



BOVINE LEUKAEMIA VIRUS IN MILK SAMPLES: A SYSTEMATIC REVIEW AND META-ANALYSIS

Veterinary Science

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ABSTRACT

Bovine leukaemia virus (BLV), a relative of viruses such as HTLV-1 and -2, is a leading causative agent of enzootic bovine leucosis (EBL). This affects both dairy and beef cattle worldwide. It's essential to understand the BLV prevalence in milk for reasons spanning livestock health, food safety, and international trade. This systematic review and meta-analysis sought to determine the prevalence of BLV in bovine milk based on current primary research. A literature search was carried out across several databases, including PubMed, EMBASE, and Web of Science up to 3 October 2023 identify primary research reporting BLV counts in milk samples. The modified Newcastle-Ottawa scale was employed to gauge the quality of the incorporated studies. We conducted the meta-analysis using a random effects model. All statistical evaluations were carried out using R software, version 4.2. From an aggregate of 14,472 samples taken from 7 distinct studies, we determined a pooled BLV prevalence of 32% (95% CI: 0.23 – 0.42) in milk samples of dairy cow. However, there was significant heterogeneity, as indicated by an I^2 value of 99%. Sensitivity analyses revealed that specific studies exerted a considerable influence on the overall results. Utilizing both Doi plots and the LFK index, no significant publication bias was detected. The considerable presence of BLV in milk samples underscores necessitates further extensive research to grasp its broader implications and potential risks in animal and milk consumer health.

KEYWORDS

Bovine leukaemia virus, Milk, systematic review, One health

INTRODUCTION

Bovine leukaemia virus (BLV) is a member of the delta retrovirus group within the *Retroviridae* family. Its relation to human T-cell leukaemia virus types 1 and 2 (HTLV-1 and -2) and simian T-cell leukaemia viruses (STLVs) has been established (1, 2). There are suggestions that certain viruses in this group may contribute to growth-related or neurological disorders in both humans and other primates (2, 3). BLV is recognized as the primary pathogen responsible for enzootic bovine leucosis (EBL), commonly referred to as bovine leucosis, marking it as a leading neoplastic ailment in both dairy and meat-producing cattle (4-6).

In cattle afflicted with the virus, there's a noticeable compromise in their immune functions, even in the dormant phases of leukaemia. This diminishes the animals' capability to perform optimally (7). As a consequence, BLV not only detrimentally impacts the health of the livestock but also their overall productivity. Globally, the economic repercussions of this ailment are substantial. Directly, it leads to diminished milk yields, severely affects reproductive capabilities, and mandates the early culling of some affected cattle. Indirectly, the disease imposes trade restrictions, limiting the import of cattle from regions known to have BLV outbreaks (8-11). The presence of BLV-infected cells in the milk and colostrum of affected animals serves as a primary transmission route of this retrovirus to subsequent generations. To mitigate the spread, early identification and subsequent segregation of infected livestock from their healthy counterparts is crucial (12, 13). Studies suggest that ingesting a mere 2,000 BLV-infected cells can result in infection in a new host. Tragically, this infection culminates in fatal outcomes for approximately 1 to 5 % of the affected animals, manifesting as acute lymphocytosis and eventually progressing to lymphatic cancer (14).

The transmission and subsequent spread of BLV within cattle populations pose a significant threat to the livestock industry. Given the intricate relationship between livestock health, productivity, and economic outcomes, understanding the prevalence of BLV in animal milk is important. This not only pertains to the health of the livestock but also has implications for food safety and international trade. While several studies have been conducted on this matter, results tend to vary based on region, methodology, and sampling sizes (15-18). A comprehensive review and synthesis of these findings would offer more conclusive insights into the true prevalence of BLV in animal milk. The aim of this systematic review and meta-analysis is to quantify the prevalence of BLV in animal milk based on the synthesized data from the available primary studies.

METHODS

This systematic review is conducted as per PRISMA guidelines and registered in PROSPERO.

Inclusion Criteria: Primary studies employing any observational

design were eligible for inclusion in this review. Commentaries, narrative reviews, conference abstracts, and the like were excluded. Only articles published in the English language were considered. Suitable studies for this review were those that reported both the total number of samples and the samples in which the presence of BLV was identified. We placed no restrictions on the method used for BLV detection. Studies from any geographical location and involving any type of animal where BLV was detected were included.

Literature Search

A comprehensive literature search was conducted on PubMed, EMBASE, and Web of Science up to 3 October 2023. The search strategy incorporated various keywords related to BLV and animal populations. Only articles in English were considered. There were no restrictions regarding the location, year of publication, or type of article.

Screening And Study Selection

Screening was performed in two steps: primary screening by reading the title and abstract, followed by reading the full text to assess eligibility. The Nested Knowledge software was used for screening.

