

PREVALENCE OF EQUINE HERPESVIRUSES (EHVS) INFECTION IN EGYPT: SYSTEMATIC META-ANALYSIS

M. Marzok¹, A. Al-mubarak², M. Elgiouhy³ and S. El-khodery⁴

¹Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia

²Department of Microbiology, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia

³Department of Animal Medicine, Faculty of Veterinary Medicine, Aswan University, Aswan 37916, Egypt.

⁴Department of Internal Medicine, Infectious Diseases and Fish Diseases, Faculty of Veterinary Medicine, Mansoura University, Manosura 35516, Egypt

Corresponding author's email: mmarzok@kfu.edu.sa ; marzok2000@hotmail.com

ABSTRACT

This report aimed to perform a meta-analysis on the occurrence of Equine herpesvirus (EHV) infection in Egypt. This systematic meta-analysis was performed in accordance with the PRISMA guidelines. Data were retrieved after a complete search, and eligible articles were identified. Data analysis with random-effects model was performed using a comprehensive meta-analysis software program. The results were presented as effect size, confidence intervals (CI), heterogeneity, and publication bias. A total of 1760 horses from 20 accepted studies were investigated for EHV infection. Of these, 740 horses were found positive for EHV, with a prevalence of 42%. The highest prevalence (100%) was during 2016 and 2020. EHV-1 was the most prevalent strain affecting horses in Egypt ($p \leq 0.05$). The prevalence of isolated strains was as follows: EHV-1 (492, 66.48 %), EHV-2 (151, 20.40 %), EHV-4 (105, 14.19 %), and EHV-5 (88, 11.89 %). The mixed EHV-1 and EHV-4 infections were predominant. However, the EHV-3 was not detected in any of these studies. At random effects, the Z-value was -1.539 (p -value = 0.124). The Q-value (373.103), I-squared (95.17), and p-value (0.000) were the final heterogeneity variables. The Egger's linear regression test did not imply a publication bias, and its outcomes were intercept (-3.66), and 95% confidence interval (-8.23 to -0.9). The results of the present meta-analysis indicated a high prevalence of EHV infection in Egypt, particularly EHV-1. Therefore, more attention should be paid to the prevention and control of this disease.

Keywords: Horse, Epidemiology, EHV, Systematic review

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INTRODUCTION

Equine herpesviruses (EHVs) are infectious agents that have deleterious effects on equine health (Lunn *et al.* 2009). They are large, enveloped, linear, double-stranded DNA viruses of the *Herpesviridae* family (Gatherer *et al.* 2021). Nine strains of herpesvirus have been recorded (Davison *et al.* 2009). EHV-1 and EHV-4 are considered the most important herpesviruses because of their high prevalence, ability to reactivate latent infections, and different clinical presentations (Pavulraj *et al.* 2021).

Clinical signs in infected animals vary depending on the type of EHV involved. Both EHV-1 and EHV-4 can cause severe respiratory syndromes. Horses infected with EHV-1 can show abortions in pregnant mares, neurological manifestations with high mortality rates in foals (Pavulraj *et al.* 2021; Slater *et al.* 2006). In addition to lymphadenopathy in foals, the most common clinical signs of EHV-2 in foals and adult horses

include upper respiratory tract symptoms and keratoconjunctivitis (Borchers *et al.* 2006). The EHV-3 causes equine coital exanthema, an acute venereal mucocutaneous disease in mares and stallions (Vissani *et al.*, 2021).

Equines can acquire EHV horizontally through direct contact with the nasal discharge of infected animals or EHV-1-positive aborted fetuses or indirectly through contact with contaminated fomites (Allen *et al.* 2004). Vertical transplacental transmission from infected mares to their offspring or through the venereal route via semen from an infected stallion has been identified as a method of transmission of EHV-3 (Negussie *et al.* 2016). The herpesviruses often remain latent in the host brain and lymphoid tissues after infection with no lytic viral replication (White *et al.* 2012). The compromised immune system of the host may cause the virus to reactivate, leading to viremia and shedding of the virus into the environment (Reese, 2016).

The clinical manifestations of EHV infections are not reliable tools for accurate diagnosis as they may

mimic those of other infectious diseases. Therefore, EHV-1 is diagnosed using several laboratory serological tests for antibody detection, including enzyme-linked immunosorbent assay (ELISA), viral neutralization, and complement fixation (Bannai *et al.* 2013; Lang *et al.* 2013). Viral isolation is considered the standard diagnostic procedure for EHV-1 (Hartley *et al.* 2005). Recently, several molecular techniques have been used to detect the genomic materials of EHV-1, including polymerase chain reaction (PCR) (Pusterla *et al.* 2009) and real-time PCR, which are considered the most sensitive molecular techniques for EHV-1 detection (Smith *et al.* 2012).

The study population and presence of risk factors are determinants of the prevalence of infectious diseases (Buitrago-Garcia *et al.* 2022). Variables such as age and sex of the horses studied have been linked to a higher risk of EHV-1 infection. Young foals are more likely to be infected with EHV-2/-5 than adult horses (Hue *et al.* 2014) while equids aged 3–8 years have a higher prevalence of EHV-1/-4 than those younger than 3 years (Worku *et al.* 2024). The gender differences have also been observed, with males being more likely to contract EHV-1 and-2 (Bolfá *et al.* 2017). However, several studies have recorded higher infection rate of EHV-1 and-4 among females than among males (de Souza *et al.* 2017; Negussie *et al.* 2016).

