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[Review Article]

Prevalence of Streptococcus Species in Horses with Upper Respiratory Tract Infections. Systematic Meta-Analysis

Helmy K. Elnafarawy, Mohamed Youssef, Maged El-Ashker, Hussam Ibrahim, Sabry El-Khodery and Alshimaa M. M. Farag *
Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.

IN THIS study, according to the guidelines of PRISMA, the meta-analysis on prevalence of Streptococcus species in horses with respiratory tract infections was performed. After compete search, data extraction and selection of studies, data were analyzed using comprehensive meta-analysis. The results of meta-analysis of selected studies were 95% confidence intervals, effect size, heterogeneity, weight, and publication bias. A total of 1831 (32.25 %) out of 5678 diseased horses in 20 study with upper respiratory symptoms were found positive for Streptococcus species. The final meta-analysis model of the size effect and null test at fixed and random effect showed the effect has a Z-value of -3.371 (P-value = 0.001) as opposed to the fixed effect's Z-value of -26.024 (P-value = 0.000). The degree of heterogenicity revealed Q-value (926.104), I-squared (97.948), P-value (0.000), and the Tau-squared of 0.989 with a 0.519 Standard Error are the final heterogeneity variables. Egger's linear regression test for asymmetry did not indicate publication bias, intercept (-0.53), 95% confidence interval (-6.45-5.39), t-value (0.18), df = 18. The 1-tailed P-value is 0.426, and the 2-tailed P-value is 0.852. The outcome of Kendall's tau with continuity correction (-0.00526), with a 1-tailed P-value of 0.487 and 2 -tailed P-value of 0,974. Duval and Tweedie's trim-and-fill method (only one study trimmed at the left side) resulted in an adjusted correlation from − 0.857 to − 0.737 with 95% CI: − 1.345 to − 0.428. The results of the present study indicate the association of Streptococcus species with clinical upper respiratory signs in horses.

Keywords: Epidemiology, Equine, Respiratory diseases, Streptococcus, Bacteria.

Introduction

Respiratory issues are among the most serious health threats encountered in equines with an incidence rate of 30% among horses [1]. Infections of the upper respiratory tract are frequent in horses and may be brought on by a number of pathogens, such as bacteria, fungi, and viruses [2] Streptococci are significant class of Gram-positive cocci bacteria that are typically seen in long chains [3]. Streptococcus equi, (Streptococci of Lancefield Group C), is a prominent equine pathogen which has two subspecies particularly clinically important for horses [2], in addition to Streptococcus dysgalactiae subsp. equisimilis, they are three B-haemolytic Streptococci that are quite significant in horses. By impairing pulmonary functions and decreasing their performance [4-6]. In a study of British National hunt racehorses, Streptococcus species (non-haemolytic and Viridans group) was most commonly isolated from the respiratory tract [7, 8, 9]. Streptococcus equi subspecies equi is the notable pathogen to the horse husbandry industry [5]. It was the causative agent of strangles, which was the most often diagnosed infectious illness in horses globally, causing substantial

*Corresponding author: Alshimaa M.M. Farag, E-mail: dr_alshimaafarag@mans.edu.eg. Tel.: 01002299710
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health problems as well as financial losses to the equestrian sector [10, 11]. Infections of the upper respiratory tract are significant source of financial loss for the horse breeding populations. They primarily cause mortality in young age of foals and difficult-to-treat illnesses in horses of all ages [12]. Infections, whether acute or chronic, can affect the pulmonary function and can lower performance and exercise tolerance. Worldwide, respiratory infections in horses are a common occurrence, causing poor respiratory health and exercise intolerance, including Inflammatory airway diseases (IAD) which is similar to asthma in humans [13, 14]. Meta-analysis is a statistical method that enables the researcher to synthesize and assess the findings of individual studies that have been conducted, published, and then critically retrieved, reviewed, and assessed after publication. All of the meta-analysis's findings are relevant to the set of results that were produced and retrieved. Because populations (or "samples") are arbitrarily chosen for the various studies, these results are inherently prone to selection bias to an unknown extent. [15]. In fact, extensive meta-analysis studies on upper respiratory tract infections in humans have been carried out [16, 17], whereas no available studies in the literature on the systematic review meta-analysis on the prevalence of Streptococcus species in equines with upper and lower respiratory tract infections based on the author's 'knowledge'. Consequently, the present systematic review with meta-analysis of the prevalence of Streptococcus species in horses aimed to review the most recent scientific evidence on the association with upper respiratory manifestations.

Material and Methods

Ethical approval

The authors conducted analyses of all scientific articles with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) standards. Consequently, this research did not require ethical approval for using animals in scientific experiments. Prior to starting the trial, the protocol of study was developed.

Selected studies

The present review adopted all published studies describing the prevalence of Streptococcus species based on number of isolates from horses with upper respiratory symptoms.

Types of reference individuals

All examined animals displayed respiratory symptoms such as pyrexia, nasal discharge, coughing, sneezing, aberrant respiratory sounds, and swollen lymph nodes. The study's sample sizes were not restricted.

Inclusion and exclusion criteria

Inclusion criteria

- The publication's English version is accessible when articles are written in another language.
- Publication of studies between 1991 and 2023.
- Studies on the isolation of Streptococcus species from horses with respiratory conditions have been conducted.
- Studies indicating the incidence of respiratory bacterial infections regardless of the laboratory diagnostic method utilized are examples of outcomes.
- The number of animals with respiratory tract infections among which specific bacteria were screened will be used to determine prevalence.

Exclusion criteria

- Streptococcus species isolation from healthy equine either horses or donkeys.
- Techniques for identifying Streptococcus species other than prevalence.
- Bacterial species other than Streptococcus species were isolated.
- Foreign-language publications.
- Data from articles that have been published are incomplete.

Search strategy and selection of studies

We searched through all publications written about prevalence of Streptococcus species from horses with upper respiratory tract infections at the PubMed, Ovid, Web of Science, Sage, BESCO, CABI, Scopus, Mendeley, and ISI web of knowledge database with a combination of the following search terms ("RESPIRATORY" "DISEASES") (title/ abstract) OR ("RESPIRATORY" "TRACT" "SYMPTOMS") (title / abstract) AND ("HORSES") (title / abstract) AND ("PREVALANCE") (title/ abstract) OR ("ISOLATION OF STREPTOCOCCUS SPP.") (title / abstract) from the earliest data available until March, 2023. This procedure was supplemented by hand-searching, Google Scholar searching, expert recommendations, and citation reviews. We integrated the database outputs using a referencing program, EndNote (version X9; Thomson Reuters). The studies' articles were shown in Figure 1.

Data extraction and analysis

The following data were extracted: The study's year, sample size, and positive cases with reported summary statistics (such as prevalence, standard error, variance, and confidence interval at 95%), country, and various streptococcus species subspecies types (Table 1).

Quality assurance

According to (PRISMA) [18], the current systematic review and meta-analysis was carried out. All the available published publications on the assessment of the prevalence of Streptococcus species based on number of isolates from horses with upper respiratory tract infections were incorporated, to diminish the publication bias.

