

Seroprevalence of enzootic bovine leukosis (EBL): a systematic review and meta-analysis

Soroprevalência de leucose bovina enzoótica (LEB): revisão sistemática e meta-análise
Seroprevalencia de la leucosis bovina enzoótica (LEB): revisión sistemática y metanálisis

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Abstract

Enzootic bovine leukosis (EBL) is a viral disease with negative impact on the economy, and the virus has been linked to breast cancer in women and its DNA detected in fresh milk and raw beef for human consumption. In this context, epidemiological surveys allow the knowledge of epidemiological indicators of infection, guiding control programs and consequently making it possible to control and/or eliminate the virus in cattle populations. Therefore, the objective of the present study was to carry out a systematic review with meta-analysis on EBL seroprevalence. Complete articles and brief communications from indexed journals that contained data on the seroprevalence of EBL and described the diagnostic methods used to identify the infection were selected. The study followed the recommendations of the PRISMA methodology - Preferred Reporting Items for Systematic Reviews and Me¹ta-Analyzes. In turn, from a total of 581 studies 15 met the eligibility criteria, and the meta-analysis showed a combined prevalence of 31% (95% CI = 25 – 37%),

although with high heterogeneity among the studies, which was attributed to research designs, years of publication of the studies, quantity and age of the animals sampled, exclusive use of dairy herds and heterogeneity among countries. Therefore, this scenario suggests the need for standardization of researches related to epidemiological studies for EBL, specifically cross-sectional surveys, with the use of planned sampling, adjustment of rates according to parameters that may influence the prevalence and specific analyzes that provide the determination of reliable epidemiological indicators.

Keywords: Cattle; Enzootic bovine leucosis; Cross-sectional surveys; Prevalence; Meta-analysis.

Resumo

A leucose enzoótica bovina (LEB) é uma doença viral com impacto negativo na economia, o vírus tem sido associado ao câncer de mama em mulheres e seu DNA detectado no leite fresco e na carne crua para consumo humano. Nesse contexto, pesquisas epidemiológicas permitem o conhecimento de indicadores epidemiológicos de infecção, orientando programas de controle e, conseqüentemente, possibilitando o controle e / ou eliminação do vírus nas populações de gado. Dessa forma, o objetivo do presente estudo foi realizar uma revisão sistemática com meta-análise sobre soroprevalência de LEB. Foram selecionados artigos completos e comunicações breves de periódicos indexados que continham dados sobre a soroprevalência da LEB e descreveram os métodos de diagnóstico utilizados para identificar a infecção. O estudo seguiu as recomendações da metodologia PRISMA - itens de relatóriferenciais para revisões sistemáticas e metanálises. Por sua vez, de um total de 581 estudos 15 atenderam aos critérios de elegibilidade, e a meta-análise mostrou uma prevalência combinada de 31% (IC 95% = 25 - 37%), embora com alta heterogeneidade entre os estudos, atribuída à desenhos de pesquisa, anos de publicação dos estudos, quantidade e idade dos animais amostrados, uso exclusivo de rebanhos leiteiros e heterogeneidade entre os países. Portanto, esse cenário sugere a necessidade de padronização de pesquisas relacionadas a estudos epidemiológicos para LEB, especificamente pesquisas transversais, com o uso de amostragem planejada, ajuste de taxas de acordo com parâmetros que possam influenciar a prevalência e análises específicas que proporcionam a determinação de indicadores epidemiológicos confiáveis.

Palavras-chave: Bovino; Leucose enzoótica bovina; Estudos transversais; Prevalência; Meta-análise.

