Urmia University

Food Safety and Packaging

2026, 2 (2): 99-115

Journal Homepage: fsp.urmia.ac.ir

FOOD Safety and Packaging

Review Article

Prevalence of *Clostridium perfringens* in livestock, foodstuffs, and related samples in Iran: A systematic review and meta-analysis

Ghader Jalilzadeh-Amin[1], Somayeh Kouhestani[2], Mahyar Hanafi[1], Hamidreza Ebrahimi Gharaghozlou[3], Hadi Keshipour[3]*

[1]Department of Internal Medicine and Clinical Pathology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran; [2] PhD Graduate, Department of Animal Sciences and Marine Biology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran; [3]Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Abstract

Clostridium perfringens remains a persistent public health challenge, being a leading cause of foodborne intoxications and zoonotic infections. In this study, we systematically searched major academic databases for all eligible studies published up to June 10, 2026, with no restrictions on the publication date. Following the PRISMA guidelines, 23 eligible studies were selected. To stabilize variance (particularly for proportions approaching zero or one hundred), a random-effects model utilizing the Freeman–Tukey double arcsine transformation was employed. Subgroup analyses were conducted to investigate potential variations based on sample type, diagnostic methodology, and study origin. The pooled prevalence of *C. perfringens*, adjusted via the Freeman–Tukey transformation, was estimated at 18% (95% CI: 12% - 25%). Subgroup analysis indicated that the prevalence was significantly higher in intestinal and fecal samples compared to other sources ($p < 0.05$). Furthermore, studies employing molecular detection methods (e.g., PCR) reported consistently higher prevalence rates than those using traditional culture techniques. Substantial heterogeneity was observed across studies ($I2 > 90\%$), which was further corroborated by meta-regression analysis showing an inverse relationship between sample size and reported prevalence, suggesting that smaller studies may overestimate the true infection rate. These findings indicate that *C. perfringens* remains an important foodborne pathogen in Iran. The observed prevalence patterns highlight the need for strengthened surveillance and more standardized detection approaches to support more accurate risk assessment and food safety planning.

Keywords: *Clostridium perfringens*, Enterotoxemia, Food poisoning, Foodborne pathogens, Livestock, Necrotic enteritis.

Introduction

Clostridium perfringens (*C. perfringens*) is an important zoonotic pathogen, with major implications for humans (Yan et al., 2025), animals (Fohler et al., 2016; Keerqin et al., 2022), and food safety due to its ability to produce various toxins and cause various diseases (Jang et al., 2020). This Gram-positive, anaerobic, spore-forming bacillus (McClane et al., 2012) is ubiquitous in various environments, including soil, water, and the digestive tract of

* **Correspondence:** H. Keshipour (h.keshipour@urmia.ac.ir).
https://doi.org/10.30466/fsp.2026.57335.1033
Received: 03 July 2026 **Accepted:** 12 August 2026 **Available online:** 15 August 2026

humans and animals (Khiav et al., 2025; Takanosu et al., 2024). An important feature of *C. perfringens* is the high resistance of its spores to adverse environmental conditions, as well as the ability to survive in the food chain and contaminated environments (Chowdhury et al., 2026; Li et al., 2016). For this reason, this microorganism can easily play a role in the transmission cycle from the environment to materials, from livestock to food, and finally to humans (Firmesse et al., 2025).

Clinically, *C. perfringens* is associated with a wide range of diseases, including food poisoning (Holtby et al., 2008), enterotoxemia (Giannitti et al., 2023), necrotic enteritis (Katalani et al., 2024), gas gangrene (Ghezel & Arfaee, 2025), and even some severe cases of septicemia (Fujikawa & Araki, 2020). Disease severity and clinical manifestations are largely influenced by toxin production. *C. perfringens* strains may produce alpha, beta, epsilon, iota, and enterotoxin, and toxinotyping has been used to classify strains with distinct pathogenic potential in humans and animals (Afshari et al., 2015; Ahsani et al., 2011; Alimolaei, 2023; Shahdadnejad et al., 2016).

From a public health perspective, the consumption of food contaminated with *C. perfringens* is a serious concern. This bacterium can proliferate rapidly in cooked foods that are improperly stored, particularly in large quantities, thereby causing food poisoning (Seman & Milkowski, 2026). Meat, meat products, ready-to-eat foods, and milk and some dairy products are among the sources that have been introduced in various studies as reservoirs or vehicles for transmission of this bacterium (Ahmadi et al., 2021; Doosti et al., 2017; Hassani, Pakbin, et al., 2022; Hosseinzadeh et al., 2018; Jang et al., 2020). On the other hand, the presence of *C. perfringens* in livestock and poultry is also important, because these animals can act as both a reservoir of the bacteria and a source of contaminated food (Ahsani et al., 2011; Alimolaei, 2023; Gharibi et al., 2017; Hayati et al., 2020; Poursoltani et al., 2013). Therefore, investigating the distribution of this microorganism in different sources is essential for control and health promotion planning.

