 1.41]
Goodman 2006-b	5	5	5	5	9.3%	1.00 [0.71, 1.41]
Hannant 1993	9	9	6	6	10.8%	1.00 [0.78, 1.28]
Heldens 2001-a	3	10	4	5	2.6%	0.38 [0.13, 1.06]
Minke 2006-1a	5	5	5	5	9.3%	1.00 [0.71, 1.41]
Minke 2006-1b	5	5	5	5	9.3%	1.00 [0.71, 1.41]
Minke 2006-1c	4	5	5	5	6.5%	0.82 [0.49, 1.38]
Minke 2006-2a	5	5	5	5	9.3%	1.00 [0.71, 1.41]
Minke 2006-2b	5	5	5	5	9.3%	1.00 [0.71, 1.41]
Minke 2006-2c	5	5	5	5	9.3%	1.00 [0.71, 1.41]
Minke 2006-2d	4	5	5	5	6.5%	0.82 [0.49, 1.38]
Perkins 2019	1	5	5	5	1.5%	0.27 [0.07, 1.11]
Schnabel 2019	1	8	8	8	1.4%	0.18 [0.04, 0.77]
Total (95% CI)		93		85	100.0%	0.88 [0.73, 1.05]
Total events	63		80			

Heterogeneity: Tau² = 0.07; Chi² = 33.99, df = 14 (p = 0.002); I^2 = 59%

Test for overall effect: Z = 1.40 (p = 0.16)

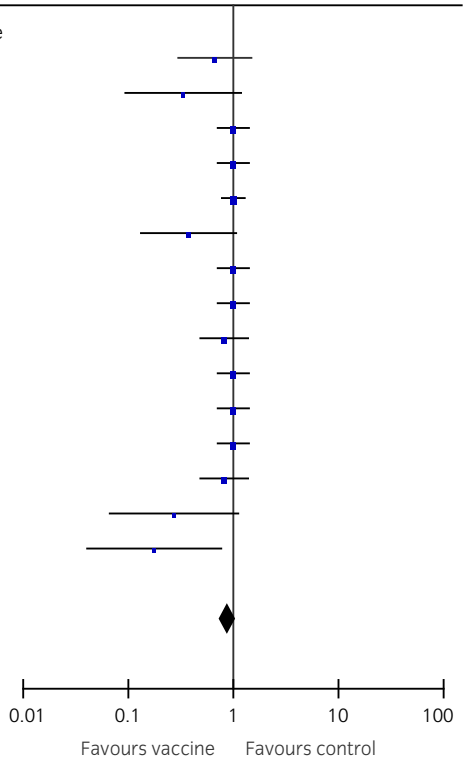

FIGURE 6 Efficacy of vaccination in reducing EHV-1 viraemia after EHV-1 challenge

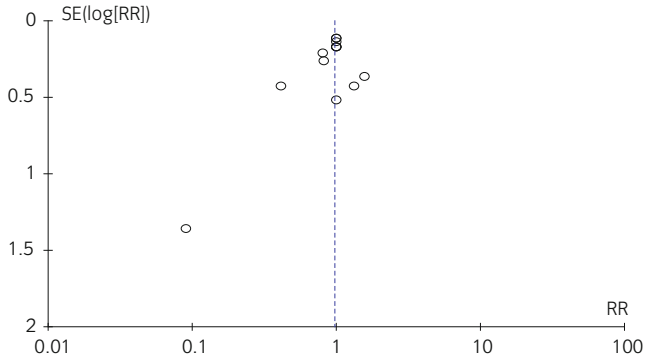

FIGURE 7 Funnel plot obtained by combining studies reporting the primary outcome (efficacy in reducing EHV-1 disease, here reflected by the presence of at least one clinical sign in the vaccinated and control group)

possible way to assess data when presented in a fragmentary fashion. It seems there is greater difference in these parameters between vaccinated and control groups in the different studies, with a reduction in the mean period of viral shedding and viraemia in vaccinated animals (Tables 3 and 4).

However, as expected and already hypothesised,[2,19,21] when considering all the studies, the findings indicate that vaccination helps to reduce the viral load in the environment, minimises virus replication in the respiratory tract, reduces nasal shedding and the magnitude and duration of viraemia. That said, the evidence is weak when the magnitude and quality of the study is considered.

Regretfully, the reduction in viraemia, that is the prerequisite to prevent abortion and neurological disease, could not be evaluated and, moreover, no pregnant mares were used in any of the selected studies. It is possible that using more refined diagnostic tests that were employed in the most recent studies,[33,34] it may be better able to assess the efficacy of vaccination in reducing viraemia following infection.