Resumen

La leucosis enzoótica bovina (LEB) es una enfermedad viral con impacto negativo en la economía, el virus se ha asociado al cáncer de mama en mujeres y su ADN se detecta en la leche fresca y la carne cruda para consumo humano. En este contexto, la investigación epidemiológica permite conocer los indicadores epidemiológicos de la infección, orientando los programas de control y, en consecuencia, posibilitando el control y / o eliminación del virus en las poblaciones bovinas. Así, el objetivo del presente estudio fue realizar una revisión sistemática con metaanálisis sobre la seroprevalencia de LEB. Se seleccionaron artículos completos y breves comunicaciones de revistas indexadas que contenían datos sobre la seroprevalencia de LEB y describieran los métodos de diagnóstico utilizados para identificar la infección. El estudio siguió las recomendaciones de la metodología PRISMA: elementos de presentación de informes diferenciales para revisiones sistemáticas y metanálisis. A su vez, de un total de 581 estudios 15 cumplieron los criterios de elegibilidad, y el metaanálisis mostró una prevalencia combinada del 31% (IC 95% = 25 - 37%), aunque con alta heterogeneidad entre estudios, atribuida a la diseños de investigación, años de publicación de estudios, cantidad y edad de los animales muestreados, uso exclusivo de rebaños lecheros y heterogeneidad entre países. Por tanto, este escenario sugiere la necesidad de estandarizar la investigación relacionada con los estudios epidemiológicos para LEB, específicamente encuestas transversales, con el uso de muestreo planificado, ajuste de tasas según parámetros que puedan influir en la prevalencia y análisis específicos que brinden la determinación de indicadores. datos epidemiológicos fiables.

Palabras clave: Bovino; Leucosis bovina enzoótica; Estudios transversales; Prevalencia; Metanálisis.

1. Introduction

Enzootic bovine leukosis (EBL) is a transmissible disease caused by the enzootic bovine leukosis virus (EBLV), an oncogenic retrovirus of the genus *Deltaretrovirus*, family *Retroviridae*, and subfamily *Oncovirinae*. The infection has a worldwide distribution with different epidemiological characteristics in each country and with variable prevalence among herds, being up to four times higher in dairy cattle when compared to beef cattle (Frie & Coussens, 2015; Polat, Takeshima, & Aida, 2017; OIE, 2018). Animals that present the clinical form are discarded from the herd due to disorders such as infertility, decreased milk

production and impairment of organs and systems (Tsutsui, Kobayashi, Hayama, & Yamamoto, 2016; Kathambi, Gitau, Muchemi, Van Leeuwen, & Kairu-Wanyoike, 2019).

The EBLV can be carried by free particles and by the transfer of cells carrying genetic material. Because it is unstable in the environment, transmission occurs directly between animals or through newly contaminated materials, through body fluids that contain blood or exudate since they are inoculated into the host or in contact with mucous membranes (Hirsch & Leite, 2016). In this context, transmission can occur by rectal palpation, immunization, blood transfusion and surgeries with the use of non-sterilized materials, milk ingestion, iatrogenic transmission and bites of hematophagous arthropods. Mismanagement practices and deficient hygienic conditions can act as risk factors (Kohara, Takeuchi, Hirano, Sakurai, & Takahashi, 2018; Konishi, Ishizaki, Kameyama, Murakami, & Yamamoto, 2018; Ruiz, Porta, Lomónaco, Trono, & Alvarez, 2018; Panei *et al.*, 2019). Vertical transmission has been proven through the detection of antibodies in newborn calves without previous ingestion of colostrum, and this transmission route is responsible for 3 to 20% of infections in calves born to positive cows (Leuzzi Junior, Alfieri, & Alfieri, 2001).

The diagnosis of EBL can be performed using direct and indirect methods. Direct methods include viral isolation by in vitro culture, and molecular detection of the agent by polymerase chain reaction (PCR), which are very efficient techniques for detecting the agent, however, they are expensive and require qualified inputs and labor. The main indirect methods are the agar gel immunodiffusion test (AGID), officially recognized as the gold standard test, presenting good specificity and sensitivity, relatively simple and low cost, and the enzyme immunoabsorbent assay (ELISA), which presents the best sensitivity and good specificity, ability to detect antibodies in serum and milk, and enable the testing of a high number of animals, presenting as a disadvantage the high cost with equipments for reading and interpretation and the import of diagnostic kits (Hirsch & Leite, 2016; OIE, 2018).