In Egypt, many epidemiological studies have investigated the prevalence of EHV-1 in horses, showing clinical signs indicative of EHV-1 infection. The prevalence of EHV-1 infection varies spatially and temporal variation (A Salib *et al.* 2016; Ata *et al.* 2020; Abdel-Rady *et al.* 2022; Ahdy *et al.* 2022; Khattab *et al.* 2022). Moreover, there was variation in the clinical findings and identified strains of EHV-1 (A Salib *et al.* 2016; Azab *et al.* 2019). On the other hand, other studies could detect EHV-1 in apparently healthy horses (Ata *et al.* 2020; Meselhy *et al.* 2019).

A meta-analysis is an epidemiological study designed to systematically assess the results of previous investigations to reach a conclusion about this research topic (Haidich 2010). It includes a combined and quantitative review of a large, frequently complex, and occasionally apparently conflicting body of literature (Moher *et al.* 2010b). Additionally, the results of the meta-analysis may provide a more accurate estimation of the effect of risk factors or treatment for specific diseases or other outcomes than any individual study participating in the pooled analysis (Moher *et al.* 2009). Thus, the objective of this investigation is to conduct a meta-analysis on the prevalence of EHV-1 infection in Egypt.

MATERIALS AND METHODS

Ethical approval : Based on guidelines of PRISMA, this study was conducted. Consequently, it is not necessary to

have approval from the ethical committee for animal use in scientific research.

Reference horses: The present meta-analysis included all articles focused on the prevalence of EHV-1 infections in Egypt using different techniques. All horses examined, characterized by symptoms of respiratory, neurological, and abortion, were also included. However, the sample size of this study was not limited.

Selection criteria

Inclusion criteria

- Only acceptable papers those are in English language.
- All publications on Equine herpesviruses (EHVs) in Egypt
- A reputable journal publication
- Case-control and cross-sectional studies
- Studies on the seroprevalence of EHV-1 were included.
- Studies imply the prevalence of EHV-1 with any diagnostic technique used.

Exclusion criteria

- Techniques for identifying EHV-1 other than prevalence.
- Arabic-language publications or languages other than English.
- Preprint and review articles.

Study selection: The objective of this study was to search all publications that had been written about the prevalence of EHV-1 infection in Egypt. We searched the PubMed, Web of Science, Sage, BESCO, Ovid, CABI, Scopus, database with a combination of the following search terms ("EGYPT" "EQUINE HERPESVIRUS") (title/ abstract) ("EQUINE HERPESVIRUS ") (title/ abstract) AND ("EGYPT") (title / abstract) AND ("PREVALANCE" "INCIDENCE") (title/ abstract) OR ("EGYPT") (title / abstract). Preliminary screening of the articles was based on the title and abstract from the earliest data available by May 2024. This procedure was supplemented by manual searching, Google Scholar searching, expert recommendations, and citation reviews. Database outputs were integrated using EndNote software. The standard identification, selection, and eligibility criteria of the selected studies are described (Figure 1).

Data extraction: The extracted data included the year of publication, study area, diagnostic method, sample size, EHV strain, and positive animals (Table 1).

Quality control: According to PRISMA (Moher *et al.* 2010a), the current meta-analysis was carried out. All available published publications on the assessment of the prevalence of EHV-1 infection in Egypt based on blood

sample collection from infected horses were included to reduce publication bias.

Data analysis: First, the prevalence of EHV was estimated and the Chi-square test was used to assess significant variation among infections with different strains of EHV (GraphPad Prism for Windows version 9, USA). The commercial meta-analysis software was used (Comprehensive Meta-Analysis software version 2, Biostat, Englewood, NJ, USA). At random effects model, effect size, 95% confidence intervals, variance, heterogeneity, relative weight, and publication bias were the main tests. To assess the significance of variation between strains of EHV, the chi square test was applied and at $p \leq 0.05$ the result was considered significant.

RESULTS

A total of 875 items were identified from database search. After applying the exclusion criteria, 20 acceptable studies were included in this meta-analysis (Table 1, Figure 1). A total of 1760 horses from 20 accepted studies were investigated for EHV infection. Of these, 740 horses were found positive for EHV, with a prevalence of 42%. EHV-1 was the most prevalent strain affecting horses in Egypt ($p \leq 0.05$).

There was a significant variation in the prevalence of different strains of EHV ($p \leq 0.01$). Infection rate with EHV-1 was significantly higher than EHV-4 ($p \leq 0.001$, Odds ratio: 12.0), EHV-2 ($p \leq 0.001$, Odds ratio: 7.73), and EHV-5 ($p \leq 0.001$, Odds ratio: 14.7). Moreover, the

infection rate with EHV-2 was significantly higher than EHV-5 ($p \leq 0.05$, Odds ratio: 1.8)

The prevalence of isolated strains was as follows: EHV-1 (492, 66.48 %), EHV-2 (151, 20.40 %), EHV-4 (105, 14.19 %), and EHV-5 (88, 11.89 %). Mixed EHV-1 and EHV-4 infections were predominant. However, EHV-3 was not detected in any of these results.

Meta-analysis

The effect of size on the prevalence of EHV infection at random effect model is presented in Table 2. At random effects, the Z-value was -1.539 (p -value = 0.124). To illustrate the degree of heterogeneity, a forest plot was constructed for random effect. The outcome of event rate with confidence interval of 95%, logit event rate, and relative weight are shown in figures 2-4. The Q-value (373.103), I-squared (95.17), and p -value (0.000) are the outcome of heterogeneity at fixed effect, but not at random effect. Results of publication bias were presented in Figure 5 and 6. The funnel plot was asymmetric, and there was no indication of publication bias. The Egger's linear regression test did not imply a publication bias, and its outcomes were intercept (-3.66), 95% CI (-8.23 to -0.9), t -value (1.96), $df = 17.00$. The 1-tailed p -value was (0.05), and the 2-tailed p -value was 0.1. The result of Kendall's tau with continuity correction was -0.18, with a 1-tailed p -value of 0.13 and 2-tailed p -value of 0.26.