Various studies have shown that factors such as Iran's large geographical area and climatic diversity, different livestock farming patterns, food storage methods, and variations in health infrastructure have contributed to the endemic occurrence of this infection in Iran (Azimi et al., 2024; Azimirad et al., 2021; Ghourchian et al., 2021; Hashemi et al., 2025; Hayati et al., 2020; Katalani et al., 2024). Other factors may also explain the different results reported for this bacterium in Iran, including sample type, detection method, sample size, study period, climatic conditions, and even differences in laboratory protocols (Ahmadi et al., 2021; Ahsani et al., 2011; Alimolaei & Ezatkhah, 2022; Azimirad et al., 2021). Therefore, interpreting the results of scattered studies alone cannot provide an accurate picture of the true status of this bacterium in the country.

In recent years, antibiotic resistance and the emergence of resistant strains of pathogenic bacteria have increased significantly. Various reports have described antimicrobial resistance patterns and the presence of toxin-related genes in *C. perfringens*, which have further highlighted the importance of a comprehensive investigation of this bacterium (Ahsani et al., 2010; Hassani, Pakbin, et al., 2022; Hosseinzadeh et al., 2018; Katalani et al., 2024; Poursoltani et al., 2013). Therefore, studying the prevalence of this pathogen is not merely a simple laboratory investigation, but can also provide a basis for risk assessment, the design of preventive programs, and health-related decision-making at the national level.

Despite numerous studies conducted in Iran, a coherent and quantitative summary of the prevalence of *C. perfringens* from different sources across the country has not yet been provided; if such summaries have been reported, they have generally been limited to certain sample types or specific regions. The absence of a comprehensive and reliable estimate means that research and policy decisions are often made on the basis of scattered and sometimes contradictory data. Meta-analysis, as a powerful statistical approach, makes it possible to combine the findings of different studies and obtain a more accurate estimate of the true prevalence. In addition, through subgroup analysis and meta-regression, sources of heterogeneity among studies can be identified, and the roles of factors such as

sample type, study year, geographical region, and diagnostic method can be examined.

Therefore, the aim of this study was to estimate the overall prevalence of *C. perfringens* in studies conducted in Iran and to examine patterns of heterogeneity among those studies. In addition, this study aimed to identify factors affecting differences in study results through complementary analyses, such as subgroup analysis and meta-regression, to provide a more accurate picture of the status of this bacterium in the country.

Materials and Methods

Search strategy and data sources

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A comprehensive literature search was performed to identify all studies reporting the prevalence of *C. perfringens* in Iran. International electronic databases, including PubMed/MEDLINE, Scopus, Web of Science Core Collection, CAB Abstracts/CAB Direct, and Google Scholar, as well as Iranian databases, including Scientific Information Database (SID), Magiran, and IranDoc, were systematically searched. The search covered all records published up to June 10, 2026, without restriction on publication year. Searches were conducted in both English and Persian. No restriction was initially applied regarding sample type to include studies conducted on food products, animal samples, human samples, environmental samples, water, dairy products, poultry, meat products, and other related matrices.

The search strategy was developed using combinations of Medical Subject Headings (MeSH) terms, free-text keywords, synonyms, and Boolean operators ("AND", "OR"). The main search terms included: *Clostridium perfringens*, *C. perfringens*, *Clostridium welchii*, prevalence, occurrence, Frequency, isolation, Detection, contamination, Iran, Iranian. The full PubMed/MEDLINE search strategy was as follows: ((*Clostridium perfringens* [Title/Abstract] OR *C. perfringens* [Title/Abstract] OR *Clostridium welchii* [Title/Abstract]) AND (Iran [All Fields] OR Iranian [All Fields]) AND (prevalence [Title/Abstract] OR occurrence [Title/Abstract] OR frequency [Title/Abstract] OR isolation [Title/Abstract] OR detection [Title/Abstract] OR contamination [Title/Abstract])). Equivalent search strategies were used for the other databases. In addition, the reference lists of all eligible articles and relevant review papers were manually screened to identify further potentially eligible studies that were not captured during the electronic database search. Duplicate records were identified and removed before screening.