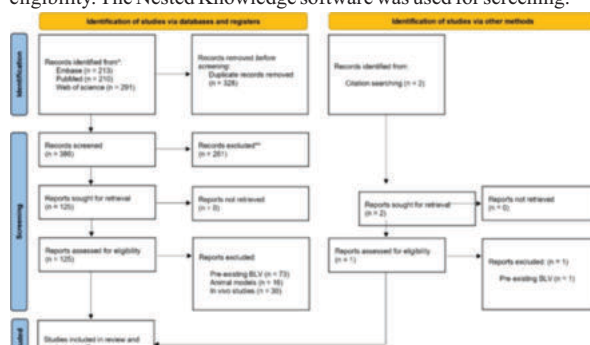


Figure 1. Prisma flow chart depicting the screening and selection process of studies.

Data Extraction And Quality Assessment

The extracted data encompassed the author's name, year of publication, geographic region, type of animal, number of samples where BLV was detected, total samples tested, and the method employed for BLV detection. We performed a quality assessment of the included studies using the Modified Newcastle-Ottawa Scale (NOS) with a total score of 6.

Statistical Analysis

Meta-analysis was performed to determine the pooled prevalence of BLV presence in cattle milk. The number of BLV positive samples and

the total samples tested were used to perform the meta-analysis. A random effects model was used to pool the results. The degree of heterogeneity in findings among studies was measured using I^2 and tau-squared metrics (19). Both metrics can range between 0% and 100%, with ascending values indicating higher inconsistency (20). For indicating this inconsistency, the 95% prediction interval is used. A p-value below 0.05 is viewed as an indicator of statistical significance. The calculation of the tau-squared value was performed using the maximum likelihood approach. The Doi plot was utilized to identify potential publication biases. Sensitivity analysis was performed to identify the individual study effect on the overall result and to identify outliers. R software, version 4.2, was used for all statistical analyses.

RESULTS
Literature Search Result

A total of 714 records were identified from a database search, out of which 328 were duplicates. We screened 386 articles by title and abstract, with 261 records being excluded. Subsequently, 125 records underwent full-text screening, of which 119 records were excluded for various reasons, leaving 6 eligible for inclusion. Additionally, 2 studies were identified through citation searching, and one study was found to be eligible. In the end, a total of 7 studies were included in this review. Figure 1 depicts the overview of screening and selection process of the studies.

Characteristics Of Included Studies

A total of 7 studies were included in our review. All the studies were published in 2016 – 2023. Two studies were from Iran (15, 21), and one each from Canada (22), Chile (16), England (23), Brazil (18), and the USA (17). All of the studies analysed milk samples from dairy cows. The total sample size for the included studies varied, with the smallest sample size being 36 and the largest being 8358 from Canada. Three studies used Nested polymerase chain reaction (PCR) for BLV detection, and three studies used Enzyme linked immune-assay (ELISA) and one with RT-PCR. The important characteristics of the included studies are presented in Table 1. Two studies scored 5, 3 scored 4, two scored 3. Overall studies scored average on Modified NOS.

Table 1. Characteristics Of Studies Included In The Review.

Study	Geographical location	Animal characteristics	Total sample size	No. of BLV positive samples	Test used to detect BLV	Study quality score
Barzegar et al. 2021(15)	Iran	Dairy cow	403	40	Nested-PCR	5
Benavides et al. 2022 (16)	Chile	Dairy cow	1527	459	ELISA	3
Benitez et al. 2022 (17)	USA	Dairy cow	3,611	1701	ELISA	4
de Quadros et al. 2023 (18)	Brazil	Dairy cow	36	14	Nested-PCR	5
Nekouei et al. 2016 (22)	Canada	Dairy cow	8358	2661	ELISA	4
Pakbin et al. 2022 (21)	Iran	Dairy cow	492	63	Nested-PCR	4
Stobnicka-Kupiec et al. 2020 (23)	England	Dairy cow	45	34	RT-PCR	3

Prevalence of BLV in milk sample

We conducted a meta-analysis to determine the overall prevalence of BLV in milk samples. Our analysis included data from 7 studies, comprising a total of 14,472 samples that were tested. The pooled prevalence we observed was 32% (95% CI: 0.23 – 0.42), with a prediction interval of 0.05 to 0.68. Notably, significant heterogeneity was observed among the studies ($I^2 = 99\%$). Figure 2 illustrates the forest plots depicting the pooled prevalence of BLV in the tested milk samples.

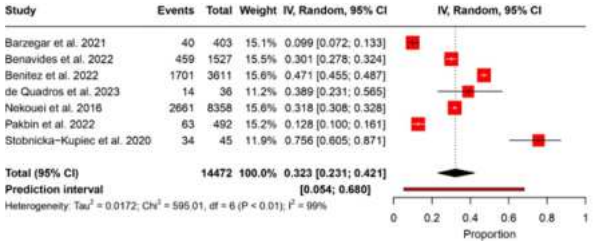


Figure 2. Forest plot illustrating the pooled prevalence of BLV positive samples.