The classic fail-safe N proposed that 98000 missing studies are wanted to determine the significance of the results of this meta-analysis. In addition, Orwin's fail-safe N suggested a -0.47-event rate in observed studies and a 0.50 mean event rate in missing studies.

Table 1. Descriptive data regarding Equine herpesvirus (EHV) infection in horses in Egypt.

Author	Location	Samples	Positive	Technique	Virus type	Signs
Amer <i>et al.</i> (2011)	Cairo	93	34	Polyclonal antibody pool against EHV-1, 2, and 4	EHV-1 (n=3) EHV-2 (n=17) EHV-4 (n=7) EHV-1 and EHV-4 (n=1)	Abortion and respiratory signs
Kalad <i>et al.</i> (2013)	Cairo	21	9	Compliment fixation test	EHV-1	Respiratory and/or nervous manifestation and contact horses.
Al-Shammari <i>et al.</i> (2016)	Cairo	12	12	Nested-PCR	EHV-4	Abortion
A Salib <i>et al.</i> (2016)	Cairo, Giza, Kafr Elsheikh, Monofeia, Beni-Suef, El Sharkia and Behira	182	9	Indirect ELISA	EHV-1	Abortions, respiratory diseases, fever and limb edema, Nervous manifestation
El Moghazy	Qalubiah	77	33	Indirect ELISA	EHV-1	No signs

<i>et al.</i> (2017)						
(Ghoniem <i>et al.</i> 2018)	Cairo	152	18	Multiplex real-time PCR	EHV-1 (n=12) EHV-4 (n=6)	Respiratory signs
Azab <i>et al.</i> (2019)	Cairo, Alexandria, Giza, Sharkia, Gharbia and Monufia	192	133	Virus-specific qPCR	EHV-1 (n=64) EHV-2 (n=63) EHV-4 (n=5) EHV-5 (n=37)	Abortion, respiratory diseases, fever and limb edema, and nervous manifestation
Meselhy <i>et al.</i> (2019)	Egypt	62	12	Nested PCR	EHV-1	No clinical signs mentioned
(Ali <i>et al.</i> 2020)	Cairo and Giza	4	4	PCR and cytopathic effect	EHV-1 (n=4)	Abortion and neonatal death
Ata <i>et al.</i> (2020)	Monufia	270	173	Indirect ELISA	EHV-1	No clinical signs
Hassanien <i>et al.</i> (2020)	Cairo and Giza	20	4	Consensus nested PCR	EHV-1 (n=3) EHV-5 (n=1)	Abortion Abortion (n=14) Stillbirth (n=3) Early neonatal deaths (n=2) Respiratory affections (n=5) Myeloencephalopathy (n=1)
Ahdy <i>et al.</i> (2022)	Cairo and Giza	66	25	Real-time PCR	EHV-1 (gB, ORF33)	Abortion
Khattab <i>et al.</i> (2022)	Cairo	20	16	Real-time PCR	EHV-4	Abortion and respiratory affections
Emam <i>et al.</i> (2022b)	Giza	72	9	CPE (Cytopathic effect)	(EHV-1)	Acute early febrile phase of respiratory infection
Abdel-Rady <i>et al.</i> (2022)	El-Menia, Assiut, Sohage and Luxor	115	92	Multiplex PCR	EHV-2 (n=71) EHV-5 (n=50) EHV-1 (n=23) EHV-4 (n=15)	abortion, respiratory illness and neurological signs
Mohammed <i>et al.</i> (2022)	Cairo and Giza	80	6	Real-time PCR	EHV-1 (n=1) EHV-4 (n=5)	Abortion and respiratory manifestations
Emam <i>et al.</i> (2022a)	Giza	72	9	PCR	EHV-1	Respiratory manifestations and abortion
El-Zayat <i>et al.</i> (2023)	Cairo, Giza, Dakahlia, Sharkia, Gharbia	120	63	PCR	EHV-1 (n=48) EHV-4 (n=15) Mixed infection (5)	No
Ali <i>et al.</i> (2024b)	Cairo, Dakahlyia and Qalyubia	80	53	Consensus PCR	EHV-1 (n=29) EHV-4 (n=24) Mixed infection (5)	Abortion
Ali <i>et al.</i> (2024a)	Dakahlyia and Qalyubia	50	26	Consensus PCR	EHV-1	

Table 2. Final Meta-analysis results of the effect of size and test of null (2-tail) for 19 studies on the prevalence of Equine herpesvirus in Egypt.