Data extraction

Study selection and data extraction were independently conducted by three authors. First, duplicate records were removed. The remaining studies were screened based on titles and abstracts according to the predefined inclusion and exclusion criteria. Potentially relevant articles were then evaluated through full-text review. Each reviewer independently performed the screening process and extracted the relevant data. After completion of these steps, the results obtained by the three reviewers were compared. Any discrepancies in study selection or extracted data were resolved through joint re-evaluation of the original articles to ensure that no eligible studies were missed and that all extracted information was accurate. Final decisions regarding study inclusion and data extraction were made by consensus among the three reviewers.

The process of study selection is summarized in the PRISMA flow diagram (**Fig. 1**). In total, 414 records were initially identified through database searching. After the removal of 88 duplicate records, 326 articles remained and were screened based on their titles and abstracts. During this stage, 158 records were excluded because they did not meet the inclusion criteria. The full texts of the remaining 168 articles were retrieved and assessed for eligibility. Following detailed evaluation, 145 studies were excluded due to reasons such as irrelevant study design, insufficient data, or lack of relevance to the research objectives. Ultimately, 23 studies fulfilled the eligibility criteria and were included in the final systematic review and quantitative meta-analysis.

Eligibility Criteria

To be included in this systematic review and meta-analysis, primary studies had to meet the following inclusion criteria: Study Design: Peer-reviewed cross-sectional or descriptive studies reporting the prevalence or isolation rates of *C. perfringens* in Iran. Target Population/Samples: Foodstuffs (including meat, dairy, vegetables, etc.) and livestock (including clinically healthy or diseased cattle, sheep, goats, and poultry, etc.). Diagnostic Methods: Studies employing standard microbiological culture/biochemical identification and/or molecular methods (e.g., polymerase chain reaction [PCR]) for *C. perfringens* detection. Sample Size: Studies with a minimum sample size of $n \geq 10$ to ensure statistical stability. Language: Articles published in English or Persian.

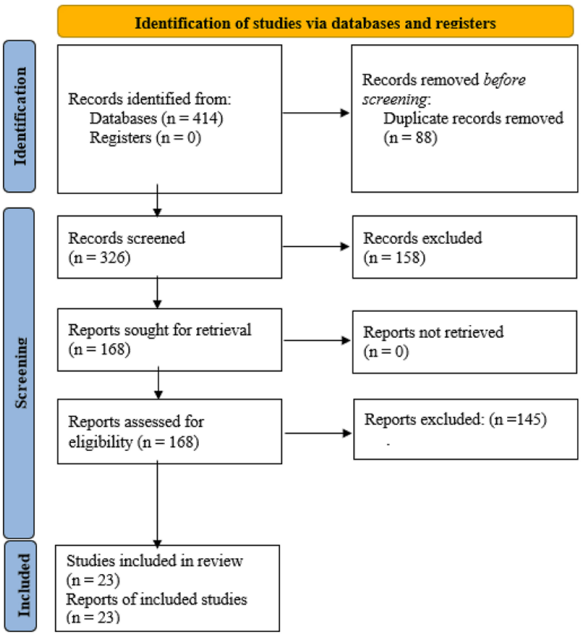

Figure 1. Flow diagram of the study selection process for the systematic review and meta-analysis of *C. perfringens* prevalence in Iran.

Studies were excluded if they met any of the following criteria: Publication Type: Review articles, systematic reviews, meta-analyses, conference abstracts, editorials, and case reports. Data Inadequacy: Studies lacking clear denominators (total sample size) or numerators (number of positive samples), and those in which data could not be extracted even after contacting the authors. Duplicate Publications: Studies presenting identical data from previously published articles.

Statistical analysis

All statistical analyses were performed using Stata version 19.5 (StataCorp, College Station, TX, USA). The pooled prevalence of *C. perfringens* was estimated using a random-effects model based on the Restricted Maximum Likelihood (REML) method, considering the expected heterogeneity across studies due to differences in geographic location, study period, and sample characteristics. To address the issue of variance stabilization and to include studies with 0% or 100% prevalence, the Freeman–Tukey double arcsine transformation was applied before pooling. Proportions were transformed using the Freeman–Tukey double-arcsine transformation for pooling and were subsequently back-transformed to the original prevalence scale for interpretation.

Statistical heterogeneity among studies was assessed using Cochran's Q test and quantified using the I^2 statistic, where I^2 values of approximately 25%, 50%, and 75% were considered to represent low, moderate, and high heterogeneity, respectively. To explore potential sources of heterogeneity, subgroup analyses and meta-regression were conducted based on selected study characteristics, including publication year and sample size. Potential publication bias was evaluated visually using funnel plots and statistically using Egger's regression and Begg's rank correlation tests, with results interpreted cautiously due to the nature of the prevalence meta-analyses. A leave-one-out sensitivity analysis was performed to assess the influence of individual studies on the pooled estimate by sequentially removing each study and recalculating the overall prevalence.