Statistical analysis

Firstly, the overall pooled prevalence, the number of positive cases divided by the number of all animals tested. A Meta-Analysis software was used for all data analysis. (Comprehensive Meta-Analysis software version 2, Biostat, Englewood, NJ, USA). In the current study, the fixed and random effect model, 95% confidence intervals, effect size, heterogeneity between-study variance were assessed using the tau-square (t2), and thus the relative weight and publication bias were the main outputs of the analysis. These variables were used in our study to assess the prevalence of streptococcus species based on the number of isolates from horses with upper respiratory tract infections. Effect size, heterogeneity, and the percentage of true heterogeneity were assessed [19, 20]. Low, moderate, and high degrees of heterogeneity were identified with values of 25%, 50%, and 75%, respectively. [21]. The relative weight of study was calculated. With the use of meta-regression, the forest plot was used to examine the degree of heterogeneity. [22]. To determine the degree of publication bias, the funnel plot was investigated [23]. Egger's linear regression intercept [24], Begg–Mazumdar rank correlation test (Kendall's statistic (P-Q) with continuity correction were used to assess the significance of funnel plot asymmetry [25]. Duval and Tweedie's trim and fill method was used to estimate potentially missing studies to modify the overall effect estimate for both fixed and random effects [26]. The fail-safe N was used to calculate the number of studies with a zero effect size that are necessary to eliminate the funnel plot's overall effect size [27]. Chi square and fisher's exact tests were conducted to determine the prevalence of streptococcus subspecies; a result was judged significant at a P-value of ($P < 0.05$) [28].

Results

Search results and eligible studies

From databases and published articles, a total of 534 items were discovered. After exclusion criteria, 20 acceptable studies were selected (Table 1, Figure 1).

Results of meta-analysis

The overall prevalence of Streptococcus spp. was 32.25 % in all 20 acceptable studies in the present meta-analysis (Table 1). The examination of 5678 diseased horses with upper respiratory symptoms revealed 1831 to be positive for Streptococcus spp. (Table 1). The lowest prevalence (2.37%) was recorded in the study of Libardoni et al. (2016b). However, the highest prevalence (100%) was reported by Båverud et al. (2007a) (Table 1). The final meta-analysis model of the size effect and null test for the prevalence of Streptococcus spp. at fixed and random effect is shown in Table 2. At random, the effect has a Z-value of -3.371 (P-value = 0.001) as opposed to the fixed effect's Z- value of -26.024 (P-value = 0.000).

Degree of Heterogeneity

A forest plot was used to evaluate and illustrate the degree of heterogeneity in the selected research on both fixed and random effects. Additionally, the outcome of relative weight for both fixed and random effects is shown in figure 2. The Q-value (926.104), I-squared (97.948), and P- value (0.000) are the final heterogeneity variables. Additionally, the Tau-squared is 0.989 with a 0.519 Standard Error (Table 3).

Publication bias

Effect sizes (x axis) are represented against their standard errors and precisions (y axis) (the inverse of standard errors) in the funnel plot by logit event rate for the prevalence of Streptococcus spp. in horses with upper respiratory symptoms (Figure 4,5).The Egger's regression intercept, Begg's rank test, Duval and Tweedie's trim-and-fill method and conducted a classic fail-safe N analysis are designed to adjust estimates for the potential impact of publication bias under some explicit model of publication selection. Egger's linear regression test for asymmetry did not indicate publication bias, Intercept (-0.53), 95% confidence interval (-6.45- 5.39), t-value (0.18), df = 18. The 1-tailed P-value (recommended) is 0.426, and the 2-tailed P-value is 0.852. The outcome of Kendall's tau with continuity correction (-0.00526), with a 1-tailed P-value (recommended) of 0.487 and 2 -tailed P-value of 0,974. Duval and Tweedie's trim-and-fill method (only one study trimmed at the left side) resulted in an adjusted correlation from − 0.857 to − 0.737 (95% CI) [from − 1.345 to − 0.428] (Figure 4, 5).

The distribution of Streptococcus species in horse with upper respiratory tract varies varied significantly (p<0.001). Where Streptococcus equi subspecies equi was more prevalent than Streptococcus equi subspecies zooepidemicus (P-value 0.005, RR: 0.87, 95% CI 0.79 to 0.96), Streptococcus equisimilis, (P-value <0.0001, RR: 8.24, 95% CI 6.55 to 10.35), and other Streptococcus spp. (P-value <0.0001, RR: 2.11, 95% CI 1.85 to 2.40). Moreover, the prevalence varied between Streptococcus equi subspecies zooepidemicus and Streptococcus equisimilis (P-value <0.0001, RR: 9.47, 95% CI 7.55 to 11.88), and between Streptococcus equi subspecies zooepidemicus and other Streptococcus spp. (P-value <0.0001, RR: 2.43, 95% CI 2.14 to 2.76).

Discussion

The prevalence of inflammatory airway disorders in horses is high and can affect horses at different ages and discipline [29, 31, 32]. It is the cause of cute infectious disease named Strangles, [33, 34], with characteristic clinical findings [34, 35]. The current study's objective was to use meta-analyses to determine the pooled prevalence of Streptococcus spp. in horses with upper respiratory manifestations. To reduce the publication bias, the current systematic meta-analysis was carried out in accordance with the PRISMA guidelines.