Model	Effect size and 95% Confidence Interval				Test of null (2-Tail)	
	Number of studies	Point estimate	Lower limit	Upper limit	Z-value	P-value
Random	20	-0.445	-0.11	0.122	-1.539	0.124

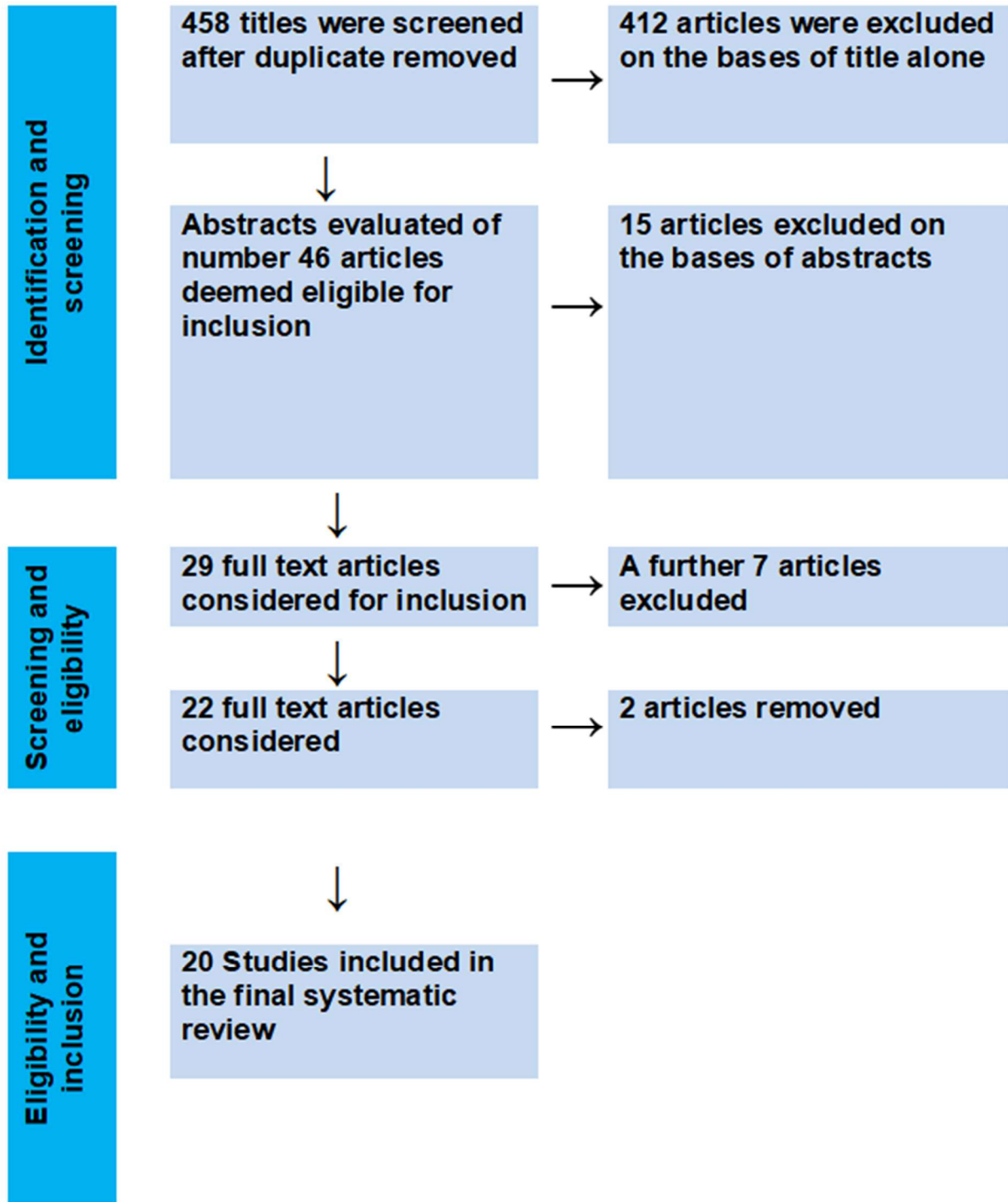


Figure 1. Results of the literature search and inclusion regarding prevalence of Equine herpesvirus (EHV) infection in Egypt.