Results and Discussion

A total of 23 studies were included in the meta-analysis, comprising 4,821 examined samples, of which 772 were positive for *C. perfringens*. These studies were conducted in different regions of Iran, including Tehran, Mashhad, Kerman, Shiraz, Hamedan, Qazvin, Ahvaz, and Gorgan, and investigated a range of sample types such as food

products, water, intestinal contents, meat, offal, and vegetables (**Table 1**).

Table 1. Characteristics of studies included in the meta-analysis of *C. perfringens* prevalence in Iran.

	Authors	Region	Sample type	Sample size	Positive	Prevalence	Diagnostic method
1	Hashemi et al., 2025	Mashhad	Food (chicken kubideh, chicken barbecue, kubideh, lentilpilaf with minced meat, vegetable pilaf with fish, fried chicken pilaf, …)	360	2	0.56	Culture
2	Shahryari et al., 2024	Gorgan	Water	98	12	12.24	Culture
3	Azimi et al., 2024	Tehran	Intestinal (rat)	100	15	15	PCR
4	Alimolaei., 2023	Kerman	Intestinal (sheep)	192	55	28.65	PCR
5	Alimolaei et al., 2023	Kerman	Intestinal (quail)	120	37	30.83	PCR
6	Hassani, Mahmoudi, et al., 2022	Qazvin	Meat (cattle)	133	24	18.04	PCR
7	Alimolaei et al., 2022	Kerman	Intestinal (goat)	142	27	19.01	PCR
8	Hassani, Pakbin et al., 2022	Qazvin	Meat (cattle)	133	18	13.53	PCR
9	Azimirad et al., 2021	Tehran	Vegetable	366	66	18.03	PCR
10	Ghourchian et al., 2021	Tehran	Vegetable	140	13	9.29	PCR
11	Ahmadi et al., 2021	Hamedan	Food (sausage, bologna, hotdog, Kebab, and hamburger)	834	0	0	PCR
12	Hayati et al., 2020	Shiraz	Intestinal (cow, sheep, goat)	459	142	30.94	PCR
13	Fahimeh et al., 2018	Mashhad	Intestinal (sheep)	40	25	62.5	PCR
14	Hosseinzadeh et al., 2018	Shiraz	Carcass (cow, sheep)	200	61	30.5	PCR
15	Doosti et al., 2017	Shahrekord	Intestinal (chicken)	100	55	55	PCR
16	Gharibi et al., 2017	Ahvaz	Intestinal (sheep)	369	54	14.63	PCR
17	Shakerian et al., 2017	Kerman	Food (curd), Meat (sheep, cow)	143	16	11.19	PCR
18	Shahdadnejad et al., 2017	Kerman	Intestinal (chicken)	122	50	40.98	PCR
19	Afshari et al., 2015	Mashhad	Carcass (chicken)	200	31	15.5	PCR
20	Zandi et al., 2014	Kerman	Intestinal (ostrich)	120	11	9.17	PCR
21	Poursoltani et al., 2013	Mashhad	Carcass (chicken)	180	6	3.33	Culture
22	Ahsani et al., 2011	Kerman	Intestinal (sheep)	140	29	20.71	PCR
23	Ahsani et al., 2010	Kerman	Intestinal (sheep)	130	23	17.69	PCR
		Over all		**4821**	**772**		

In most of the included studies, PCR was used as the primary detection method, whereas only a few studies relied on culture-based methods. The reported prevalence of *C. perfringens* varied

substantially between studies, ranging from 0% in some investigations to more than 60% in studies such as those by Fahimeh et al. (2018) and Doosti et al. (2017). This wide variation in the reported prevalence suggests considerable heterogeneity among the studies included in the meta-analysis.