The occurrence of the disease has a negative impact on the economy, since the infection can cause reduced fertility, decreased milk production, increased costs with replacement of heifers, loss of income resulting from the premature slaughter of animals and commercial restrictions (Bartlett *et al.*, 2013; Khudhair, Hasso, Yaseen, & Al-Shammari, 2016; Norby, Bartlett, Byrem, & Erskine, 2016). It's worthy mentioning that BLV has been linked to breast cancer in women (Baltzell *et al.*, 2018; Schwingel, Andreolla, Erpen, Frandoloso, & Kreutz, 2019) and BLV DNA detected in fresh milk and raw beef for human consumption (Olaya-Galán *et al.*, 2017).

Taking into account the economic impacts, the implications for animal health and the possible impact on public health, added to the lack of vaccines or effective treatment, it is extremely important that government entities and policy makers consider adopting a control program for the disease. Once implemented, the control program facilitates the diagnosis of infected animals and allows the adoption of measures that hinder the spread of the virus, minimizing its impact on the cattle production and, eventually, on humans who consume cattle food products (Olaya-Galán *et al.*, 2017). In this context, epidemiological surveys allow the knowledge of epidemiological indicators of infection, guiding control programs and consequently making it possible to control and/or eliminate the virus in cattle populations. Thus, the objective of the present research was to carry out a survey of the prevalence of enzootic bovine leukosis in a worldwide scenario through a quantitative synthesis with a systematic literature review and meta-analysis.

2. Methodology

A systematic literature review was carried out with emphasis on the seroprevalence of enzootic bovine leukosis, followed by a meta-analysis of quantitative data available in articles from indexed journals. To prepare the study, the recommendations of the PRISMA methodology - Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Moher, Liberati, Tetzlaff, & Altman, 2009) were observed.

Complete articles and brief communications from indexed journals that contained data on the seroprevalence of enzootic bovine leukosis and described the diagnostic methods used to identify the infection were selected. There was no restriction on the year of conduction or publication, the language in which the article was published or the country in which the survey was developed. Literature reviews, research notes, editorials and experimental essays and other types of publications not included in the inclusion criteria were excluded. Studies containing other animal species, case reports, phylogenetic analysis of the virus and clinical aspects of the disease were also excluded.

Based on the pre-established inclusion criteria, the search for the articles was carried out in the PubMed, Scielo, ScienceDirect, Scopus and Web of Science databases, using the following terms in English: {leukosis} AND {enzootic} AND {bovine} AND {prevalence}. The citations of the identified studies containing the title and abstract were saved in BibTex format or text document and exported to a bibliographic manager for later selection. The searches were conducted between August 26 and 30, 2019.

To exclude duplicate articles, the tool provided by the bibliographic manager was used. Then, two researchers independently carried out a selection of the studies, initially by analyzing the title and abstract, and later by reading the full papers. After evaluating the texts, other studies were excluded because they did not meet the eligibility criteria. Divergent cases were resolved by consensus.

The survey by Meirelles, Dittrich, Cipriano, & Ollhoff (2009) was subdivided for analysis in three years, in view of the methodology used by the authors. Data extraction was performed individually by two researchers and the information was entered into a previously prepared spreadsheet. Data extracted from the articles were: references (authors and year of publication), type of sampling, total number of animals, number of positive animals, frequency (%) of positive animals, country of study, and diagnostic method.

For the analysis of the quantitative data the 95% confidence interval (95% CI) was considered. Heterogeneity was assessed by Cochran's Q test and quantified by Higgins and Thompson's I^2 test. The combined estimates and the 95% CI were calculated based on the random effects model by the inverse of the variance using the DerSimonian-Laird method. Visual assessment of the funnel plot and the Egger test were also used as alternatives to identify possible biases. All analyzes were performed on the R environment (R CORE TEAM, 2019), RStudio interface (version 1.1.463).

3. Results

The initial search in the databases and the selection process of the studies are presented in Figure 1. Of the total number of studies surveyed ($n = 581$) 13 met the eligibility criteria. These papers consisted of cross-sectional surveys (prevalence surveys) with sufficient data for a quantitative synthesis and performance of meta-analysis. The studies included in this stage were carried out in Brazil ($n = 2$), Bulgaria ($n = 1$), Canada ($n = 1$), Chile ($n = 1$), Colombia ($n = 1$), United States ($n = 1$), Iran ($n = 1$), Iraq ($n = 1$), Japan ($n = 1$), Peru ($n = 1$), Turkey ($n = 1$), and Venezuela ($n = 1$). In one of these studies (Meirelles *et al.*, 2009), the prevalence of EBL was analyzed in three different years, being, therefore, considered as three independent studies. Thus, 15 studies were used for the meta-analysis.