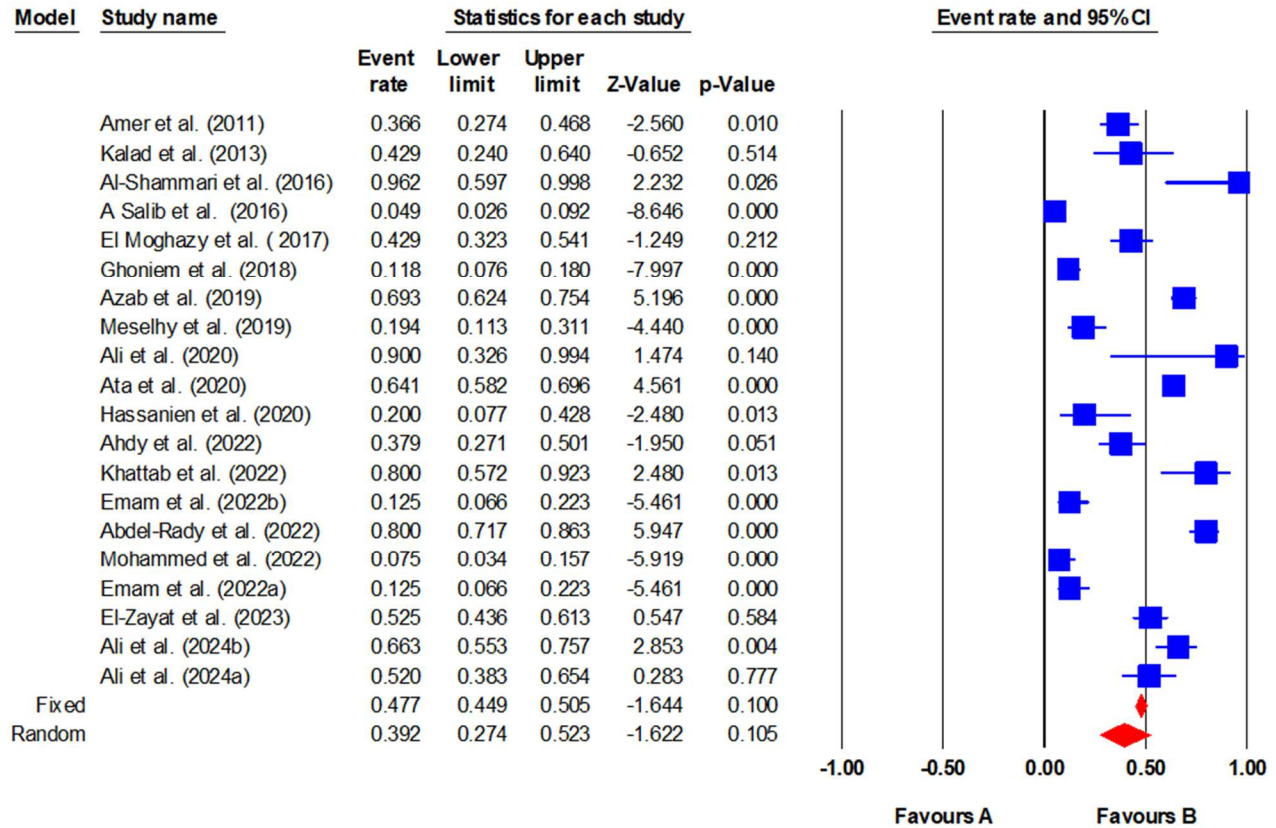


Figure 2. Forest Plot on the prevalence of EHV infection shows the event rate, 95% confidence interval, Z- value, P- value, and relative weight of 20 observed studies.

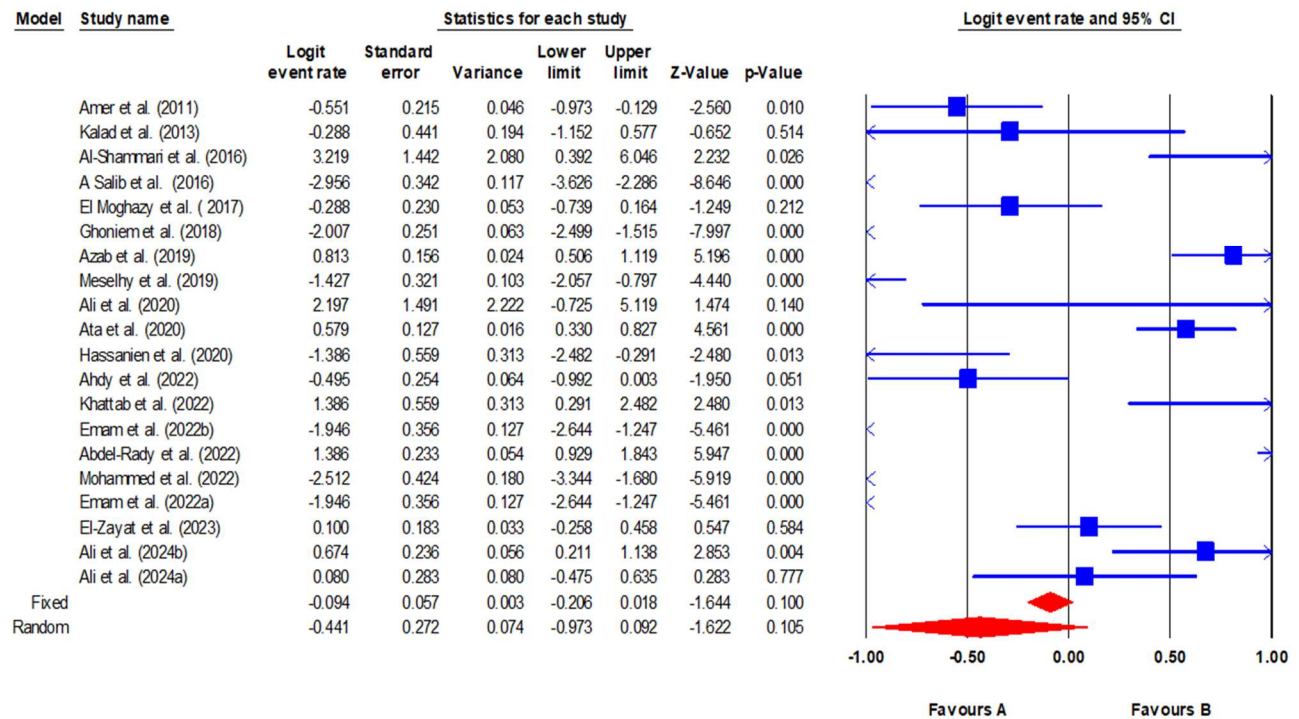


Figure 3. Forest Plot of the prevalence of EHV infection shows the logit event rate, 95% C, standard error, and variance on the random effect model of 20 observed studies.