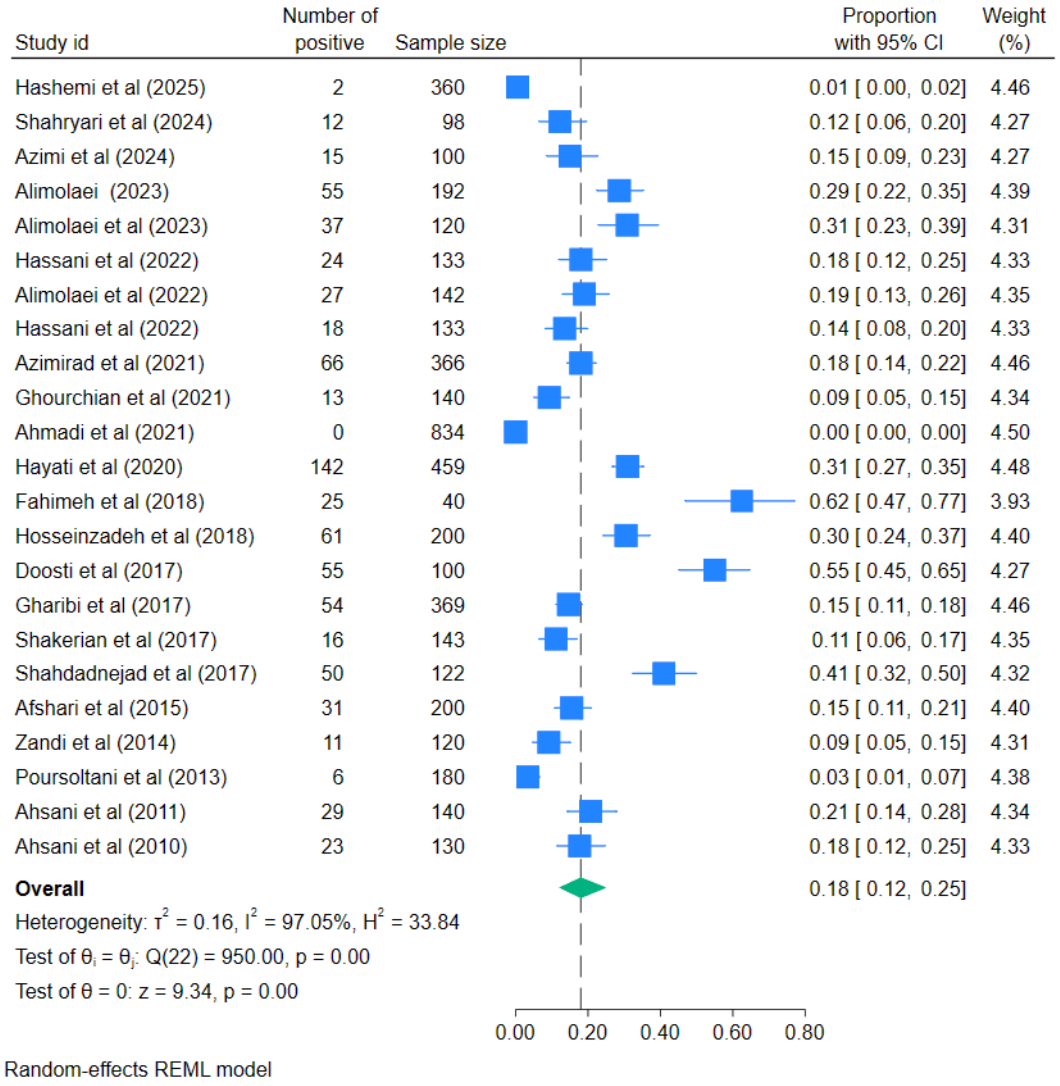

Figure 2. Forest plot showing the pooled prevalence estimates of *C. perfringens* based on 23 studies included in the meta-analysis. The analysis was conducted using the REML random-effects model with Freeman–Tukey transformation. The pooled prevalence was 18% (95% CI: 12%–25%). A very high level of heterogeneity was observed among the studies (I^2 = 97.05%).

In this meta-analysis, the pooled prevalence of *C. perfringens* was estimated to be 18% (95% CI: 12%–25%) using a REML-based random-effects model with Freeman–Tukey double-arcsine transformation (**Fig. 2**). Substantial heterogeneity was observed across studies (I^2 = 97.05%, τ^2 = 0.16, H^2 = 33.84; Q (22) = 950.00, p < 0.001), indicating considerable variability in prevalence estimates.

Publication bias was assessed using a funnel plot (**Fig. 3**). Visual inspection of the plot revealed an asymmetric distribution of studies, with several studies located outside the triangular region representing the pseudo 95% confidence interval. Formal statistical tests confirmed the visual asymmetry observed in the funnel plot. Egger's regression test (random-effects REML model)

indicated significant funnel-plot asymmetry (β_1 = –4.31, SE = 0.498, z = –8.64, p < 0.0001), providing strong evidence for potential small-study effects. Begg's rank-correlation test also showed significant asymmetry (Kendall's score = – 355.00, SE = 95.532, z = – 3.73, p = 0.0002). Collectively, these findings suggest that smaller studies reporting lower prevalence or null findings may be underrepresented in the published literature, and the pooled estimate may therefore overestimate the true prevalence to some degree.

