Meta-Analysis
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A Meta-Analysis of Whole Blood Cholinesterase Activity in Healthy Ruminants Using a Modified Electrometric Measurement Method

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Abstract

Objective. This meta-analysis aimed to compile and analyze WBChE activity using the modified electrometric method to establish normal or baseline enzyme activity values in ruminants (sheep, goats, cattle, and buffalo). **Materials and Methods.** This is a one-group randomized-effect-size meta-analysis model applied to the mean ± SD of WBChE activities in sheep, goats, cattle, and buffaloes. **Results.** Using PRISMA selection, we identified 16 records from five studies that measured WBChE activity in sheep (N=267), goats (N=165), cattle (N=197), and buffaloes (N=31) using a modified electrometric method. The studies included both sexes and were published between 2007 and 2025. The forest plot showed that the WBChE activities of sheep, goats, cattle, and buffaloes were 0.40, 0.32, 0.51, and 0.42 Δ pH/enzymatic reaction incubation time, respectively. Their 95% CI were 0.19-0.60, 0.16-0.49, 0.31-0.70, and 0.39-45, respectively. The weights of the individual records across the four species varied from 0.91% to 14.77%. The I^2 heterogeneity index of 61% was moderate and significant (Q=38.35; P=0.001). Despite the low pseudo-R^2 value (24.24%), the I^2 of subgroup analysis was significant only in sheep (64.2%, Q=14, P=0.016). The funnel plot showed the possibility of publication bias in five imputed studies. However, Egger's regression analysis was not statistically significant (t=1.91, P=0.077). The overall risk of bias in the studies was low. **Conclusion.** This study is the first meta-analysis to establish the reference WBChE activity in healthy ruminants, including sheep, goats, cattle, and buffaloes. This is an additional contribution to the existing literature, as the modified electrometric method is recommended for measuring blood or tissue ChE activity in various animal species. These findings provide valuable reference points for the clinical and toxicological assessment of poisoning by cholinesterase-inhibiting pesticides in these species.

Key Words: Blood Cholinesterase ▪ Insecticide Exposure ▪ Sentinel Species ▪ Organophosphate ▪ Carbamate.

Introduction

Organophosphate (OP) and carbamate (CA) insecticides are used to control ectoparasites in ruminants (1-3). The toxicity of these insecticides in animals is caused by the inhibition of cholinesterase (ChE) activity in the nervous system, either irreversibly (OP) or reversibly (CA) (4, 5). This enzyme-inhibitory action of insecticides leads to the buildup of acetylcholine at synapses and neuromuscular junctions, inducing a toxidrome of cholinergic poisoning, which is characterized by nicotinic, muscarinic, and central nervous system manifestations (4-7). The measurement of blood ChE activity is the key diagnostic assessment of poisoning by OP and CA insecticides (6, 7). Blood ChE activity comprises butyrylcholinesterase (E.C. 3.1.1.8) found in the plasma, serum, and liver, and acetylcholinesterase (E.C. 3.1.1.7) in erythrocytes, and the whole blood ChE (WBChE) activity is the net of these two enzyme entities (6-8).

Ruminants are characterized by low plasma ChE activity compared to erythrocytes (9-13).

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Therefore, measuring WBChE activity in ruminants under field conditions would be beneficial, as it would skip the stage of separating blood constituents by centrifugation, allowing for rapid monitoring of the enzyme activity in cases of insecticide poisoning (13-15). Several studies have advocated the measurement of WBChE activity to assess exposure to OP and CA insecticides in humans and animals (14-19). This would be especially beneficial in ruminants, as whole blood contains the majority of acetylcholinesterase in erythrocytes (>90%) compared to butyrylcholinesterase (8, 9, 11, 13, 20). Studies have also identified ruminants, particularly sheep, as sentinel species for monitoring exposure to ChE-inhibiting insecticides, as these animals are raised close to human agricultural settings where pesticides are commonly used (21-23). Measuring WBChE activity would reflect the functional status of ChE activity in the nervous system following exposure to OP and CA insecticides (13-15, 18).

Various ChE assay methods are available to measure the enzyme activity in biological fluids (9, 20-28). One of the simplest and most reliable ChE methods is the electrometric method of Michel (26, 28), which was modified specifically for ruminants to accommodate the inherently low blood ChE activities (9, 13, 24, 25, 29). Recent studies have used the modified electrometric method to determine WBChE activity (19, 20, 23). WBChE activity reference values are required to assess animal exposure to OP and CA insecticides (6, 7, 9, 12, 13, 25), similar to the reference values used for humans (30).