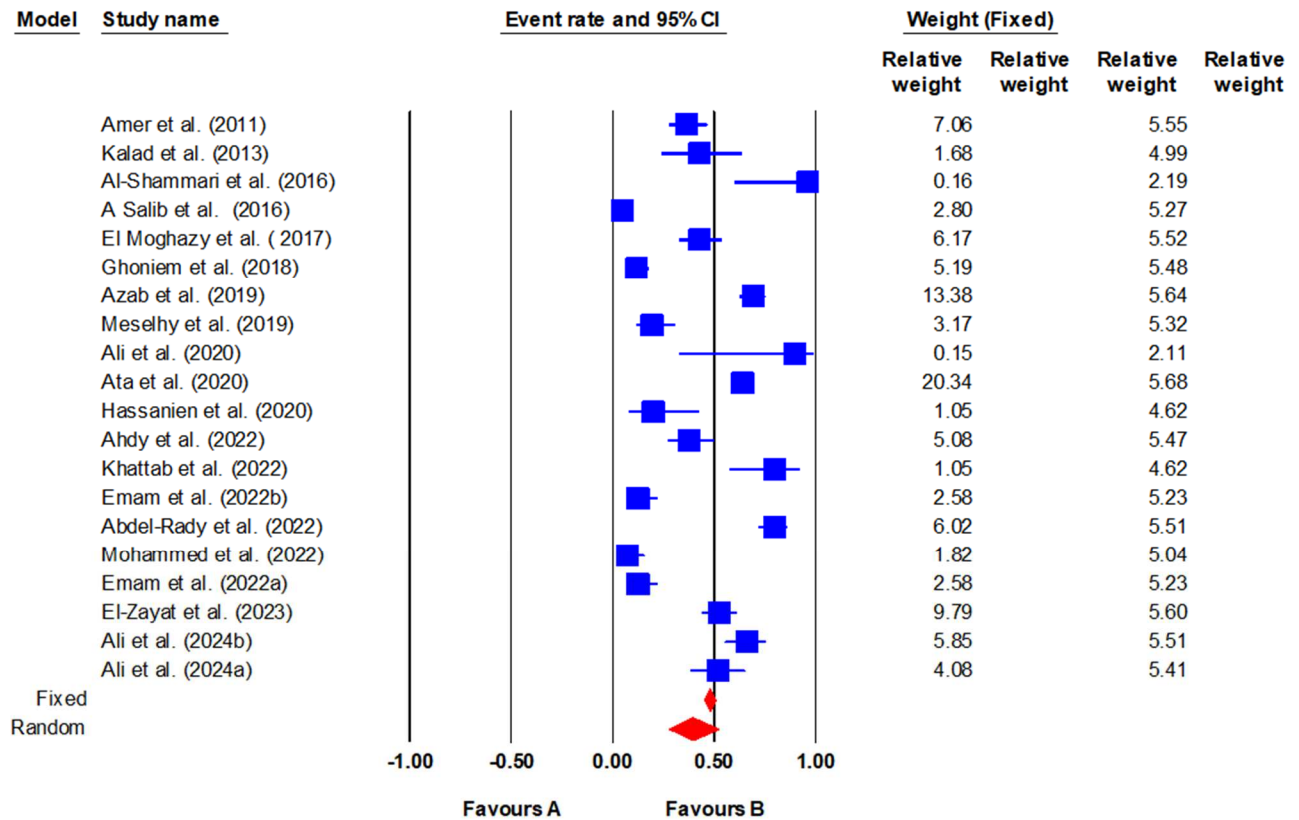


Figure 4. Forest Plot of the prevalence of EHV infection shows the weight on the random effect model of 20 observed studies.

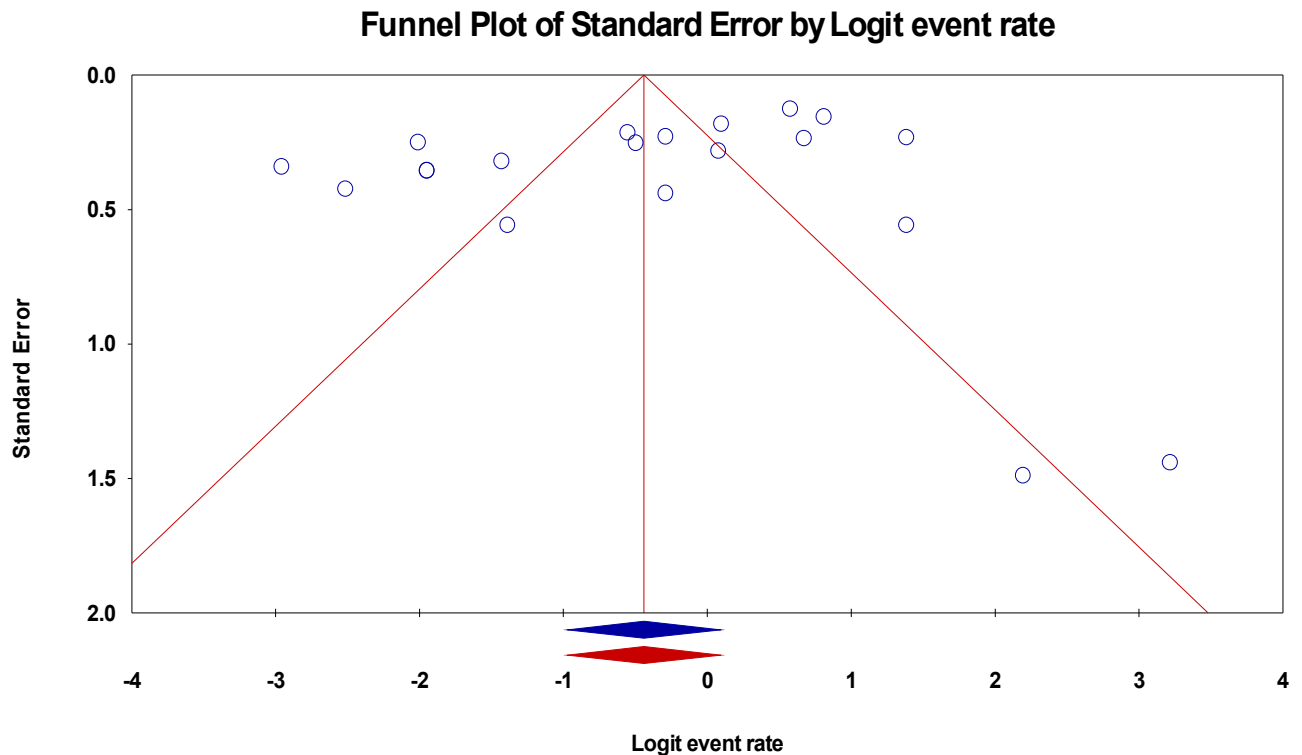


Figure 5. Funnel plot of the prevalence of EHV infection shows standard error by logit event rate on the random effect model of 20 observed and imputed studies.