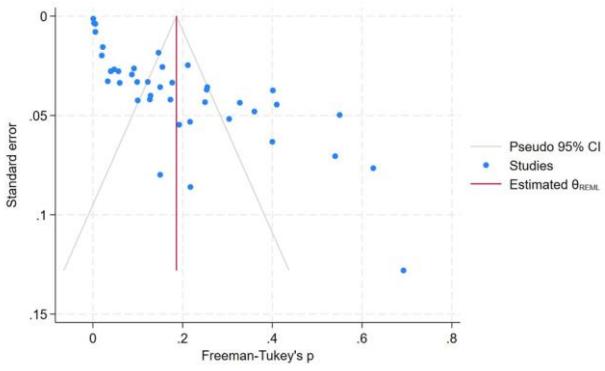

Figure 3. Funnel plot used to assess potential publication bias among the included studies. The horizontal axis represents the prevalence estimates (Freeman–Tukey transformation), and the vertical axis indicates the standard error. Each blue dot represents an individual study, and the vertical red line indicates the pooled estimate obtained using the REML model. The asymmetrical distribution of points may suggest the presence of publication bias or reflect a high level of heterogeneity among the included studies.

Subgroup analysis showed that host type, sample type, and detection method were the main sources of heterogeneity in the prevalence of *C. perfringens* (**Fig. 4**).

Host: The highest prevalence was observed in quail (30%) and sheep (28%), while the lowest prevalence was reported for food groups (2%). The difference between host subgroups was statistically significant (Q = 35.76, p < 0.001). Although a high prevalence was observed in the quail subgroup (**Fig. 4**), this estimate was based on a single study (Alimolaei et al., 2023). Therefore, this finding is statistically unstable and should be interpreted with caution as an exploratory observation rather than a definitive epidemiological estimate.

Sample type: The highest prevalence was observed in the intestinal samples (27%), whereas the lowest was observed in the "other" category (2%). This difference was also statistically significant (Q = 29.83, p < 0.001).

Method: Studies using PCR reported a higher prevalence than those using culture-based methods (19% vs. 4%), and this difference was statistically significant (Q = 17.76, p < 0.001).

Province: Unlike the other variables, the geographical distribution of studies across provinces (analyzed as East, West, North, South, and Center) did not reveal a statistically significant difference in *C. perfringens* prevalence (Q = 9, p= 0.11). While notable numerical variations were observed, with the highest point estimate appearing in the southern regions (23%), subgroup analysis was likely constrained by low statistical power. This limitation stems from the small number of eligible studies per province, which results in wide 95% confidence intervals and substantial overlap between regional estimates. Therefore, despite the numerical differences, we cannot confidently conclude significant regional heterogeneity in prevalence based on the current evidence.

The sensitivity analysis using the leave-one-out method showed that removing individual studies did not substantially affect the overall estimate of *C. perfringens* prevalence (**Fig. 5**). The pooled estimates remained within a narrow range of 15% to 17% after sequential removal of each study, and the confidence intervals showed only minimal variation. The highest estimate was observed after excluding the studies by Hashemi et al. (2025) and Ahmadi et al. (2021), while the lowest estimate was associated with the removal of the studies by Fahimeh et al. (2018), Doosti et al. (2017), one entry from Alimolaei (2023), and one entry from Ahsani et al. (2011). These findings indicate that no single study had a disproportionate influence on the overall meta-analysis results, suggesting that the findings are reasonably robust.

To investigate the effect of time on changes in the prevalence of *C. perfringens* in Iran, a random-effects meta-regression analysis (REML method) was performed using the publication year as the

moderator variable. According to the bubble plot (**Fig. 6**), the prediction line was nearly horizontal with only a slight, statistically non-significant positive slope (β= 0.0031, SE = 0.0175, 95% CI: -0.031 to 0.037, z =0.18, p = 0.858). This suggests that the prevalence of this bacterium has not changed markedly from 2010 to 2026, and the overall trend has remained relatively stable over time. Furthermore, the publication year failed to account for the observed heterogeneity among studies (R^2 = 0.00%, residual I^2 = 95.45%, residual Q = 989.71, df = 41 p < 0.0001). Most studies fall within the 95%confidence interval (represented by the shaded gray area), although the heterogeneity of studies appears to increase in more recent years, particularly after 2020 (**Table 2**).