In the present study, twenty studies met the criteria of selection. Out of 5678 diseased horses with upper respiratory symptoms examined, the pooled prevalence of Streptococcus spp. was determined to be 32.42%. According to the analysis's findings, Båverud et al., 2007a in Sweden had the greatest prevalence (100%, and 95% CI: 0.85-0.99). But Libardoni et al.'s 2016b study recorded the lowest prevalence (2.37%, 95% CI: 0.01-0.03). Studies on the prevalence of Streptococcus spp with respiratory manifestation in horses have been conducted with varying degrees of results [31, 36-54]. The high prevalence of Streptococcus spp. with respiratory symptoms in horses may be due to the endemicity of these bacteria in the horse population [55]. Due to the fact that *S. equi* can be found in the respiratory tracts of both healthy and horses exhibiting symptoms of respiratory disease [56]. Factors favor increase or restrict the prevalence of Streptococcus in equine were previously studied and stated [57]. In the selected studies, the prevalence of Streptococcus spp. in horses with respiratory manifestation was examined using conventional and molecular methods (nasopharyngeal swab, bacterial culture and identification, and PCR) both fixed and random effects were assessed [46]. This research included 20 studies; 8 out of 20 depended on traditional techniques for Streptococcus identification and 12 out of 20 depended on molecular ones. Molecular diagnostics, which are known to have a high sensitivity [58, 59]. According to the meta-analysis's findings, from the logit of the fixed effect model in the present study, large studies by some studies[44, 45, 54] have a relative weight of 55.23 %, while the small studies by several [36, 37, 39, 48, 52] are given only about 1.99 % of the relative weight. The common effect is well estimated by the larger studies, while the common effect is poorly estimated by the small study, hence the small studied are given a low weight. Small studies have a negligible effect on the total value, which is calculated in the range of 0.00 to 0.50, with only 1.99% of the relative weight. In contrast, each study's effect size under the random effect model is estimated for a specific population, hence each study's estimate must be assigned the proper weight in the analysis. In the current analysis, as shown in the column of the "Relative weights" under random effect each of the large studies carried out by some investigators [44, 45, 54] are given about 16.58 % of the relative weight (rather than 55.23 %), even though the small studies that carried out by some studies [36, 37, 39, 48, 52] are given about 20.27 % of the relative weight (rather than 1.99 %). In contrast to the fixed effect model, when the small study had essentially no influence, it has a much greater impact effect. Concretely, it receives a relative weight of 20.27%, which is virtually the same as the weight given to any of the larger studies (16.58%). As a result, studies that are larger and have smaller standard errors are given greater weight than those that are smaller and have larger standard errors. The pooled effect estimate's uncertainty is reduced by this selection of weights. For heterogenicity in our study, the Q-value (926.104), I-squared (97.948), and P- value (0.000) are the final heterogeneity variables. Additionally, the Tau-squared is 0.989 with a 0.519 Standard Error. It is crucial to determine the magnitude of the variance between the distributions since the studies included in the meta-analyses have varying effect sizes [60, 61, 62]. Statistical methods, such as the Cochran's Q test or the index of heterogeneity I2 (I-squared), can be used to quantify the level of heterogeneity in meta-analysis research. The eyeball test (graphical method at forest plot) is a less formal alternative to assess the heterogeneity [63]. In the present study, the z-values of the prevalence of Streptococcus spp. in horses with upper respiratory symptoms were -26.024 (P -value < 0.000) and -3.371 (P-value < 0.001) for both the fixed and random effects, respectively. In the current study, the Q-statistic for the prevalence of Streptococcus spp. with upper respiratory manifestations in horses was 926.104, compared with the expected value of 19 (P-value < 0.000) [64, 65]. The tau-squared of the prevalence of Streptococcus spp. with upper respiratory manifestations in horses was 0.989. This is the variance "between studies" that was utilized to calculate the weights. Fixed-effect model often includes Q- statistic and tau-squared, whereas random-effect model does not typically provide the Q-statistic or tau-squared. According to the current statistical analysis, there is >75% great heterogeneity in the prevalence of Streptococcus spp. with upper respiratory symptoms in horses, and only three individual studies have an overall effect that indicates there is data heterogeneity. The overall effect estimated by the random effects model corresponds to the mean of the distribution of the true effect. The sample size, sampling technique, age, breed, and gender of the animals in our study may be responsible for the statistical discrepancies between the studies. Publication bias is based on the assumption that not all studies on a study's results are published depending on the direction or significance of the study's findings. Studies with small sample sizes, insufficient power, no discernible difference between groups, and a higher incidence of complications or adverse events in the research study are thought to have a detrimental impact on the overall effect or to introduce bias by increasing the average effect size [66]. Therefore, detecting of the publication bias is a crucial issue because such bias may lead to incorrect conclusions of systematic reviews [67]. A funnel plot is frequently used in systematic reviews and meta-analyses to examine the existence of publication bias or systematic heterogeneity in individual studies. Effect size is typically displayed against standard errors or precisions in this type of funnel plot [68]. Because the funnel plot in the current study is asymmetric, there is a systematic distinction between studies with higher and lower precision. Several statistical tests, including the Egger's regression test [24] and Begg's rank test [25], have been proposed to examine publication bias in the funnel plot. Egger's regression test is a statistical tool for measuring funnel plot asymmetry through the standardized effect sizes on their precisions; in the absence of publication bias, the regression intercept is expected to be zero [24, 69]. The Begg and Mazumdar rank correlation test examines the correlation between the effect sizes and their corresponding sampling variances; a strong correlation implies publication bias [25]. The outcome of rank test in the present study is Kendall's Tau (-0.0052), z-value for Tau (0.0324) and P-value (0.487). This outcome provides the Egger regression test results with no indication of publication bias. In addition, the trim and fill method are another attractive tool that modifies the predicted total effect size in addition to testing for publication bias [70]. It uses a repeated method to remove small studies at the extreme ends of the positive end of the funnel plot. The trimming and filling process is repeated until the funnel plot is symmetric in regard to the effect size [71]. In the present study, the results of the trim-and-fill method appear as the closed dots indicate the missing studies (only one study), and the open dots indicate the observed studies (about 19 studies) imputed which depend greatly on the selected estimator (R0, L0, or Q0) for imputing missing studies. The fail-safe N or file drawer number strategy is another tool created by Rosenthal in 1979 [72] to counteract publication bias. It assumes that it is possible to calculate the actual number of missing studies and argues that finding studies to include in a meta-analysis is necessary before determining whether the p value is significant. The use of fail-safe N assumes that the main effect of missing studies has no effect. In the present study of that meta- analysis incorporates data from 20 studies, which yield a z-value of -22.18542 and corresponding 2-tailed p-value of 0.00000. The fail-safe N is 2543. This means that we would need to locate and include 2543 'null' studies for the combined 2-tailed P-value to exceed 0.050. From another way, there would need to be 127.2 missing studies for every observed study for the effect to be nullified. Despite being frequently used in meta-analysis applications, these publication bias tests may have high type I error rate or low power in certain simulation settings [73-77]. The limitations of the current study should be identified. Generally, the protocol was carried out in accordance with PRISMA guidelines to minimize the limitations. Unfortunately, there is no available publication of prevalence of the streptococcus spp in horses with upper respiratory manifestation all over the world wid. Therefore, we included published peer-reviewed publications, which could have limited the number of studies included in the meta-analysis (20 individual studies).

Conclusion

The results of the present meta-analysis provide an idea about the prevalence and distribution of streptococcus spp in horses with upper respiratory tract diseases.

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

Acknowledgment: NA

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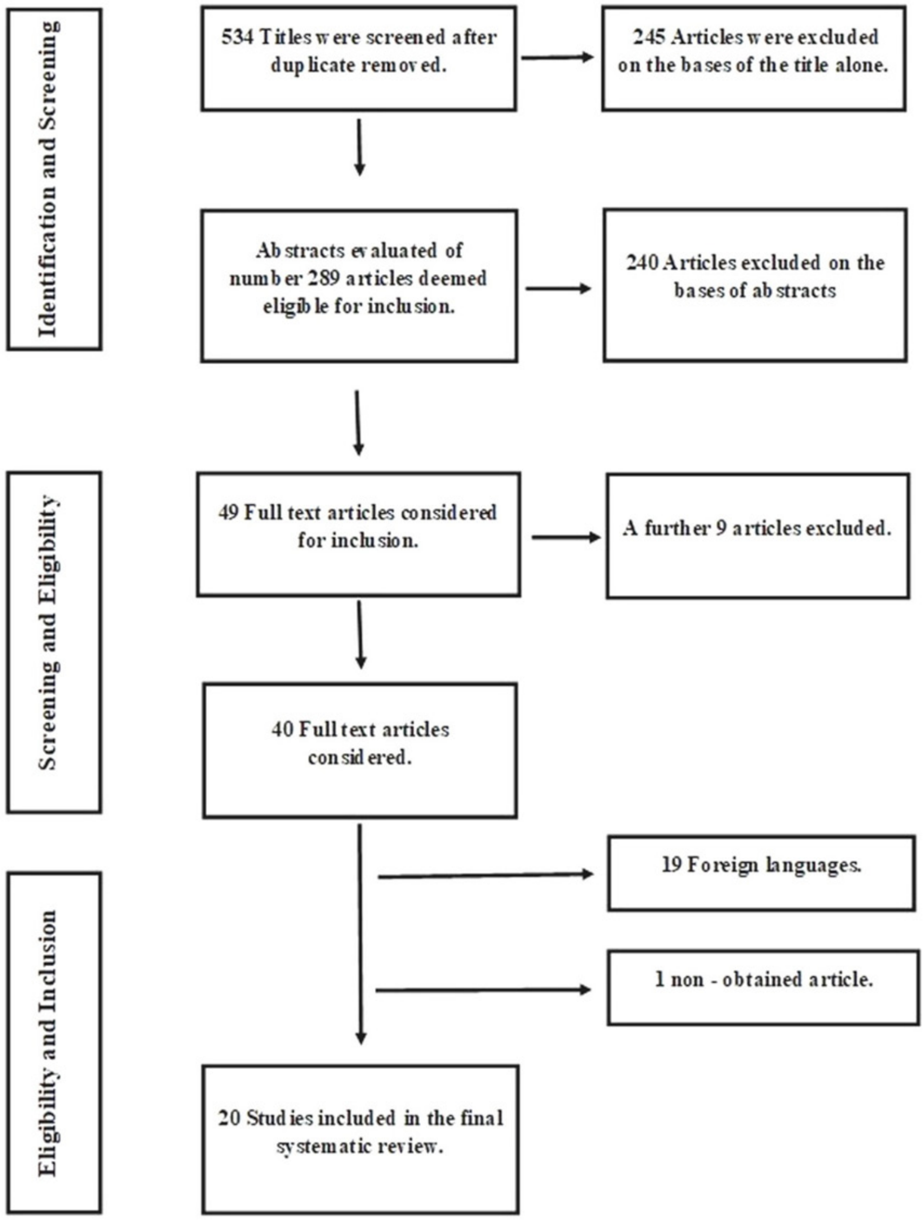

Fig.1. Identification, screening, and eligibility of literature on the prevalence of streptococcus species in horses with upper respiratory tract infection.