Sensitivity Analysis

We conducted a sensitivity analysis to assess the impact of individual studies on our results. The Baujat plot indicated that the study by Benitez et al. was influencing the overall outcome and contributing to heterogeneity. Subsequently, we performed a Leave-one-out analysis to detect the effect of each study on both the overall result and heterogeneity. When we omitted the study by Benitez et al., the pooled prevalence decreased to 29% (95% CI: 0.20 - 0.39). Similarly, omitting the study by Stobnicka-kupiec et al. reduced the pooled prevalence to 27% (95% CI: 0.18 - 0.37). However, there was no significant change in heterogeneity observed during this analysis.

Publication Bias

We employed Doi plots and the LFK index to assess the presence of publication bias. Fortunately, our analysis did not reveal any indications of potential publication bias, as evidenced by the absence of skewness in the Doi plot and the LFK index value of 0.22 (Figure 3).

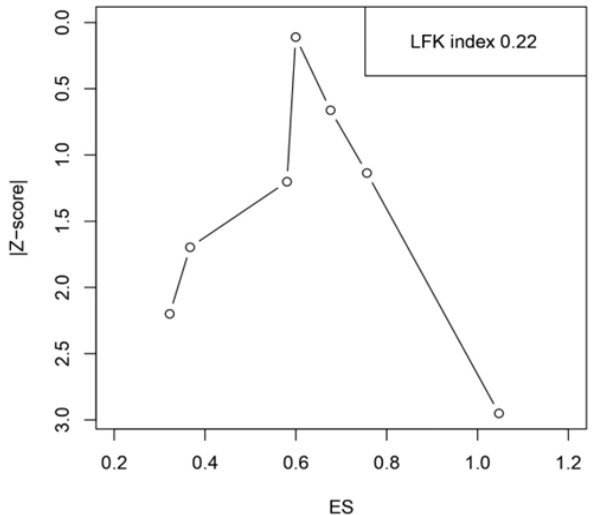


Figure 3. Doi plot illustrating the publication bias of the studies

DISCUSSION

To the best of our knowledge, this is the first systematic review and meta-analysis that has examined the prevalence of BLV in milk samples. Our findings revealed a pooled prevalence of 32% for BLV presence in milk samples from tested dairy cows. However, the significant heterogeneity observed is a matter of concern. We were unable to pinpoint the source of this heterogeneity, but several possibilities exist. One potential factor may be the geographical locations where the studies were conducted. Additionally, variations in sampling techniques used across the studies could contribute to the heterogeneity. The possibility of selection bias cannot be ruled out. Unfortunately, due to the limited number of studies available, we were unable to conduct any subgroup analysis.

A previous meta-analysis investigated the prevalence of BLV in humans, encompassing samples like peripheral blood and paraffin-embedded breast tissue collected from a total of 10,398 individuals (24). Their analysis revealed an overall virus frequency of 27%, with a range spanning from 17% to 37%. The authors of that systematic review underscored the importance of conducting additional studies to determine the route of transmission to humans. Given our study's findings, which indicate a significant prevalence of BLV in milk samples, dairy milk could potentially serve as a transmission route. However, further targeted studies are warranted in this area to gain

deeper insights.

Our findings have significant implications for the livestock industry, food safety, and international trade. The presence of BLV in milk samples highlights the potential risk to consumers, as well as the importance of effective control measures to prevent its spread within cattle populations. It is worth noting that BLV-infected cells in milk and colostrum serve as a primary transmission route for this retrovirus to new hosts (25). There are some emerging evidences that BLV causes breast cancer in humans (26). For example, a study revealed that one of the risk factors associated with breast cancer in women involves the presence of BLV, which can accumulate in breast tissue due to consumption of contaminated food from livestock (27). This signifies of early identification and segregation of infected livestock to mitigate the spread of BLV.

Our study has some limitations. We only included English language articles, potentially excluding relevant studies in other languages and introducing language bias. Moreover, the limited number of available studies constrained our ability to conduct more extensive subgroup analyses or identify the precise sources of the significant observed heterogeneity. Additionally, there is the possibility of selection bias in the sampling methods used by the included studies, which could impact the representativeness of our findings for the broader population of dairy cows. These limitations emphasize the need for caution when interpreting our results and highlight the importance of future research with larger sample sizes, diverse geographic representation, and rigorous methodologies to provide a more comprehensive understanding of BLV in milk samples.

CONCLUSION

Our study revealed a considerable prevalence of BLV in milk samples, highlighting potential risks to consumers and the livestock industry. This necessitates further research with broader sample sizes to better understand BLV prevalence and its health implications.

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