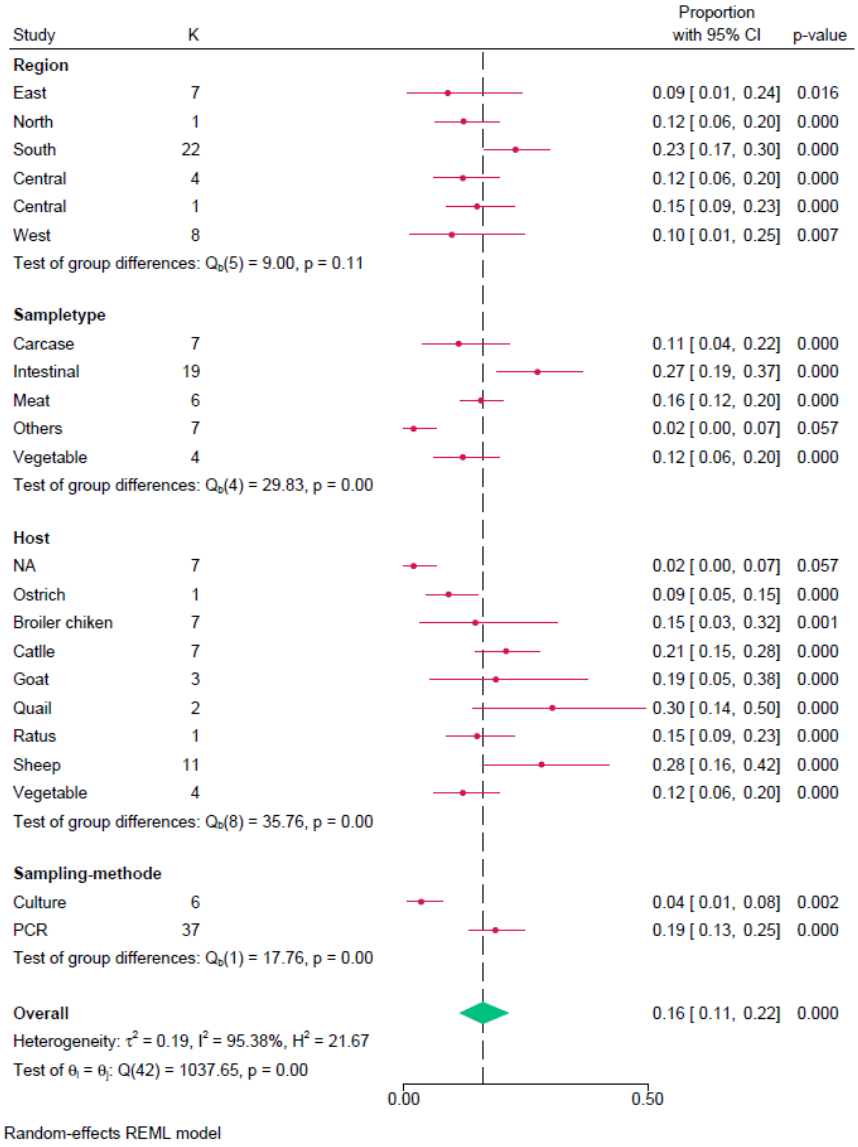

Figure 4. Forest plot of subgroup analyses based on host, region, sample type, and detection method. The plot presents the pooled prevalence of *C. perfringens* across these four variables. Analyses were performed using a random-effects model (REML) with the Freeman–Tukey double-arcsine transformation. Blue squares indicate the prevalence estimate for each subgroup, and diamonds at the end of each section represent the pooled estimate for that subgroup. Horizontal lines show the corresponding 95% confidence intervals. Overall heterogeneity among the included studies was high (I^2 = 95.38%). The test for subgroup differences was statistically significant for all variables except the geographic region.

To assess the potential influence of sample size on reported prevalence, a meta-regression analysis was conducted using sample size as the predictor variable (**Fig. 7**). The bubble plot reveals a

decreasing trend (negative slope) in prevalence estimates as sample size increases, suggesting that studies with smaller sample sizes tend to report higher prevalence values. Although this trend is visually apparent, the relatively wide 95% confidence interval (shaded gray area) indicates substantial heterogeneity among studies with smaller sample sizes.