The aim of the present study was to conduct, for the first time, a meta-analysis of the existing literature that used the modified electrometric method to determine baseline (e.g., 9, 12, 13, 20, 24, 25) WBChE activity in ruminants (sheep, goats, cattle, and buffaloes). This sets the basis for comparing WBChE activity in animals exposed to OP or CA insecticides.

Materials and Methods

Study Approval

Study approvals were provided by the Directorate of Postgraduate Studies and Scholarships at the University of Duhok, Kurdistan Region, Iraq (Number 9327, dated September 10, 2024).

Literature Search

Using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (31), different databases (PubMed, Google Scholar, Science Direct, Directory of Open Access Journals, Iraqi Academic Scientific Journals, and Iraqi Digital Repository) were searched for the use of the modified electrometric method (9, 13, 20, 23-25, 29, 32, 33) in measuring WBChE activity in ruminants until December 30, 2025. A manual search was also conducted to identify published academic articles and theses in the above-mentioned databases. The main keywords used to retrieve articles were: "whole blood cholinesterase activity + electrometric method + ruminants or sheep, goats, cattle, buffaloes, camels", "whole blood cholinesterase activity + modified electrometric method + ruminants, or sheep, goat, cattle, buffalo, camel", "cholinesterase + modified electrometric method + ruminants", "blood cholinesterase + modified electrometric method + ruminants", "modified electrometric method for blood cholinesterase measurement in ruminants", or "normal whole blood cholinesterase activity in ruminants + modified electrometric method" with continuous refinements of the search as needed. The search also included the citations of articles that qualified for inclusion in the meta-analysis. This study did not impose any language restrictions. Both authors searched the data, and any discrepancies were resolved by consensus with the first author (FKM).

Meta-Analysis Duration and Location

The study was initiated on August 1, 2024, and continued until the conclusion of the analysis on December 30, 2025, at the Department of Pharmacology, College of Pharmacy, University of Duhok, Kurdistan Region, Iraq.

Inclusion Criteria

The published studies or academic theses included in this study comprised records of WBChE activity, as measured by the modified electrometric method, in apparently healthy ruminants. The PRISMA advocated identification, screening, and inclusion criteria were employed to finalize the study selection (Figure 1). Data were extracted from each study to include animal species, sex, number of animals, mean, and standard deviation (SD) of normal or baseline WBChE activity of ruminants not exposed to pesticides (including OP and CA) (9, 13, 20, 23, 24, 29, 32, 33). The unit of enzyme activity was Δ pH per incubation time, which usually ranged between 20 and 40 minutes at 37 °C. The typical steps of the modified electrometric method for measuring WBChE activity in ruminants are shown in Table 1.

Table 1. Steps for the Determination of Whole Blood Cholinesterase (WBChE) Activity in Ruminants*

Assay mixture	Animal blood	Blank
Distilled water	3 ml	3.2 ml
Whole blood	0.2 ml	None
Buffer, pH 8.1: 1.237 g sodium barbital, 0.163 g potassium dihydrogen phosphate, and 35.07 g sodium chloride/L distilled water	3 ml	3 ml
Measure pH1 with a pH meter	pH1	pH1
Acetylcholine iodide 7.1%	0.1 ml	0.1 ml
Incubate in a water bath at 37 °C	30 min	30 min
Measure pH2	pH2	pH2
WBChE activity: Δ pH/incubation time=(pH1–pH2)–Δ pH of blank		

*References: 9, 13.

The initial search identified 100 studies; after applying PRISMA screening, the pool was refined to 16 records drawn from five published studies. The mean WBChE activity values (± SD) for sheep, goats, cattle, and buffaloes were extracted from carefully selected studies (13, 20, 23, 32, 33) and included in the meta-analysis in accordance with PRISMA (Figure 1).