The informations included in the meta-analysis are shown in Table 1. After the analysis was carried out, it was possible to found the presence of high heterogeneity among the studies, which was detected by performing the Cochran's Q test ($P < 0.001$) and the Higgins and Thompson's I^2 statistic ($I^2 = 100\%$). Thus, the random effects model was used,

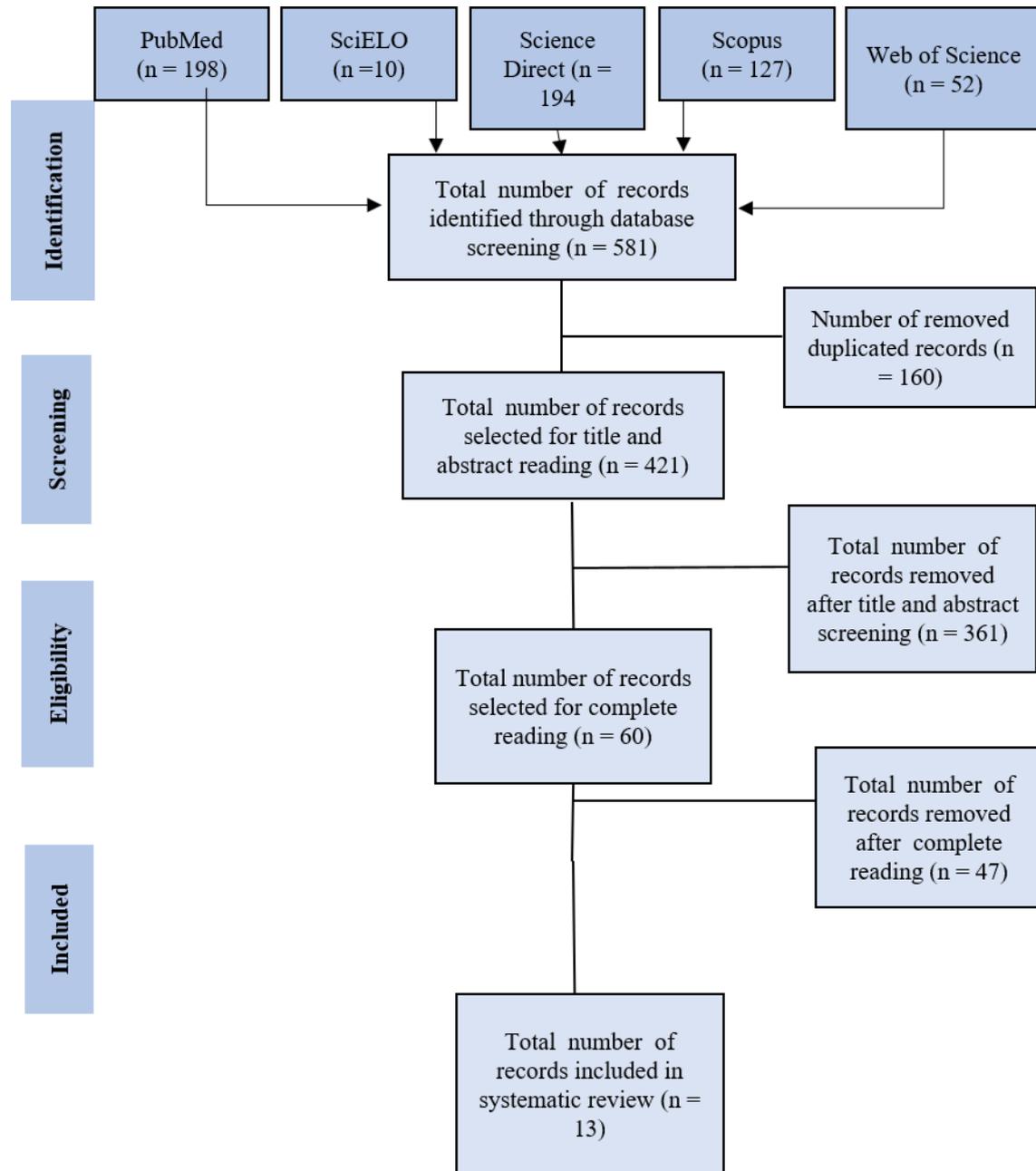
by subgroups, to perform the meta-analysis. Using this model, the combined prevalence of the surveys 31% (95% CI = 25 – 37%) (Figure 2).