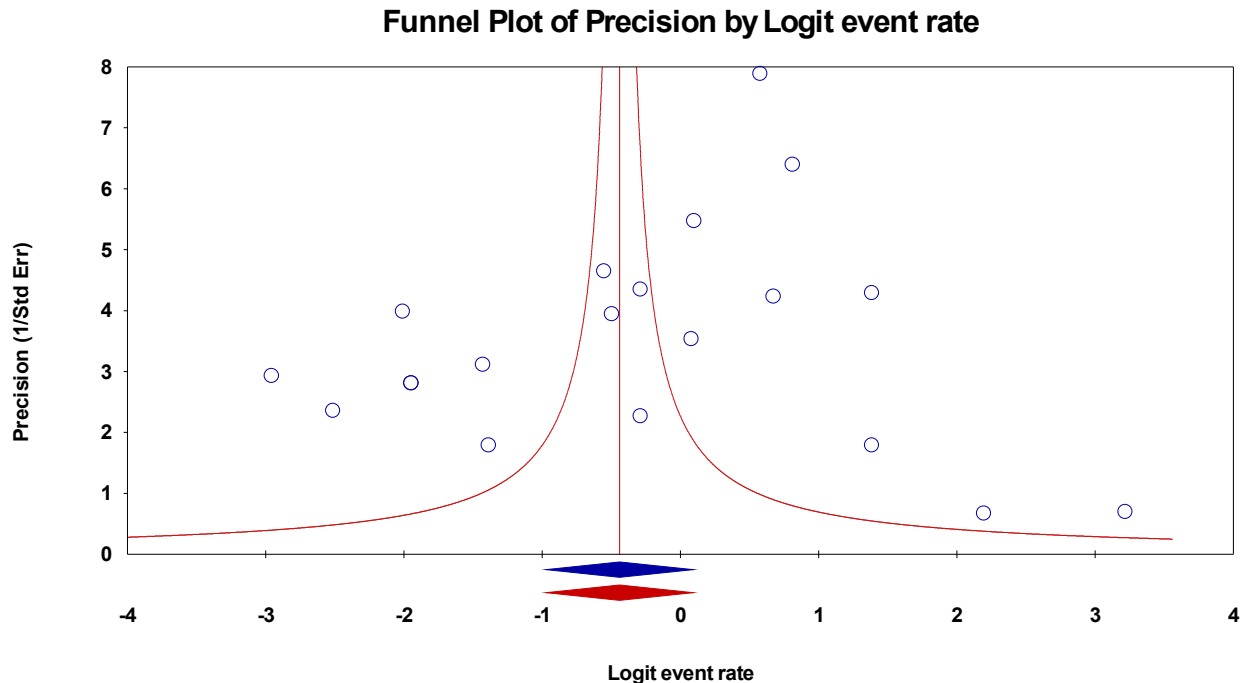


Figure 6. Funnel plot of the prevalence of EHV infection shows precision by logit event rate on the random effect model of 20 observed and imputed studies.

DISCUSSION

Egypt is one of the most famous countries and centers for breeding and exportation of pure Arabian horses (Kalad *et al.* 2013). In the absence of any previous systematic meta-analysis on the prevalence of EHV infection in Egypt, we conducted this study. This meta-analysis provides the results of electronic and print sources of peer-reviewed publications without restrictions on the publication date. Conference proceedings, technical reports, and other grey literature were not included in this meta-analysis.

Twenty studies fulfilled the selection criteria for the present study. In these studies, a total of 1683 horses were tested for EHV infection with a prevalence of 42%. The prevalence of EHV infection varied widely among selected studies. This finding may be attributed to the used diagnostic test, age and gender of tested horses. The high prevalence reflects the importance of the EHV infection to cause multiple clinical manifestations, especially respiratory signs and abortion. Similar prevalence has been recorded in Tunisia (Badr *et al.* 2022). In the present study, the prevalence varied among studies even with using the same diagnostic technique. The prevalence of EHV-1 was the highest among the Arabian horses (Abdel-Rady *et al.* 2022; Ahdy *et al.* 2022). This finding is supported by findings of outbreaks caused by EHV-1 (El-Hage *et al.* 2021).

The study population and presence of risk factors are determinants of the prevalence of infectious diseases

(Buitrago-Garcia *et al.* 2022). Variables such as age and sex of the horses studied have been linked to a higher risk of EHV infection. Young foals are more likely to be infected with EHV-2/-5 than adult horses (Hue *et al.* 2014), whereas equids aged 3–8 years have a higher prevalence of EHV-1/-4 than those younger than 3 years (Worku *et al.* 2024). Gender differences have also been observed, with males more likely to contract EHV-1-2 (Bolfá *et al.* 2017). On the other hand, many researchers have reported higher rates of EHV-1 and -4 among females than among males (de Souza *et al.* 2017; Negussie *et al.* 2016).