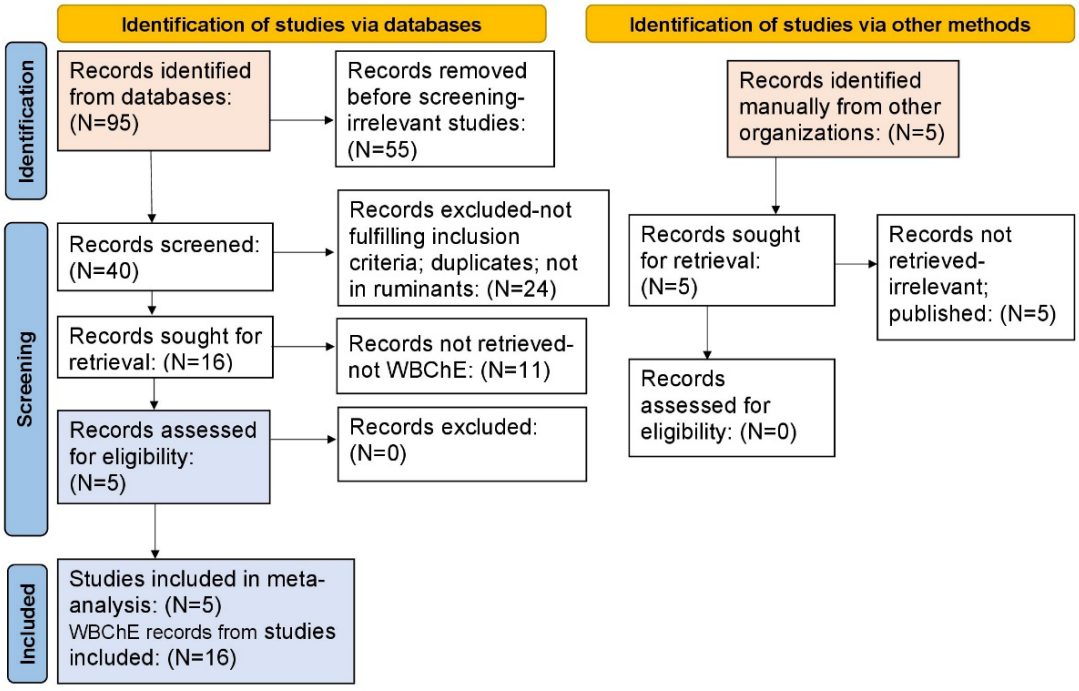

Figure 1. Studies on whole blood cholinesterase (WBChE) activity in ruminants were selected for the meta-analysis following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.

Exclusion Criteria

Studies that did not report normal or baseline WBChE activity in ruminants or those that used other electrometric methods were excluded. Studies that did not report the SD or standard error (SE) of WBChE activity were also excluded. Academic theses or dissertations were excluded because of duplication, as their findings were subsequently published in peer-reviewed journals.

Meta-Analysis and Statistics

This is a single-group randomized-effect-size meta-analysis applied to the mean WBChE activity (±SD) in sheep, goats, cattle, and buffaloes, and was conducted using Meta-Essentials, Version 1.5, from the Erasmus Research Institute of Management (ERIM) in Rotterdam, Netherlands (34). The analyses included a forest plot showing the WBChE effect size, 95% confidence intervals (CI), each study's weight (percentage) in the overall estimate, and the Z-test ($P<0.05$), subgroup analysis, and moderator effect (34-37). Heterogeneity was assessed using Cochran's Q-test ($P<0.10$) and Higgins' I^2, categorized as low (25%), moderate (50%), and high (75%) heterogeneity (35-38). Additionally, a pseudo-R^2 was estimated in the subgroup analysis to quantify the extent to which heterogeneity across studies is explained by subgroup/moderator factors (34, 38). A small R^2 value (e. g., 0.25) explains only a modest portion of the between-study heterogeneity.

Publication Bias

Publication bias was analyzed using a funnel plot, which examines the effect size against the SE, and by Egger's regression test at $P<0.05$ (34, 39, 40). Additionally, Galbraith regression and quantile plots were used to assess data homogeneity and outliers (34, 39, 40).

Risk of Bias

The method of scoring potential bias (risk assessment) in animal studies was applied to the present data by allocating ten questions within six bias items (selection, performance, detection, attrition, reporting, and other problems) (41, 42). Scores of 2, 1, and 0 indicated low, unclear (moderate), and high risk of bias, respectively. Unclear or moderate bias was indicated when a score of one was given to one or more of the sub-questions or when there was "no information" (41, 42). The online Risk of Bias Visualization (robvis) tool was used to assess the scores (43).