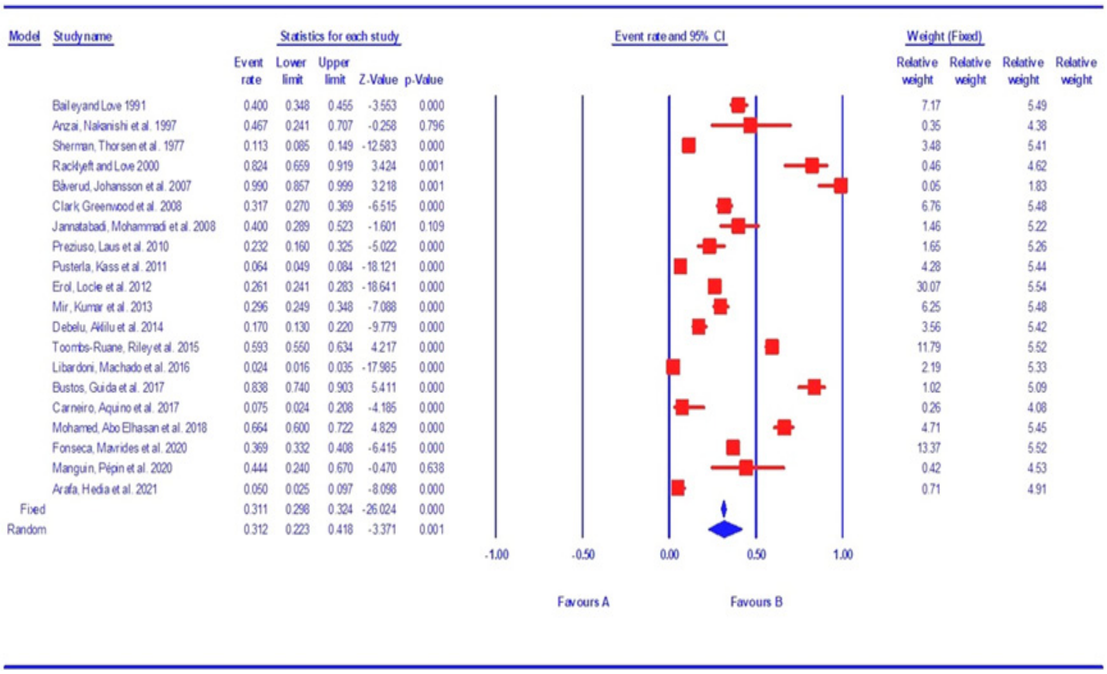

Fig. 2. Forest Plot of the prevalence of *Streptococcus spp*. in horses with upper respiratory manifestations shows the event rate, 95% confidence interval, Z- value, P- value, and relative weight on both Fixed and Random models of 20 observed studies.

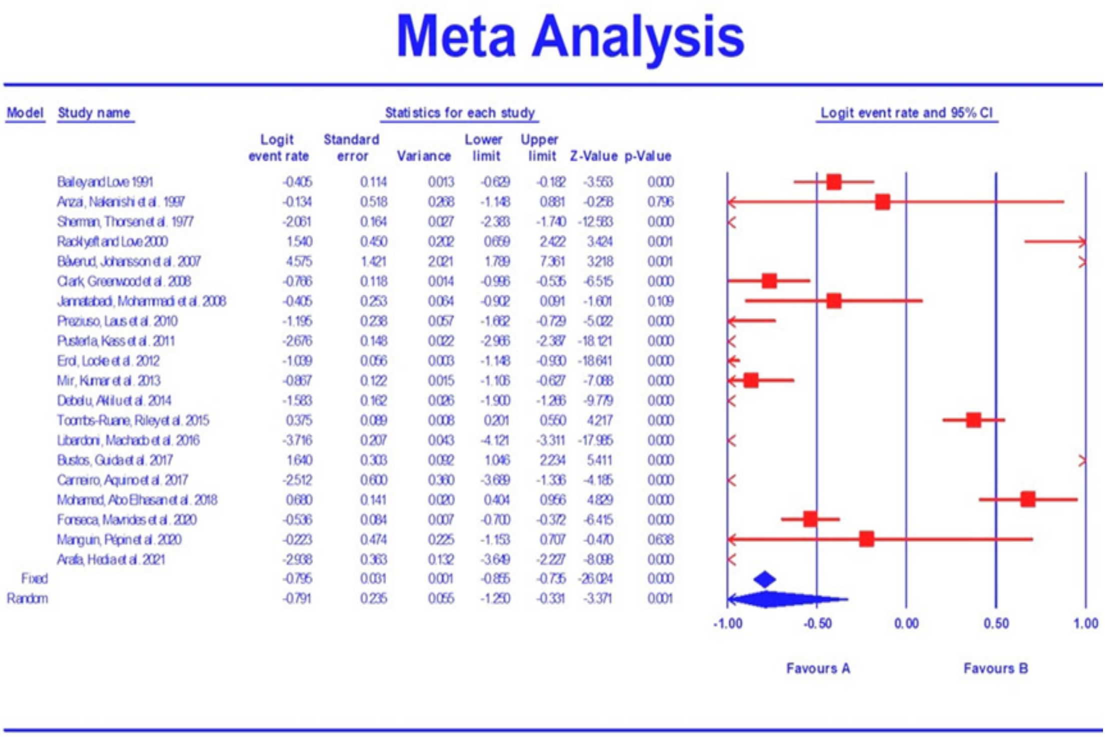

Fig. 3. Forest Plot of the prevalence of *Streptococcus spp*. in horses with upper respiratory manifestations shows the logit event rate, 95% C, standard error, and variance on both Fixed and Random models of 20 observed studies.