Table 1 - Quantitative synthesis regarding the main characteristics of the studies included in the meta-analysis.

Study	Random sampling	Total number of animals	Number of positive animals	Frequency (%)	Country	Diagnostic method
Heald <i>et al.</i> (1992)	No	998	242	24	Canada	AGID
Uysal <i>et al.</i> (1998)	No	481	51	11	Peru	ELISA
Sandev <i>et al.</i> (2001)	No	200,518	34,119	17	Bulgaria	AGID
Amoril <i>et al.</i> (2009)	Yes	1,229	94	8	Brazil	AGID
Meirelles <i>et al.</i> (2009) – A	No	60	38	63	Brazil	AGID
Meirelles <i>et al.</i> (2009) – B	No	37	30	81	Brazil	AGID
Meirelles <i>et al.</i> (2009) – C	No	115	30	26	Brazil	AGID
Grau and Monti (2010)	Yes	4,360	637	15	Chile	ELISA
Murakami <i>et al.</i> (2011)	No	5,420	1,548	29	Japan	ELISA
Morovati <i>et al.</i> (2011)	No	403	330	82	Iran	ELISA
Nava <i>et al.</i> (2011)	Yes	360	219	61	Venezuela	ELISA
Benavides <i>et al.</i> (2013)	Yes	242	48	20	Colombia	ELISA
Şevik <i>et al.</i> (2015)	Yes	28,982	460	2	Turkei	ELISA
Khudhair <i>et al.</i> (2016)	No	400	28	7	Iraq	ELISA
Bauermann <i>et al.</i> (2017)	Yes	1,996	771	39	USA	ELISA

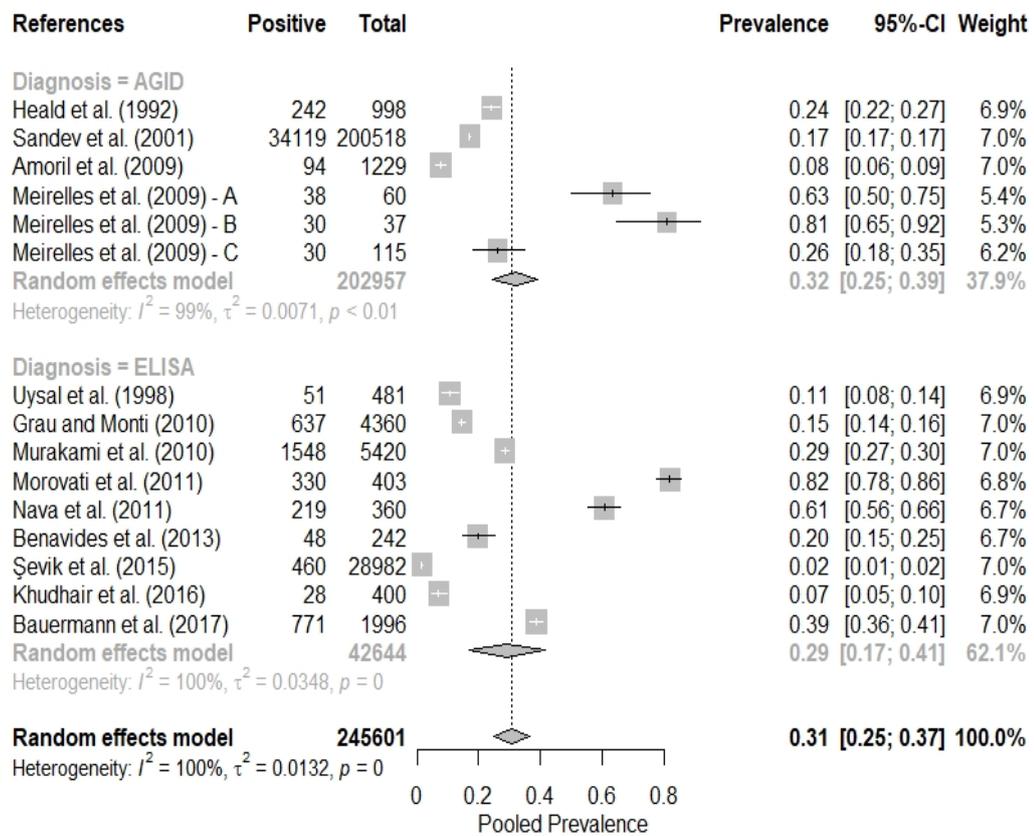
Source: Authors.