Results

Extraction of WBChE Activities of Ruminants from Studies

The literature search identified 100 studies, as shown in the PRISMA flowchart (Figure 1). After applying the inclusion and exclusion criteria, this number was finalized to 16 records of five studies showing WBChE activities as measured by the modified electrometric method in sheep (N=267), goats (N=165), cattle (N=197), and buffaloes (N=31) of both sexes, totaling 660 animals (Table 2). These studies were published between 2007 and 2025.

Meta-Analysis

The forest plots of the single-group random-effects model analysis showed that the WBChE activities of sheep, goats, cattle, and buffaloes (effect sizes) were 0.40, 0.32, 0.51, and 0.42 Δ pH/enzyme reaction incubation time, respectively (Figures 2, 3). Their 95% CI were 0.19-0.60, 0.16-0.49, 0.31-0.70, and 0.39-45, respectively. The weights of the individual records across the four species varied from 0.91% to 14.77% (Figure 2).

Analysis using the Galbraith regression (Z-score against inverse SE) indicated that the WBChE data points were within the lower and upper boundaries of the regression line, with no points below the zero (no effect) line (Figure 4). Furthermore, no outliers were observed in the normal quantile plot (Figure 5).

Table 2. Whole Blood Cholinesterase (WBChE) Activity in Ruminants (Δ pH/ Incubation Time), Measured Using a Modified Electrometric Method

Code	Authors/year	Species	Sex	N	Mean WBChE activity (Δ pH)±SD
A	Mohammad et al., 2007 (13)	Sheep	M	100	0.249±0.105
B		Sheep	F	98	0.257±0.069
C		Goat	M	59	0.234±0.058
D		Goat	F	53	0.252±0.071
E		Cattle	M	103	0.374±0.142
F		Cattle	F	43	0.450±0.075
G	Esmaial et al., 2010 (32)	Sheep	F	10	0,36±0.034
H	Alias, 2018 (33)	Buffalo	F	31	0.42±0.017
I	Ramadhan and Mohammad, 2025 (20)	Sheep	M	33	0.436±0.205
J		Sheep	F	15	0.943±0.260
K		Goat	M	31	0.409±0.202
L		Goat	F	11	0.498±0.282
M		Cattle	M	40	0.693±0.328
N	Ramadhan and Mohammad, 2025 (23)	Sheep	M	11	0.605±0.105
O		Goat	M	11	0.519±0.107
P		Cattle	M	11	0.640±0.099

M=Male; F=Female; WBChE activity: Δ pH/incubation time=(pH1−pH2)−Δ pH of blank.

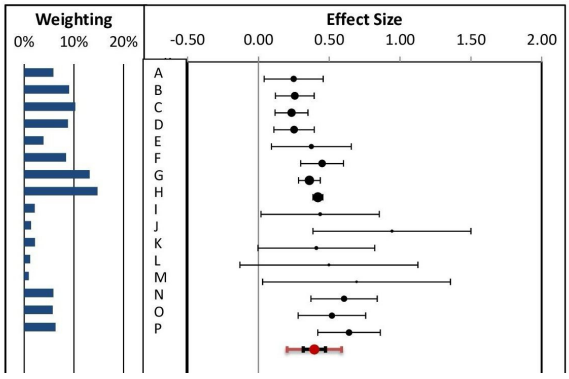

Figure 2. Forest plot of whole blood cholinesterase activity and its weighting in ruminants. The overall effect size was enzyme activity (0.40 Δ pH/incubation time, SE=0.04, 95% CI: 0.32, 0.47; Z value=10.72, P<0.0001).

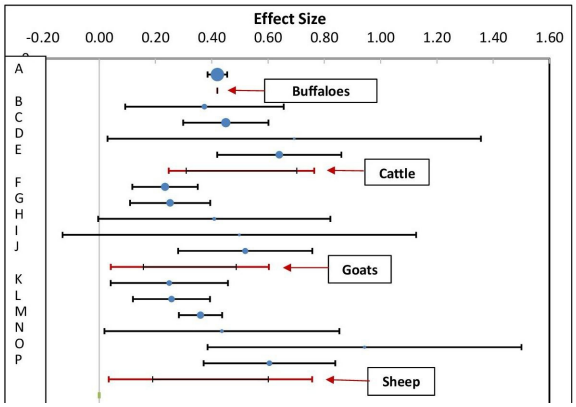

Figure 3. Forest plot of subgroup analysis of whole blood cholinesterase activity (Δ pH/incubation time) in ruminants.