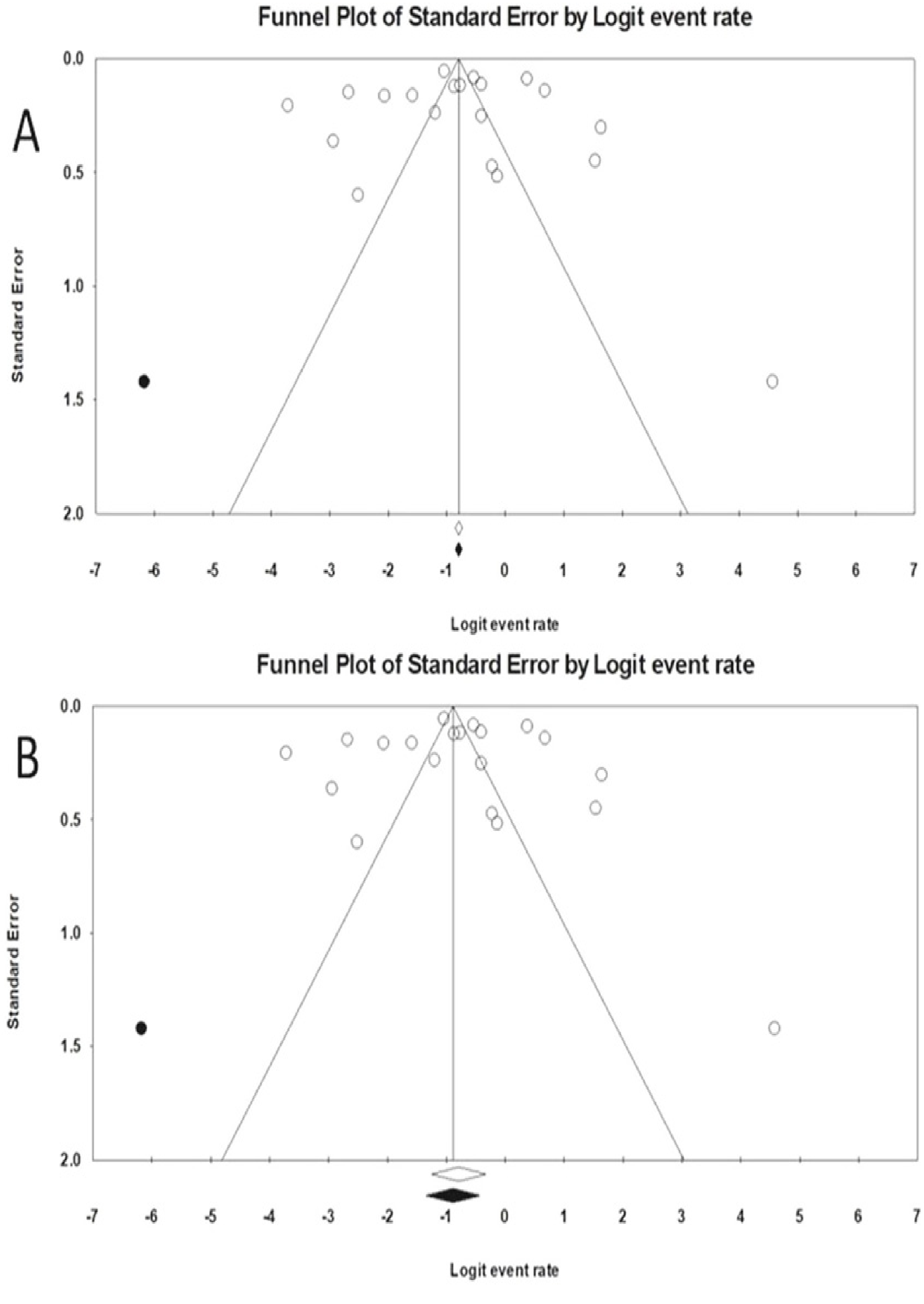

Fig.4. Funnel plot of the prevalence of *Streptococcus spp*. in horses with upper respiratory manifestations shows standard error by logit event rate on fixed (A) and random (B) models of 20 observed and imputed studies.

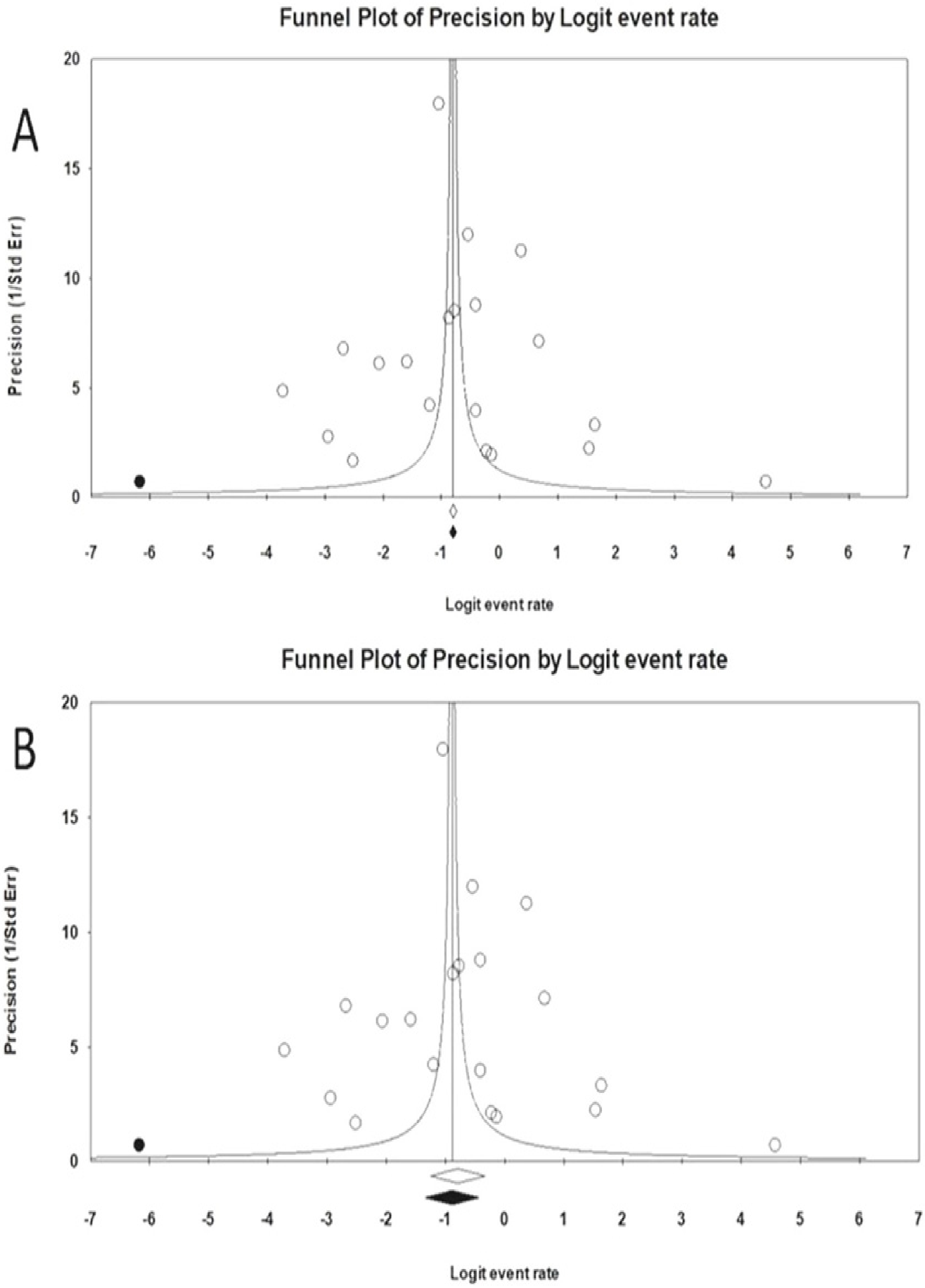

Fig.5. Funnel plot of the prevalence of Streptococcus spp. in horses with upper respiratory manifestations shows precision by logit event rate on fixed (A) and random (B) model of 20 observed and imputed studies.

TABLE 1. Descriptive statistics for the prevalence of *Streptococcus spp* in diseased horses with upper respiratory manifestations.

Study Name	Positive	Sample size	Prevalence (%)	Country	Streptococcus Species			
					Streptococcus equi subspecies equi	Streptococcus equi subspecies zooepidemicus	Streptococcus equisimilis	Other streptococcus
Bailey and Love, 1991b [38]	128	320	40	Australia	99	-	-	29
Anzai et al., 1997a [36]	7	15	46.66	Japan	2	5	-	-
Sherman et al., 1977b [53]	42	372	11.29	Ontario, Canada	-	42	-	-
Racklyeft and Love, 2000b [52]	28	34	82.35	Sydney	-	28	-	-
Båverud et al., 2007b [39]	48	48	100	Sweden	24	24	-	-
Clark et al., 2008a [42]	106	334	31.73	Western Canada	-	79	-	27
Jannatabadi et al., 2008b [46]	26	65	40	Malaysia	1	25	-	-
Preziuso et al., 2010b [51]	23	99	23.23	Central Italy	-	-	23	-
Pusterla et al., 2011b [31]	49	761	6.43	USA	49	-	-	-
Erol et al., 2012a [44]	436	1668	26.13	Kentucky	179	216	41	-
Mir et al., 2013b [49]	95	321	29.59	India	4	56	17	18
Debelu et al., 2014b [43]	46	270	17.03	Central Ethiopia	-	-	-	46
Toombs-Ruane et al., 2015 [54]	310	523	59.27	New Zealand	-	125	-	185
Libardoni et al., 2016b [47]	24	1010	2.37	State of Rio Grande do Sul, Brazil	24	-	-	-
Bustos et al., 2017b [40]	67	80	83.75	Buenos Aires Province	67	-	-	-
Carneiro et al., 2017b [41]	3	40	7.5	Brazil	-	-	-	3
Mohamed et al., 2018 [50]	150	226	66.37	Egypt	124	26	-	-
Fonseca et al., 2020a [45]	227	615	36.91	United Kingdom	86	141	-	-
Manguin et al., 2020b [48]	8	18	44.44	Montreal, Canada	-	-	-	8
Arafa et al., 2021a [37]	8	159	5.03	Giza, Egypt	8	-	-	-

TABLE 2. Final Meta-analysis model of the effect of size and test of null (2-tail) for 20 observed studies on the prevalence of *Streptococcus spp* in diseased horses with upper respiratory manifestations.