Figure 1 - Flowchart of the search, selection and inclusion of studies in the systematic review.



Source: Authors.

Figure 2 - Combination of 15 prevalence studies on enzootic bovine leukosis according to the diagnosis method used.



Source: Authors.

To assess the possible causes of heterogeneity, the studies were divided into subgroups according to serological tests used (ELISA and AGID) and continents in which the surveys were conducted (North America, South America, Asia and Europe), and the meta-analysis results are summarized in Table 2; however, high heterogeneity among the studies were also detected.

The visual analysis of the funnel plot (Figure 3) showed asymmetric distribution of the 15 studies, showing possible publication biases, however, by applying the Egger test ($P = 0.62$), the occurrence of such biases was not verified.

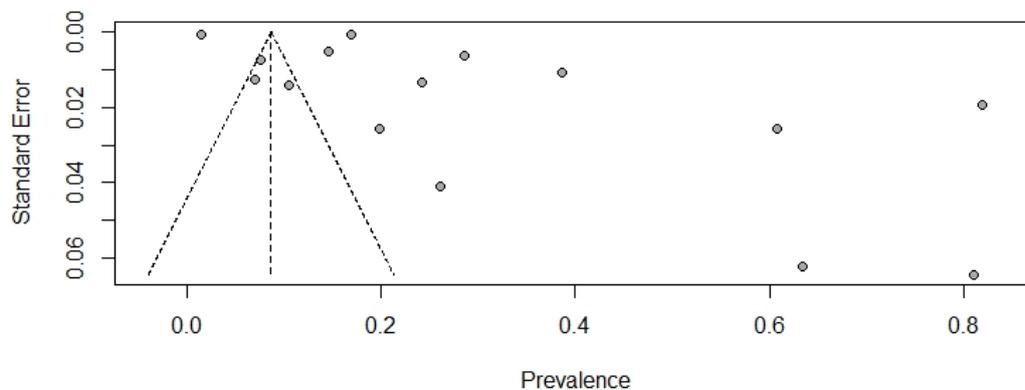
In addition, a meta-analysis (Figure 4) was performed considering age as a factor associated with EBL seropositivity, using data from four studies from which it was possible to standardize the age groups of the animals in < 4 years and > 4 years, and the results demonstrated a combined odds ratio (OR) of 2.87 (95% CI = 1.19 – 6.93).

Table 2 - Results of the meta-analysis with surveys divided into subgroups according to serological tests used (ELISA and AGID) and continents in which the surveys were conducted (North America, South America, Asia and Europe).

Parameters	Number of studies	Total number of animals	Number of positive animals	Pooled seroprevalence (95% CI)	Heterogeneity	
					<i>P</i> -value	<i>I</i> ²
Pooled effect	15	245,601	38,645	31% (25 - 37%)	<0.001	99.90%
Subgroups						
Diagnosis						
AGID	6	202,957	34,553	32% (25 - 39%)	<0.001	98.50%
ELISA	9	42,644	4,092	29% (17 - 41%)	<0.001	99.90%
Continent						
North America	2	2,994	1,013	31% (17 - 46%)	<0.001	98.50%
South America	8	6,884	1,147	34% (25 - 43%)	<0.001	98.80%
Asia	4	35,205	2,366	30% (7 - 52%)	<0.001	99.90%
Europe	1	200,518	34,119	17% (17 - 17.2%)	Not applicable	