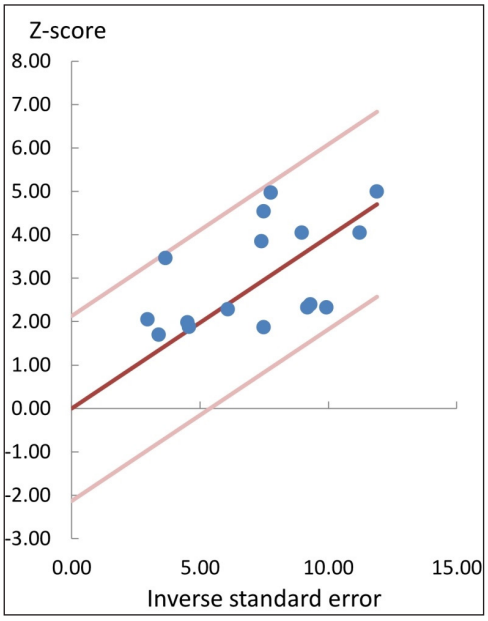

Figure 4. Galbraith regression distribution of whole blood cholinesterase activity in ruminants (Z-score vs. inverse standard error). The zero line represents the no-effect line.

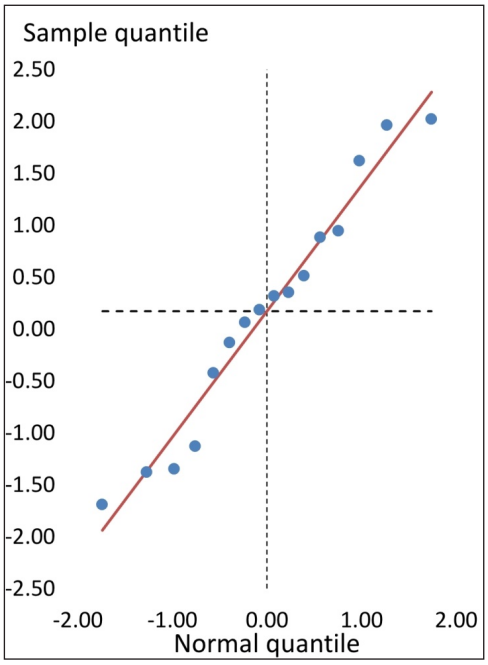

Figure 5. Normal quantile plot of whole blood cholinesterase activity in ruminants.

Data Heterogeneity

The I^2 heterogeneity index of 61% was moderate, with low T^2 (0.01) and T (0.08) values. However, the test of heterogeneity was significant (Q=38.35; P=0.001), indicating heterogeneity in the data, which were therefore subjected to a subgroup analysis.

Subgroup Analysis

Despite the low pseudo R^2 value (24.24%), subgroup analysis showed that WBChE activity (Δ pH) was highest in cattle (0.51), followed by buffaloes (0.42), sheep (0.40), and goats (0.32) (Figure 3). Within subgroups, the heterogeneity values (I^2) in sheep, goats, and cattle were 64.2% (Q=14, P=0.016), 40.2% (Q=6.69, P=0.153), and 16.1% (Q=3.6, P=0.311), respectively. The WBChE activity of buffaloes was not generated by the software because of the single record of WBChE activity in this species. The significant heterogeneity was attributed to the sheep WBChE values, as shown above. The regression analysis of the moderator (sex) on effect size was not significant (F value=0.47, P=0.502, T^2=0.01).

Funnel Plot Examining Publication Bias

The funnel plot (SE versus effect size) showed the possibility of publication bias, since effect size points were not symmetrical within the designated area of the plot, and the five imputed studies were added to the plot to adjust for symmetrical equilibrium (Figure 6). However, Egger's regression analysis was not statistically significant (t=1.91, P=0.077).