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
Fixed	20	0.311	0.298	0.324	-26.024	0.000
Random	20	0.312	0.223	0.418	-3.371	0.001

TABLE 3. Heterogeneity and Tau-squared for 20 observed studies on the prevalence of *Streptococcus spp* in diseased horses with upper respiratory manifestations.

Model	Heterogeneity					Tau-squared	
	Number of studies	Q-value	df (Q)	P-value	I-squared	Tau-squared	Standard Error
Fixed	20	926.104	19	0.000	97.948	0.989	0.519
Random	20	-	-	-	-	-	-

References

1. Newton, J., Verheyen, K., Talbot, N., Timoney, J., Wood, J., Lakhani, K. and Chanter, N. Control of strangles outbreaks by isolation of guttural pouch carriers identified using PCR and culture of Streptococcus equi. *Equine Veterinary Journal*, **32**, 515-526 (2000).

2. Paillot, R., Darby, A.C., Robinson, C., Wright, N.L., Steward, K.F., Anderson, E., Webb, K., Holden, M.T., Efstratiou, A., Broughton, K., Jolley, K.A., Priestnall, S.L., Marotti Campi, M.C., Hughes, M.A., Radford, A., Erles, K. and Waller, A.S. Identification of three novel superantigen-encoding genes in Streptococcus equi subsp. zooepidemicus, szeF, szeN, and szeP. *Infection and Immunity*, **78**, 4817-4827 (2010).

3. Stewart, G.C. Streptococcus and Enterococcus. *Veterinary Microbiology*, 240-251 (2022).

4. Halliwell, R., McGorum, B., Irving, P., Dixon, P. Local and systemic antibody production in horses affected with chronic obstructive pulmonary disease. *Veterinary Immunology and Immunopathology*, **38**, 201-215 (1993).

5. Baracco, G.J. infections caused by group C and G streptococcus (streptococcus dysgalactiae subsp. equisimilis and others): epidemiological and clinical aspects. *Microbiology Spectrum*, **7**, 32 (2019).

6. Timoney, J. The pathogenic equine streptococci. *Veterinary Research*, **35**, 397-409 (2004).

7. Cardwell, J., Smith, K., Wood, J. and Newton, J. A longitudinal study of respiratory infections in British National Hunt racehorses. *The Veterinary Record*, **172**,637 (2013).

8. Yoshii, Y., Shimizu, K., Morozumi, M., Chiba, N., Ubukata, K., Uruga, H., Hanada, S., Wakui, H., Minagawa, S., Hara, H., Numata, T., Saito, K., Araya, J., Nakayama, K., Kishi, K. and Kuwano, K. Detection of pathogens by real-time PCR in adult patients with acute exacerbation of bronchial asthma. *BMC Pulmonary Medicine*, **17**,1-8 (2017).

9. Hilty, M., Burke, C., Pedro, H., Cardenas, P., Bush, A., Bossley, C., Davies, J., Ervine, A., Poulter, L., Pachter, L., Moffatt, M.F. and Cookson, W.O. Disordered microbial communities in asthmatic airways. *PloS one*, **5**, 8578 (2010).

10. Mallicote, M. Update on Streptococcus equi subsp equi infections. *The Veterinary Clinics of North America Equine Practice*, **31**, 27-41 (2015).

11. Mani, R.J., Thachil, A.J. and Ramachandran, A. Discrimination of Streptococcus equi subsp. equi and Streptococcus equi subsp. zooepidemicus using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Journal Veterinary Diagnostic Investigation*, **29**, 622-627 (2017).

12. Boguta, L., Gradzki, Z., Borges, E., Maurin, F., Kodjo, A. and Winiarczyk, S. Bacterial flora in foals with upper respiratory tract infections in Poland. *Journal of Veterinary Medicine, Series B*, **49**, 294-297 (2002).

13. Bernard, B., Dugan, J., Pierce, S. and Gardiner, I. The influence of foal pneumonia on future racing performance. *American Association of Equine Practitioners*, 17-18 (1991).

14. Houtsma, A., Bedenice, D., Pusterla, N., Pugliese, B., Mapes, S., Hoffman, A.M., Paxson, J., Rozanski, E., Mukherjee, J., Wigley, M. and Mazan, M.R. Association between inflammatory airway disease of horses and exposure to respiratory viruses: a case control study. *Multidisciplinary Respiratory Medicine*, **10**, 1-11 (2015).

15. Borenstein, M., Hedges, L.V., Higgins, J.P. and Rothstein, H.R. Computing Effect Sizes for Meta-analysis. Introduction to meta-analysis: Chichester: John Wiley & Sons, Ltd, (2021).

16. Claassen-Weitz, S., Lim, K.Y.L., Mullally, C., Zar, H.J. and Nicol, M.P. The association between bacteria colonizing the upper respiratory tract and lower respiratory tract infection in young children: a systematic review and meta-analysis. *Clinical Microbiology and Infection*, **27**, 1262-1270 (2021).

17. Jones, E.A.K., Mitra, A.K. and Malone, S. Racial Disparities and Common Respiratory Infectious Diseases in Children of the United States: A Systematic Review and Meta-Analysis. *Diseases*, **1**, 11-23 (2023).

18. Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G. and PRISMA Group, T. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of Internal Medicine*. **151**, 264-269 (2009).

19. Duffield, T.F., Rabiee, A.R. and Lean, I.J. A Meta-Analysis of the Impact of Monensin in Lactating Dairy Cattle. Part 1. Metabolic Effects. *Journal of Dairy Science*, **91**, 1334-1346 (2008).

20. Chen, I.C., Hill, J.K., Ohlemüller, R., Roy, D.B. and Thomas, C.D. Rapid range shifts of species associated with high levels of climate warming. *Science*, **333**, 1024-1026 (2011).

21. Higgins, J.P., Thompson, S.G., Deeks, J.J., Altman, D.G. Measuring inconsistency in meta-analyses. *BMJ*, **327**,557-560 (2003).

22. Duffield, T.F., Rabiee, A. and Lean, I.J. Overview of meta-analysis of monensin in dairy cattle. *Veterinary Clinics: Food Animal Practice*, **28**, 107-119 (2012).

23. Higgins, J., Altman, D. and Sterne, J. Assessing risk of bias in included studies. In: Higgins JPT, Green S. (Ed.). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1. 0 (updated March 2011). The Cochrane Collaboration, 2011. Available from handbook cochrane org, 243-296 (2011).

24. Egger, M., Smith, G.D., Schneider, M. and Minder, C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*, **315**, 629-634 (1997).

25. Begg, C.B. and Mazumdar, M. Operating characteristics of a rank correlation test for publication bias. *Biometrics*, **50**, 1088-1101 (1994).