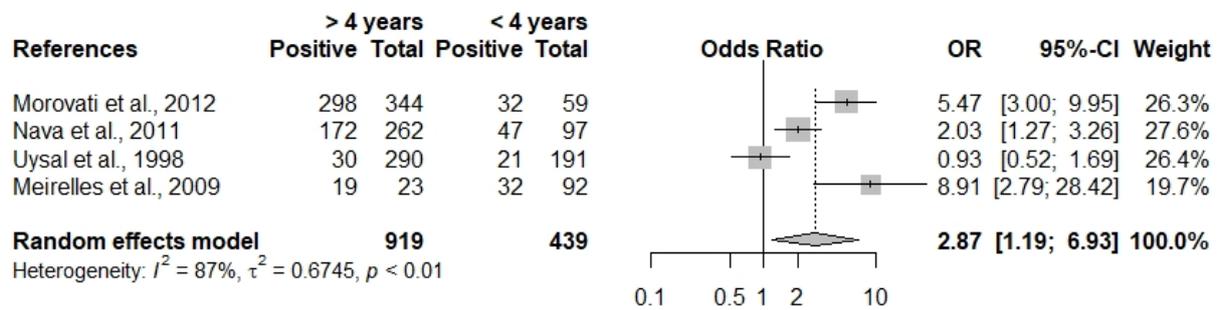
Source: Authors.

Figure 3 - Funnel plot presenting the asymmetrical distribution of studies on the prevalence of enzootic bovine leukosis.



Source: Authors.

Figure 4 - Meta-analysis of four surveys demonstrating the association of age with the seroprevalence of enzootic bovine leukosis.



Source: Authors.

4. Discussion

The combined EBL prevalence among the surveys was 31% (95% CI = 25 – 37%), but the occurrence of high heterogeneity challenges the reliability of the result. In an attempt to identify the responsible factor, an analysis by subgroup was carried out according to the diagnostic method used (ELISA and IDGA) and continents in which the surveys were conducted (North America, South America, Asia and Europe); however, the results demonstrated that the diagnostic tests employed or the continents do not justify the high heterogeneity. Despite that, the scenario of high combined prevalence raises concern because BLV can cause great economic losses to cattle production, as well as reports on the association of the BLV with cancer in women are growing. Baltzell *et al.* (2018) analyzed formalin fixed paraffin embedded breast tissue sections from 216 women were received from The University of Texas MD Anderson Cancer Center and found that women diagnosed with breast cancer were significantly more likely to have BLV DNA in their breast tissue compared with women with benign diagnoses and no history of breast cancer. Buehring *et al.* (2015) conducted a case-control study of archival formalin fixed paraffin embedded breast tissues from 239 donors, received 2002–2008 from the Cooperative Human Tissue Network, USA, and found that the frequency of BLV DNA in mammary epithelium from women with breast cancer (59%) was significantly higher than in normal controls (29%) (multiply-adjusted odds ratio = 3.07, confidence interval = 1.66–5.69, $P = .0004$). Schwingel *et al.* (2019) investigated the presence of BLV genome in healthy ($n = 72$) and cancerous ($n = 72$) paraffin-embedded samples of breast tissues from women in south Brazil, and BLV DNA was

found most frequently (30.5%) in breast cancer tissue than in healthy breast (13.9%) (odds ratio = 2.73; confidence interval = 1.18–6.29; $P = 0.027$). It has been suggested that milk consumption has been associated with a higher incidence of cancer in humans, and at the same time BLV DNA has been detected in fresh milk and raw beef for human consumption in Colombia (Olaya-Galán *et al.*, 2017).