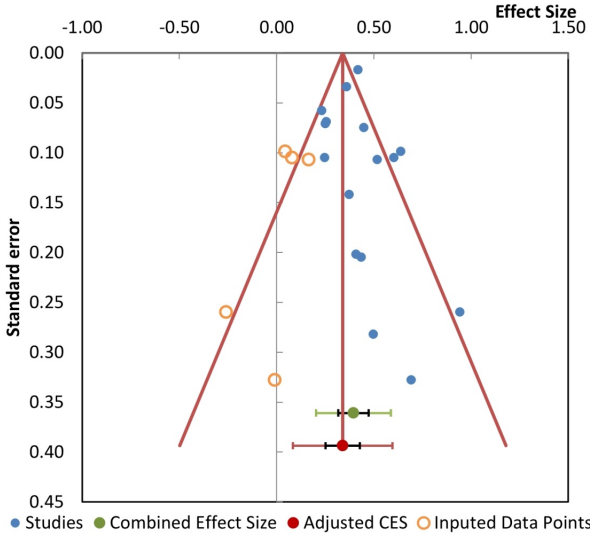

Figure 6. Funnel plot assessing publication bias in studies of whole blood cholinesterase activity in ruminants. Five imputed data points were included to achieve symmetrical distribution.

Risk of Bias

The risk of bias was low in three of the five studies in the present meta-analysis, with total scores of 20/20 (Figure 7). Two studies had a moderate risk of bias, with total scores of 16/20 (allocation of concealed selection and attrition of addressing incomplete data). However, the total score of the studies (18.4 out of 20) indicated a tendency toward a low risk of bias.

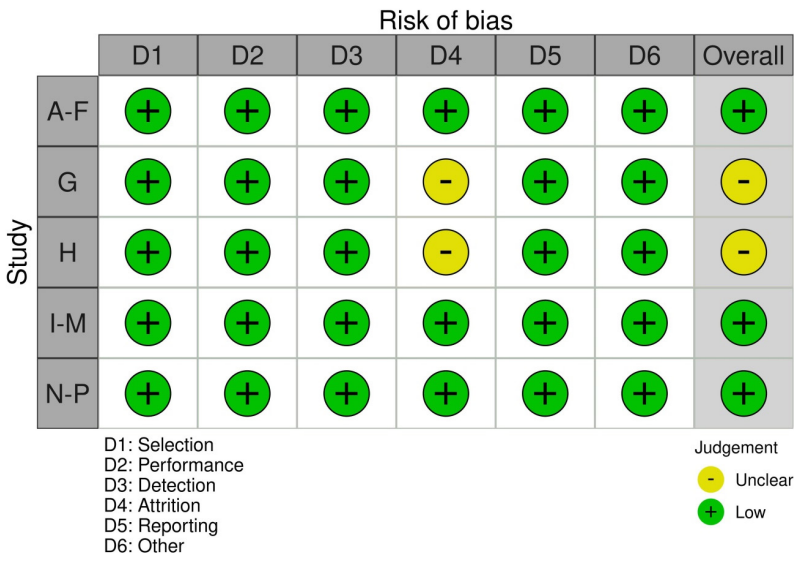

Figure 7. Risk of bias distribution across studies included in the meta-analysis of whole blood cholinesterase activity in ruminants.

Discussion

This study is the first meta-analysis to establish the reference WBChE activity in healthy ruminants, including sheep, goats, cattle, and buffaloes. It is an additional contribution to the existing literature, since the current modified electrometric method is recommended for measuring blood or tissue ChE activity in various animal species (9, 12, 13, 20, 23-25) as well as humans (16, 23, 27, 30) to evaluate exposure to OP and CA pesticides. This method has gained popularity for its simplicity, high throughput, and cost-effectiveness (9, 19, 25, 30). The use of WBChE is preferred in ruminants because of their inherently low plasma ChE activity, which is approximately 10% of the WBChE (8, 9, 11, 13, 20). The use of whole blood for measuring ChE activity is preferred under field conditions and requires minimal laboratory equipment (a pH meter and water bath) (9). Furthermore, WBChE activity suitably mirrors the functional status of ChE activity in the nervous system when assessing exposure to OP and CA pesticides (13-15, 18). Studies have also identified ruminants, particularly sheep, as sentinel species for monitoring exposure to ChE-inhibiting pesticides, as these animals are raised in human agricultural settings where pesticides are commonly used (21-23). Ruminants can be exposed to OP or CA pesticides by grazing on contaminated forage, drinking contaminated water, or inhaling airborne pesticide particles after nearby applications (1, 5, 10, 12, 21, 22).