26. Shi, L. and Lin, L. The trim-and-fill method for publication bias: practical guidelines and recommendations based on a large database of meta-analyses. *Medicine*, **23**, 98 (2019).

27. Orwin, R.G. A fail-safe N for effect size in meta-analysis. *Journal of Educational Statistics*, **8**,157-159 (1983).

28. Kim, H.Y. Statistical notes for clinical researchers: Chi-squared test and Fisher's exact test. *Restorative Dentistry & Endodontics*, **42**, 152- 155 (2017).

29. Wasko, A., Barkema, H., Nicol, J., Fernandez, N., Logie, N. and Léguillette, R. Evaluation of a risk-screening questionnaire to detect equine lung inflammation: results of a large field study. *Equine Veterinary Journal*, **43**, 145- 152 (2011).

30. Couëtil, L., Cardwell, J., Gerber, V., Lavoie, J.P., Léguillette, R. and Richard, E. Inflammatory airway disease of horses-revised consensus statement. *Journal of Veterinary Internal Medicine*, **30**,503-515 (2016).

31. Pusterla, N., Kass, P., Mapes, S., Johnson, C., Barnett, D., Vaala, W., Gutierrez, C., McDaniel, R., Whitehead, B., Manning, J. Surveillance programme for important equine infectious respiratory pathogens in the USA. *Veterinary Record*, **169**, 12 (2011).

32. Boyle, A., Rankin, S., Duffee, L., Boston, R. and Wheeler-Aceto, H. Streptococcus equi detection polymerase chain reaction assay for equine nasopharyngeal and guttural pouch wash samples. *Journal of Veterinary Internal Medicine*, **30**, 276-281 (2016).

33. Whelchel, D. and Chaffin, M. Sequelae and complications of Streptococcus equi subspecies equi infections in the horse. *Equine Veterinary Education*, **21**,135-141(2009).

34. Pusterla, N., Watson, J., Affolter, V., Magdesian, K., Wilson, W. and Carlson, G. Purpura haemorrhagica in 53 horses. *Veterinary record*, **153**,118-121 (2003).

35. Boyle, A., Timoney, J.F., Newton, J., Hines, M., Waller, A. and Buchanan, B. Streptococcus equi infections in horses: guidelines for treatment, control, and prevention of strangles-revised consensus statement. *Journal of Veterinary Internal Medicine*, **32**, 633-647 (2018).

36. Anzai, T., Nakanishi, A., Wada, R., Higuchi, T., Hagiwara, S., Takazawa, M., Oobayashi, K. and Inoue, T. Isolation of Streptococcus equi subsp. equi from thoroughbred horses in a racehorse-breeding area of Japan. *Journal of Veterinary Medical Science*, **59**,1031-1033 (1997).

37. Arafa, A.A., Hedia, R.H., Ata, N.S. and Ibrahim, E.S. Vancomycin resistant Streptococcus equi subsp. equi isolated from equines suffering from respiratory manifestation in Egypt. *Veterinary World*, **14**, 1808 (2021).

38. Bailey, G. and Love, D.N. Oral associated bacterial infection in horses: studies on the normal anaerobic flora from the pharyngeal tonsillar surface and its association with lower respiratory tract and paraoral infections. *Veterinary Microbiology*, **26**,367-379 (1991).

39. Båverud, V., Johansson, S. and Aspan, A. Real-time PCR for detection and differentiation of Streptococcus equi subsp. equi and Streptococcus equi subsp. zooepidemicus. *Veterinary Microbiology*, **124**, 219-229 (2007).

40. Bustos, C.P., Guida, N., Casamayor, A., Muñoz, A.J., Fernández-Garayzábal, J.F. and Alonso, A.I.V. First report of molecular characterization of Argentine isolates of Streptococcus equi subsp. equi by pulsed-field gel electrophoresis. *Journal of Equine Veterinary Science*, **53**, 30-37 (2017).

41. Carneiro, V.C., Aquino, M.H.C., Cerqueira, A., de Mattos Guttmann, P., Magalhães, H., de Mello Costa, M.F., Luis Eduardo, C. and Daniel, A.B. Microbiological Findings in Tracheal Wash from Mule Foals with and Without Clinical Evidence of Respiratory Disease. *Journal of Equine Veterinary Science*, **55**, 40-43 (2017).

42. Clark, C., Greenwood, S., Boison, J.O., Chirino-Trejo, M. and Dowling, P.M. Bacterial isolates from equine infections in western Canada (1998–2003). *The Canadian Veterinary Journal*, **49**,153 (2008).

43. Debelu, T., Akililu, N. and Desissa, T. Isolation and identification of aerobic bacterial species from upper respiratory tract of cart horses in Central Ethiopia. *Veterinary Medicine and nimal Health*, 239 (2014).

44. Erol, E., Locke, S.J., Donahoe, J.K., Mackin, M.A. and Carter, C.N. Beta-hemolytic Streptococcus spp. from horses: a retrospective study (2000–2010). *Journal of Veterinary Diagnostic Investigation*, **24**,142-147 (2012).

45. Fonseca, J.D., Mavrides, D.E., Morgan, A.L., Na, J.G., Graham, P.A. and McHugh, T.D. Antibiotic resistance in bacteria associated with equine respiratory disease in the United Kingdom. *Veterinary Record*, **187**,189 (2020).

46. Jannatabadi, A., Mohammadi, G., Rad, M. and Maleki, M. Molecular identification of Streptococcus equi subsp. equi and Streptococcus equi subsp. zooepidemicus in nasal swabs samples from horses suffering respiratory infections in Iran. *Pakistan Journal of Biological Sciences:PJBS*, **11**,468-471(2008).

47. Libardoni, F., Machado, G., Gressler, L.T., Kowalski, A.P., Diehl, G.N., dos Santos, L.C., Corbellini, L.G. and de Vargas, A.C. Prevalence of Streptococcus equi subsp. equi in horses and associated risk factors in the State of Rio Grande do Sul, Brazil. *Research in Veterinary Science*, **104**, 53-57 (2016).

48. Manguin, E., Pépin, E., Boivin, R. and Leclere, M. Tracheal microbial populations in horses with moderate asthma. *Journal of Veterinary Internal Medicine*, **34**, 986-995 (2020).

49. Mir, I.A., Kumar, B., Taku, A., Faridi, F., Bhat, M.A., Baba, N.A. and Maqbool, T. Bacteriological and Molecular Detection of Streptococcus equi subsp. equi and Streptococcus equi subsp. zooepidemicus in Equines of Northern India. *Journal of Equine Science*, **24**, 53-55(2013).

50. Mohamed, O., Abo Elhasan, D. and Elmolla, A. Study on β-haemolytic streptococci Infection in Equines at Different Seasons and Ages. *Egyptian Journal of Veterinary Sciences*, **49**,147-154 (2018).

51. Preziuso, S., Laus, F., Tejeda, A.R., Valente, C. and Cuteri, V. Detection of Streptococcus dysgalactiae subsp. equisimilis in equine nasopharyngeal swabs by PCR. *Journal of Veterinary Science*, **11**,67-72 (2010).

52. Racklyeft, D. and Love, D. Bacterial infection of the lower respiratory tract in 34 horses. *Australian Veterinary Journal*, **78**, 549-559 (2000).

53. Sherman, J., Thorsen, J., Barnum, D., Mitchell, W. and Ingram, D. Infectious causes of equine respiratory disease on Ontario Standardbred racetracks. *Journal of Clinical Microbiology*, **5**, 285-289 (1977).

54. Toombs-Ruane, L., Riley, C., Kendall, A., Bolwell, C., Benschop, J. and Rosanowski, S. Antimicrobial susceptibilities of aerobic isolates from respiratory samples of young New Zealand horses. *Journal of Veterinary Internal Medicine*, **29**,1700-1706 (2015).