Many studies were excluded from the qualitative analysis because of an absence of randomisation (n = 29) or of a control group (n = 3). Presence of a control group can be expensive in case of horses, but randomisation is easily achievable and inexpensive and should be widely implemented in veterinary studies. RCT is considered the gold standard for evaluating the efficacy of preventive interventions[25] because appropriate randomisation guarantees a balanced distribution of subjects among groups without bias, while the presence of a control group is necessary to have an appropriate comparison in effect. However, both randomisation and inclusion of a control group must be performed and reported according to specific criteria to be considered valid. On the contrary, biased trials have the potential to give inaccurate results and promote misleading decision making by clinicians, researchers, policy makers and finally the general public.[24,25] Moreover, veterinary studies have specificities with respect to RCTs in humans that should be considered when designing RCTs and when the presence of biases must be evaluated.[26]

The current study found that the majority of the included RCTs analysed were of poor quality based on the Jadad scale, modified according to the REFLECT Statement.[23–25] Often it was not possible to determine whether there was only a bias in the reporting or a structural bias in the study design. Only one study proved to be of good quality,[32] and only two could be considered double-blind.[32,33] Most of the included studies did not correctly perform randomisation and double blinding, which represent a guarantee for correct selection of subjects and the absence of misclassification and detection bias. This analysis confirms, as reported in the REFLECT Statement, that reporting in veterinary literature is poor.[25] As previously discussed, poor reporting also results in limitations in the quantitative analyses. The overview of extracted data from these RCTs revealed these limitations and the lack of certain relevant information. It should be emphasised that accurate reporting of RCTs is necessary if the reader is to be able to evaluate the internal and external validity of a study.[25] Well-conducted studies with poor reporting cannot be assessed appropriately and therefore lose credibility. The existing limits in veterinary medicine literature must be taken into account, especially when considering the necessity of clinicians to reach a decision in practice. On systematic review, a selection of RCTs was obtained and a limited effect of EHV-1 vaccination was observed; it does not mean that other clinical trials not included in this analysis, or other types of studies like the observational studies, are not useful when evaluating the efficacy of vaccination. In those cases, however, it is necessary to evaluate the influence that potential biases may have been in each of them. In general, any study, including RCT, should be assessed to be of good quality. Many of the 89 excluded studies, highlighted in the flowchart, have informational value, which however needs to be carefully and critically evaluated.

A further characteristic of RCTs, that influence the efficacy of a vaccine in veterinary medicine, is the use of a deliberate challenge in the case of an infectious disease.[25] For some authors, the real value of a vaccine in horses can only be assessed in virus challenge experiments.[29] For ethical reasons, however, this kind of study is not applicable in human medicine and, for the same reason, results in restricted populations in veterinary medicine. All the included studies evaluated had a limited test population, with the maximum number reached only in a single study which had 10 foals in the study group.[29] This results in studies of low statistical power. It is indeed possible to have a Type II error or false negative, which means that the study can fail to reject a null hypothesis when it is false, so that a small effect could go unnoticed. Meta-analysis represents a good tool to combine data of different studies and increase statistical power. However, to obtain this, the studies must be comparable, measure the same outcomes and have a good and standardised reporting of data.

The funnel plot used in the present study confirmed that the selected studies had generally a positive or null effect in reducing the clinical signs in vaccinated animals. However, detected asymmetry should be evaluated with caution both because challenge studies are generally carried out on a small study population and the number of the RCTs included in the meta-analysis is limited. We have to consider also that often guidelines of a journal require a specific format that does not agree with the REFLECT Statement or will not publish non-innovative or negative results, resulting in reporting or publication bias.

Qualitative data extracted from the RCTs showed relevant differences among studies of: different types of vaccines, demography of the population, schedules of vaccination, study outcomes. Some of these differences were probably determined by economic limits, initial aims of a study, practical aspects in the management of the animals, and technical aspects. The wide heterogeneity makes the findings of these studies hard to generalise (low external validity). For example, many of the studies were conducted on ponies, probably due to the lower cost compared with horses, reducing generalisation of the results. Different breeding management of the animals should be carefully evaluated because it could affect the outcome of an experiment.

Moreover, virus strains used for challenge were known to have different viral shedding profiles and duration of viraemia,[39] and some of them were even recognised as neuropathogenic strains only retrospectively with respect to the corresponding study. However, the presence of the control group can weight these differences.