Clinically, 16 out of 20 eligible studies detected EHV in horses with various respiratory manifestations, abortion, and myeloencephalopathy. Similar findings have been reported globally (Kapoor *et al.* 2014; Sutton *et al.* 1998; Van Maanen 2002). The EHV-1 infection is a cause for upper respiratory tract signs in young horses and abortion in mares (Smith *et al.* 2004). However, myeloencephalopathy in adults and placental damage without abortion during early gestation have been also demonstrated (Allen *et al.* 1999). The EHV-4 also induces mainly respiratory infections in young and rarely causes abortion (Reed and Toribio 2004). The signs of respiratory disease associated with EHV infection are difficult to detect but may be observed as an outbreak (Reed and Toribio 2004). However, the genotype of EHV-1 did not affect clinical signs (Pusterla *et al.* 2020).

The present meta-analysis included 20 studies, of which 14 used molecular techniques for the diagnosis of EHV. However, the remaining studies used

only screening tests. This finding may reflect the potential of studies using this technique. The multiplex real-time PCR is a convenient and rapid tool for investigating the clinical relevance of EHV-2 and EHV-5 (Fürer *et al.* 2022). Meanwhile, Other techniques were found to be reliable for detecting different strains of EHV (Lang *et al.* 2013; Nordengrahn *et al.* 2001).

Based on the results of meta-analysis, the study of Ata *et al.* (2020) provided a relative weight of 20.34 %, whereas the small studies by Al-Shammari *et al.* (2016) and Ali *et al.* (2020) are given approximately 0.16 % and 0.15% of the relative weight, respectively. It is known that the common effect is well assessed by larger studies but not by small studies. Studies with small sample size had a negligible effect on the total value. Consequently, larger studies with smaller standard errors have greater weight than those of smaller studies with large standard errors. The present meta-analysis provided Z-values of -1.377 ($p = 0.168$) and -1.539 ($p = 0.124$). The Z-value here does not add to the results, as it is not the effect size, but only indicates the data distribution (Hak *et al.* 2016).

Regarding heterogeneity, the Q-statistic and I^2 for the prevalence of EHV were 373.103 and 95.17, respectively (p -value ≤ 0.000). The Q-statistic includes the observed dispersion, while the null hypothesis for heterogeneity proposes that the studies assign a common effect size. Consequently, it is assumed that the degrees of freedom are equal to the Q-statistic (Thompson 1994). However, if Q-statistic provides no effect size dispersion, I^2 and tau-squared can provide alternative good interpretation (Schulz *et al.* 1995).

The I^2 concludes that the definite differences in effect sizes for 99 % of the variance among the studies, and only 1% of the observed variation can be predicted by means of random error. The tau-squared value for EHV infection was 1.383, which is the variance among studies and is utilized to calculate the weights. It has been stated that the I^2 can be used to measure the level of heterogeneity in meta-analysis, but the eyeball test is a less formal substitute to measure the heterogeneity (Huedo-Medina *et al.* 2006).

Heterogeneity analysis usually proves how the effect width varies among studies. This statistical test explains the difference among studies due to differences between the studies or to sampling errors (Borenstein *et al.* 2021). Heterogeneity tests are performed to determine the conformity of the normal distribution of effect sizes. Considering heterogeneity, the null hypothesis is that the effect will be zero for both fixed and random effects. Hedges' g /standard error for the relevant model is usually used to determine the z-value, which is used to check the null hypothesis (Higgins JP 2019). It has also been stated that the p-value is not an effect size, and therefore, is not a measure of the magnitude of heterogeneity. In this case, a low p -value indicates that there is probably some

(unidentified) degree of heterogeneity (Duffield *et al.* 2008).

Regarding publication bias, publication bias is evident in reports with small sample size (Joober *et al.* 2012). Thus, detecting bias is an important issue as it has determinantal effect on the conclusion of systematic meta-analyses (Sutton *et al.* 2000). Funnel plot, an indicator usually used to decide the evidence of bias. In this plot, the effect size is usually shown against standard errors or precision (Light and B. Pillemer 1986). In the present study, the funnel plot was asymmetric, and there was no indication of publication bias. Moreover, the Egger's test confirmed absence of publication bias (intercept: -3.66, 95% confidence interval: - 8.23 to - 0.9; t-value: 1.96; df: 17.00). From the statistical meta-analysis, lack of bias is identified by zero level of regression slope (Rothstein HR 2005). If the Begg test has strong correlation, this indicates presence of publication bias (Begg and Mazumdar 1994).

Trim and fill test evaluates the total effect size and tests publication bias (Duval and Tweedie 2000). A repeated technique was used to exclude small studies at the extreme ends of the positive end of the funnel plot. The trimming and filling processes were repeated until the funnel plot was symmetric (Duval 2005). In the present result, the trim-and-fill finding appears as the closed dots indicating the missing studies (no studies trimmed), and the open dots indicate the observed studies (about 20 studies) imputed, which depend greatly on the selected estimator (R0, L0, or Q0) for imputing missing studies and its result in an adjusted correlation from -0.19 to 0.034 (95% CI).

The fail-safe test suggests that 980000 missing studies were required to conclude the result of study is significant ($p = 0.000$). Additionally, Orwin's fail-safe N suggests a 0.47 event rate in observed studies and a 0.50 mean event rate in missing studies. Even though they are usually used in meta-analysis, these publication bias assessments could have type I error rate and/or low power (Peters *et al.* 2006; Peters *et al.* 2007; Rücker *et al.* 2008; Sterne *et al.* 2000; Terrin *et al.* 2003).

Conclusion: The results of the present meta-analysis indicate a high prevalence of EHV infection in Egypt, particularly EHV-1. Therefore, more attention should be paid to prevention and control of this disease.

Conflict of interest: Authors declare that there is no conflict of interest.

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Authors' contribution: Authors contributed equally to this paper.

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