55. Tirosh-Levy, S., Blum, S., Steward, K., Waller, A. and Steinman, A. Streptococcus equi subspecies equi in horses in Israel: seroprevalence and strain types. *Veterinary Record Open*, **3**,187 (2016).

56. Jaramillo-Morales, C., Gomez, D.E., Renaud, D. and Arroyo, L.G. Streptococcus equi culture prevalence, associated risk factors and antimicrobial susceptibility in a horse population from Colombia. *Journal of Equine Veterinary Science*, **111**,103890 (2022).

57. de Moraes, C.M., de Vargas, A.P.C., Leite, F.P.L., Nogueira, C.E.W. and Turnes, C.G. Strangles: etiology, diagnosis and control/Adenite equina: sua etiologia, diagnostico e controle. *Ciência Rural*, **39**,1944-1953 (2009).

58. Taylor, S.D. and Wilson, W.D. Streptococcus equi subsp. equi (Strangles) infection. *Clinical Techniques in Equine Practice*, **5**,211-217 (2006).

59. Tshikhudo, P., Nnzeru, R., Ntushelo, K. and Mudau, F. Bacterial species identification getting easier. *African Journal of Biotechnology*, **12**, 5975 -5982 (2013).

60. Hedges, L.V. and Olkin, I. Statistical methods for meta-analysis: Academic press (2014).

61. Borenstein, M., Hedges, L., Higgins, J. and Rothstein, H. Introduction to Meta-Analysis. John Wiley & Sons. Ltd, Chichester, UK*, (*2009).

62. Higgins, J.P., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J. and Welch, V. Cochrane handbook for systematic reviews of interventions: John Wiley & Sons; (2019).

63. Huedo-Medina, T.B., Sánchez-Meca, J., Marin-Martinez, F. and Botella, J. Assessing heterogeneity in meta-analysis: Q statistic or I^2 index? *Psychological Methods*, **11**,193(2006).

64. Thompson, S.G. Why and how sources of heterogeneity should be investigated. *Systematic reviews in health care: meta-analysis in context*, 157-175 (2001).

65. Schulz, K.F., Chalmers, I., Hayes, R.J. and Altman, D.G. Empirical evidence of bias: dimensions of methodological quality associated with estimates of treatment effects in controlled trials. *JAMA*, **273**,408-412 (1995).

66. Joober, R., Schmitz, N., Annable, L. and Boksa, P. Publication bias: what are the challenges and can they be overcome? *Journal of Psychiatry and Neuroscience*, **37**,149-152 (2012).

67. Sutton, A.J., Duval, S.J., Tweedie, R., Abrams, K.R. and Jones, D.R. Empirical assessment of effect of publication bias on meta-analyses. *BMJ,* **320**, 1574-1577 (2000).

68. Light, R. and Pillemer, D. Summing Up: The Science of Reviewing Research Harvard University Press: Cambridge. *Educational Researcher*, **15**, 16-17 (1986).

69. Rothstein, H.R., Sutton, A.J., Borenstein, M. Publication bias in meta-analysis. Publication bias in meta-analysis: Prevention, assessment and adjustments. John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England.1-7(2005).

70. Duval, S. and Tweedie, R. Trim and fill: a simple funnel-plot–based method of testing and adjusting for publication bias in meta-analysis. *Biometrics*, **56**,455-463 (2000).

71. Duval, S. The trim and fill method. Publication bias in meta-analysis: Prevention, assessment and adjustments. John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England. 127-144 (2005).

72. Rosenthal, R. The file drawer problem and tolerance for null results. *Psychological Bulletin*, **86**, 638 (1979).

73. Sterne, J.A., Gavaghan, D. and Egger, M. Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. *Journal of Clinical Epidemiology*, **53**,1119-1129 (2000).

74. Terrin, N., Schmid, C.H., Lau, J. and Olkin, I. Adjusting for publication bias in the presence of heterogeneity. *Statistics in Medicine*, **22**,2113-2126 (2003).

75. Peters, J.L., Sutton, A.J., Jones, D.R., Abrams, K.R. and Rushton, L. Comparison of two methods to detect publication bias in meta-analysis. *JAMA*, **295**,676-680 (2006).

76. Peters, J.L., Sutton, A.J., Jones, D.R., Abrams, K.R. and Rushton, L. Performance of the trim and fill method in the presence of publication bias and between-study heterogeneity. *Statistics in Medicine*, **26**,4544-4562 (2007).

77. Rücker, G., Schwarzer, G. and Carpenter, J. Arcsine test for publication bias in meta-analyses with binary outcomes. *Statistics in Medicine*, **27**,746-763 (2008).

انتشار أنواع البكتريا العقدية في الخيول المصابة بعدوى الجهاز التنفسي العلوي. التحليل التلوي المنهجي

حلمــى كمال النفــراوى ، محمــد يوسف ، ماجـد الأشقــر ، حســام إبراهيــم ، صبرى الخضرى و الشيماء محمد محمد فرج *
قسم أمراض الباطنة والمعدية- كلية الطب البيطرى - جامعة المنصورة - المنصورة ٣٥٥١٦ - مصر.

فى هذه الدراسة ، وفقًا لإرشادات PRISMA ، تم إجراء التحليل التلوي حول انتشار أنواع البكتريا العقدية في الخيول المصابة بعدوى الجهاز التنفسي. بعد البحث المتنافس واستخراج البيانات واختيار الدراسات ، تم تحليل البيانات باستخدام التحليل التلوي الشامل. كانت نتائج التحليل التلوي للدراسات المختارة ٩٥٪ فترات ثقة ، حجم التأثير ، التغايرية ، الوزن ، وتحيز النشر. تم العثور على ما مجموعه ١٨٣١ (٣٢,٢٥٪) من أصل ٥٦٧٨ من الخيول المريضة في ٢٠ دراسة مع أعراض الجهاز التنفسي العلوي إيجابية لأنواع البكتريا العقدية. أظهر نموذج التحليل التلوي النهائي لتأثير الحجم والاختبار الفارغ في التأثير الثابت والعشوائي أن التأثير له قيمة Z تبلغ -٣,٣٧١ (قيمة P = 0.001) مقابل قيمة Z للتأثير الثابت البالغة -٢٦,٠٢٤ (P -القيمة = ٠,٠٠٠). كشفت درجة التغايرية عن قيمة P-value ، I-squared (97.948) ، Q (926.104) (٠,٠٠٠) ، و Tau-squared 0.989 مع ٠,٥١٩ خطأ معياري هي متغيرات التغايرية النهائية. لم يشر اختبار الانحدار الخطي لإيجر لعدم التماثل إلى تحيز النشر ، التقاطع (-٠,٥٣) ، فاصل الثقة ٩٥٪ (-٦,٤٥- ٥,٣٩) ، df = ، t-value (0.18) 18. قيمة P أحادية الذيل هي ٠,٤٢٦ ، وقيمة P ثنائية الذيل هي ٠,٨٥٢. نتيجة Kendall›s tau مع تصحيح الاستمرارية (-٠,٠٠٥٢٦) ، بقيمة P أحادية الذيل تبلغ ٠,٤٨٧ وقيمة P ثنائية الذيل تبلغ ٠،٩٧٤. أدت طريقة التقليم والتعبئة لدوفال وتويدي (دراسة واحدة فقط تم قصها في الجانب الأيسر) إلى ارتباط معدل من - ٠,٨٥٧ إلى - ٠,٧٣٧ مع ٩٥٪ CI: - 1.345 إلى - ٠,٤٢٨. تشير نتائج الدراسة الحالية إلى ارتباط أنواع المكورات العقدية بعلامات الجهاز التنفسي العلوي السريرية في الخيول.

الكلمات الدالة: علم الأوبئة ، الخيول ، أمراض الجهاز التنفسي ، العقدية ، البكتيريا