With two exceptions,[33,34] studies were carried out within a limited period of time (protocol of vaccination plus 14–28 days of follow-up), that probably provides good observation of the animals, without lack of follow-up, but for a brief period, which may be the one with a better immune response. Furthermore, the included studies cover a period from 1993 to 2019. Diagnostic methods have changed considerably during the time frame the included studies span, in particular with the most recent ones[32–34] using sophisticated immune markers to better define the protection induced by vaccines. Generally, molecular approaches have replaced traditional methods, especially viral isolation, in definition of the viral load because they are less time-consuming. Accordingly, studies become less comparable over time.

Other points that make studies heterogeneous are represented by the timing of vaccination protocols (2 vs 3 vaccinations), but also by the time between vaccination and challenge (ranging from 14 days to 9 months), both of which could influence whether the efficacy of the vaccine response was better or worse. In the case of a long period between vaccination and challenge,[33,34] ensuring that there was no possibility of exposure or re-exposure or in monitoring reactivation of latency of the virus in the subjects and their respective controls over the course of each vaccinal study is very relevant, considering the ubiquitous nature of EHV-1. This extended period between vaccination and challenge is probably employed to assess the presence of long-term immunity from vaccination, since it is estimated that protective immunity −1 following natural infection with EHV-1 is not expected to last more than 3–6 months at most.[40]

The data on adverse reactions, already limited due to the small number of subjects observed, is reported only in three studies,[29,31,32] including five trials, while in others, it is stated in the materials and methods, but absent from the results. The adverse reactions reported were primarily local reactions, with soft tissue swelling at the vaccination site in the days that followed vaccination,[31,32] and a systemic reaction by way of pyrexia.[32]

Except in the case of four independent instances,[27,28,33,34] the studies present conflicts of interest. In situations involving a possible conflict of interest, it is especially important that the reporting is sufficiently complete to allow the reader to interpret the quality of the study. The nonindependence of a study could also result in a bias, in that studies with positive results are more frequently published; this is confirmed by results of the funnel plot.

This systematic review may have some limitations such as a potential bias in selection of the studies. Nonetheless, to offset this, the search for articles was performed using four widely accepted scientific databases, in addition to Google Scholar. All the included RCTs were present in each of the search engines used and in general a good overlap in coverage was present for these kinds of studies. However, for convenience, free access, and rapid consultation, PubMed is probably the best and most accessible search engine, and can be particularly useful in rapid scoping reviews. The other databases require an access fee. CAB Abstract is considered the most comprehensive one in the field of veterinary medicine.[41] Google Scholar, on the other hand, is free, but results are displayed in relation to numbers of visits from users and not controlled by standardised and reproducible methods. Moreover, it is not easy to manage a search based on use of single keywords.[42] Web of Science Core Collection also has a lot of content on this topic which is more manageable thanks to its advanced search strategy applications. The choice of the databases probably becomes more important depending on the topic or the necessity to retrieve studies or documents not published in a journal or the grey literature (official reports, data from database, and similar, which, however, are becoming increasingly important).

In conclusion, the main limitations of the study were the remarkable heterogeneity and poor reporting of the selected studies. Although the results obtained are not conclusive, this was the first attempt to systematise this type of literature research. It was also very useful in highlighting existing gaps, which have been discussed, in this context. The underlined critical points in the included trials will be useful in improving future studies, the goal stated by the European Food Safety Authority for a systematic review.[43] Meta-analysis, providing pooled results, is a useful tool to increase the number of observations and obtain more robust data. However, at present, this cannot always be achieved due to the numerous constrains in the performance and reporting of veterinary medicine trials, which are certainly less standardised than those performed in human medicine. The results of this study should help in the development of recommendations on the use of vaccines in the future, following the GRADE approach.[44]

There are solutions that can be applied in the future to improve such studies: publishing the data according to guidelines for reporting or making raw data from studies accessible or approval dossiers available in the case of commercial vaccines, even as grey literature; implementation of the use of randomisation in the veterinary area. On the other hand, if challenge trials are considered too expensive or not ethical, the possibility to perform multicentre, structured, standardised and comparable field trials should be considered as an alternative in proving the efficacy of vaccination.

AUTHOR CONTRIBUTIONS

Maria Luisa Marenzoni and Chiara De Waure contributed to the study design, data collection and analyses, and preparation of the manuscript. Maria Luisa Marenzoni performed the statistical analysis. Maria Luisa Marenzoni, Chiara De Waure and Peter J. Timoney contributed to verifying the data and interpreting the results. Peter J. Timoney reviewed the manuscript. All authors have read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/evj.13870.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

ETHICAL ANIMAL RESEARCH

Not applicable.

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SUPPORTING INFORMATION

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