The studies selected for the meta-analysis, despite presenting the eligibility criteria, do not share the same methodology, which is, therefore, the most plausible explanation for heterogeneity, which can be classified as methodological heterogeneity (Santos & Cunha, 2013). The non-random sampling used in part of the studies may have directly influenced the results obtained, considering that this type of selection allows the determination of important epidemiological indicators, however, when selecting the sample units according to non-probabilistic criteria, it undertakes generalization of results (external validity), thus influencing the prevalence. In addition to the type of sampling used, the number of animals sampled showed high variability, such as a high number of animals in the study by Sandev *et al.* (2001) and a small number in the study by Meirelles *et al.* (2009)

Discrepancies were found regarding the years of publication. Some research such as that by Heald, Toews, Jacobs, & McNab (1992) and Uysal *et al.* (1998) date from the last century, while others are more recent, such as that of Bauermann, Ridpath, & Dargatz (2017) and Khudhair *et al.* (2016). Regarding the age, Fig. 4 shows that cattle over 4 years of age are more likely to be seropositive to EBL (OR = 2.87; 95% CI = 1.19 – 6.93), suggesting that the age as an associated factor for the occurrence and dissemination of EBL as reported by Morovati *et al.* (2011), Nava, Obando, Molin, Bracamonte, & Tkachuk (2011) and Sevik, Avci, & Ince (2015). In those studies, there was a higher prevalence in animals aged 2 to 5 years, and the authors inferred that this result is due to the longer exposure to the virus and the clinical presentation of the disease. Thus, it can be noted that the selection of sample units without taking into account the effect of age can result in under or overestimation of prevalence.

The use of dairy herds only in some studies (Heald *et al.*, 1992; Meirelles *et al.*, 2009; Grau & Monti, 2010; Morovati *et al.*, 2011; Nava *et al.*, 2011) may be another possible explanation for heterogeneity, since infection is more frequent in these types of herds due to the animals staying longer in herds, which can lead to an overestimation of prevalence. In these studies, the prevalence ranged from 24% (Heald *et al.*, 1992) to 82% (Morovati *et al.*, 2011). In the study by Heald *et al.* (1992) a herd-level prevalence of 47% was also found. In the study by Murakami *et al.* (2011), although mixed production herds were used, the

prevalence in dairy cattle (34.7%) was higher than for both fattening beef cattle (7.9%) and breeding beef cattle (16.3%), as well as for the survey by Bauermann *et al.* (2017), in which the prevalence in dairy animals (dairy plants; 47.6%) was statistically higher than the positive rate at slaughter plants that processed mainly beef animals (beef plants; 33.6%; $P < 0.05$). It's worthy mentioning that Sevik *et al.* (2015) used animals from the Central Anatolia Region of Turkey, an important milk production centre, and found a prevalence of 2%, however, this scenario may be justified by the existence of a control program for EBL in Turkey.

The analysis by subgroup according to the continent had no influence on heterogeneity (Table 2), although there are among the countries that compose these continents differences in production systems, climatic conditions, cultural aspects and the existence of control programs, which may have an influence on prevalence. For example, mandatory notification of the disease in Japan (Kobayashi *et al.*, 2014) and the adoption of control measures in the United States (Bauermann *et al.*, 2017) and Bulgaria (Sandev *et al.*, 2001) and the realization periodic serological exams contribute to the reduction of the prevalence of EBL, which does not occur in countries that do not adopt such measures, which have higher prevalence values (Polat *et al.*, 2017).

With the application of the Egger test, it was confirmed that the meta-analysis performed does not present publication bias, and the asymmetry of the funnel plot may be attributed to other factors, such as the high heterogeneity found (Sterne *et al.*, 2011). The presence of bias can be identified through the use of the funnel plot and statistical tests, being recommended for meta-analyzes with 10 studies or more, and in the present work, 15 studies were selected, which justifies the methodology used to verify publication bias. In inaccurate studies, with small sampling, positive or negative results may occur due to casual influence (Pereira & Galvão, 2014).

5. Conclusion

The present study demonstrated a significant combined prevalence for EBL. The heterogeneity among the results of the studies included in this review is expected in view of the differences related to the research designs, years of publication of the studies, quantity and age of the animals sampled, exclusive use of dairy herds and heterogeneity among countries. Therefore, this scenario suggests the need for standardization of researches related to epidemiological studies for EBL, specifically cross-sectional surveys, with the use of planned

sampling, adjustment of rates according to parameters that may influence the prevalence and specific analyzes that provide the determination of reliable epidemiological indicators.

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