The ruminant WBChE activities presented in the current meta-analysis could be considered as pilot and initial references, especially in the absence of pre-pesticide exposure values, for normal enzyme activity in sheep, goats, cattle, and buffaloes. Nevertheless, several factors, such as age, animal species, disease conditions, concurrent exposure to other pesticides, and nutritional status, affect blood ChE activity and need to be carefully considered when interpreting pesticide exposure and its adverse effects (7, 26, 28). The pooled WBChE activity values calculated in the meta-analysis differed between ruminant species, with cattle displaying the highest mean effect size (0.51 Δ pH/enzyme reaction time), followed by buffaloes (0.42), sheep (0.40), and goats (0.32). These differences suggest species-specific reference ChE activity levels, that may reflect genetic or physiological variations inherent to each species (8, 9, 12, 13, 20-23). In the present study, the heterogeneity was mainly attributed to the WBChE activity of sheep (I^2=64.2%, Q=14, P=0.016), as the sex effect was not significant using the regression of the moderator on effect size. The ranking of WBChE activity among ruminants is in accordance with previous species-specific reports of WBChE activity in ruminants using electrometric methods (9, 12, 13, 20) or other methods of measuring enzyme activity (8, 10, 11, 21, 22). Understanding these reference values of WBChE activity using the modified electrometric method is crucial for clinical and toxicological assessments of exposure to ChE-inhibiting pesticides, where the enzyme activity deviates from normal states (9, 12, 23-25).

The relatively narrow 95% CI of WBChE activity for each species indicates the precision of the estimates. However, the expected overlap among ruminants (Figures 2, 3) requires cautious differentiation and interpretation of borderline WBChE values.

The low pseudo-R^2 value (24.24%) of WBChE activity across the species was accompanied by a moderate level of heterogeneity (I^2=61%), with no sex effects. The effect size in buffaloes was limited by a single record of WBChE activity, necessitating additional studies in this species using the present modified electrometric method. The subgroup analysis results confirmed the previously reported species variations in blood ChE activities among ruminants. This variation may lead to different interpretations of pesticide exposure, given the expected differences in how ruminant species respond to ChE-inhibiting pesticides (12, 13, 21-25). However, future studies should aim to standardize the modified electrometric method for measuring reference WBChE activity across species and control for animal-related pathophysiological variables. We recommend measuring WBChE activity in both ruminants and humans working in agricultural settings exposed to OP and CA pesticides.

The funnel plot indicated potential publication bias, as asymmetry was observed; however, Egger's regression test was not statistically significant (P=0.077). This borderline result suggests a cautious interpretation of the results; the addition of imputed data points to achieve symmetry hints that unpublished or missing data might impact the overall findings.

Risk of bias assessment revealed that the studies in the present study had a low-risk profile; two studies showed moderate risk, mainly arising from allocation concealment and incomplete data handling issues (Figure 7). However, the high combined quality score (18.4/20) supports the reliability of the studies included in this meta-analysis; however, future research should address these moderate-risk areas to reduce bias further.

Conclusion

This meta-analysis presents reference WBChE activities across four ruminant species using a reliable modified electrometric method, revealing clear species differences while inferring significant inter-study heterogeneity. These findings provide valuable reference points for the clinical and toxicological assessment of OP or CA poisoning in these species. Addressing any variability in WBChE across and within the species will improve the diagnostic utility of WBChE activity measurements in clinical veterinary medicine and toxicology.

What Is Already Known on This Topic:
The measurement of whole blood cholinesterase activity is used to evaluate exposure to organophosphate and carbamate pesticides in humans and animals. Recent studies have employed a modified electrometric method to measure WBChE activity in humans and animals because of its simplicity and reliability. Similar to humans, reference values for WBChE activity are required to assess animal exposure to organophosphate and carbamate insecticides.

What This Study Adds:
This meta-analysis established baseline whole blood cholinesterase activities using a reliable modified electrometric method for ruminant species (sheep, goats, cattle, and buffaloes). It provides a collective reference for whole blood cholinesterase values essential for diagnosing cholinergic toxidrome caused by organophosphates and carbamates in ruminants.

Authors' Contributions: Conception and design: FKM; Data collection: FKM and MAR; Acquisition, analysis and interpretation of data: FKM and MAR; Drafting the article: FKM; Revising it critically for important intellectual content: FKM and MAR; Approved final version of the manuscript: FKM and MAR.

Conflict of Interest: The authors declare that they have no conflict of interest.

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