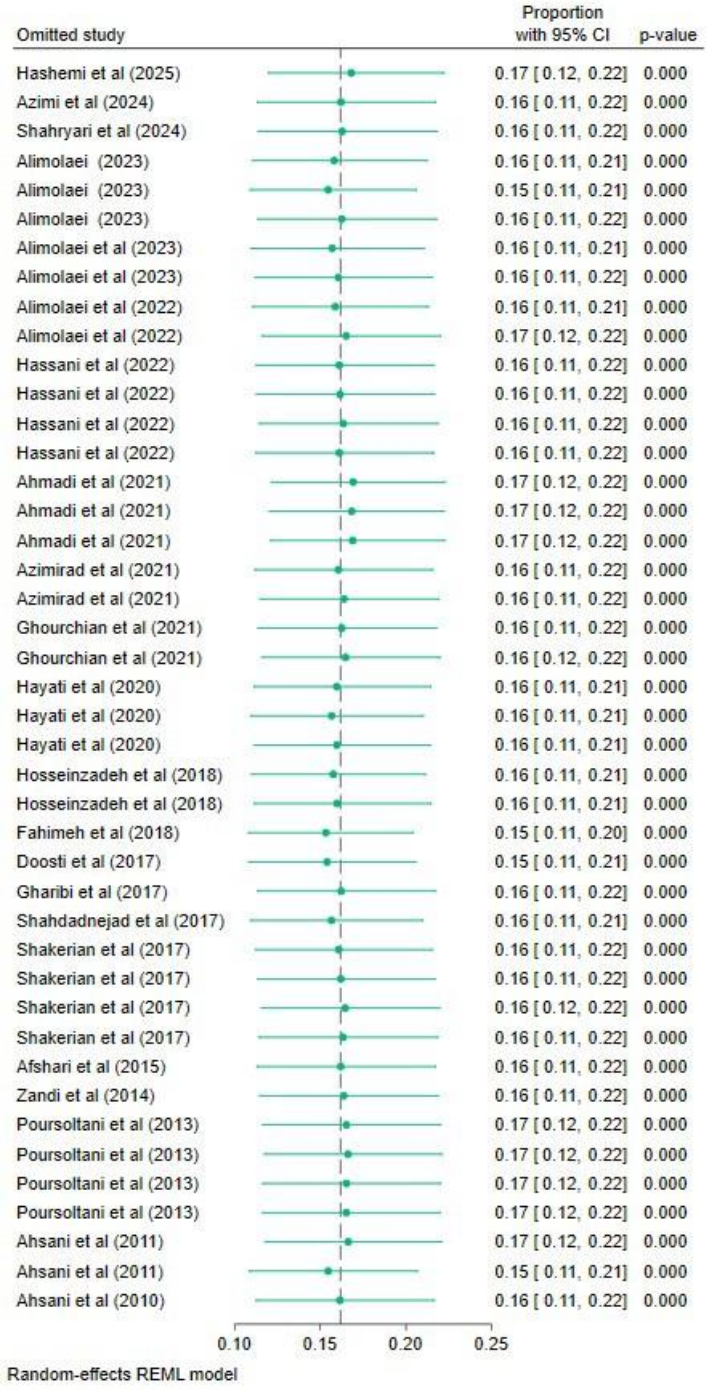

Figure 5. Sensitivity analysis using the leave-one-out method to evaluate the robustness of the pooled estimate of *C. perfringens* prevalence. In this analysis, one study was removed at a time, and the pooled estimate was recalculated using the REML random-effects model with the Freeman–Tukey transformation. The consistency of the estimates after removing different studies indicates that the overall meta-analysis result is robust and not disproportionately influenced by any single study.

The results of this meta-analysis indicate that the overall prevalence of *C. perfringens* in Iran is relatively high across various hosts and sample types. Our subgroup pooled estimates, back-transformed from the REML random-effects model using the Freeman–Tukey double-arcsine transformation, ranged between 11% and 20% across most categories. This prevalence is likely attributable to the bacterium's role as part of the normal intestinal flora of domestic animals and poultry (Alimolaei, 2023; Gharibi et al., 2017; Hayati et al., 2020), which aligns with previous findings in the region (Hakeem et al., 2024; Mohiuddin et al., 2020). Nevertheless, this remains a significant concern for animal health, food safety, and public health. Such contamination levels suggest that *C. perfringens* maintains a persistent and widespread presence throughout the food chain, particularly within the production, processing, and distribution environments of livestock products in Iran.

Table 2. Univariate random-effects meta-regression analyses of publication year and sample size as potential moderators of *C. perfringens* prevalence.

Covariate	Coefficient (β)	Standard error (SE)	Z-value	P- value	95% CI	R^2(%)	Residual heterogeneity ($I^2_{residual}$)
Year	0.0031	0.0175	0.18	0.858	-0.031 to 0.037	0.00%	95.45%
Sample size	-0.0013	0.0006	−2.13	0.033	-0.0026 to -0.0001	8.57%	94.69%

Note: Results are from separate univariate random-effects meta-regression models. Coefficients indicate the change in transformed prevalence per one-unit increase in the moderator.

The heterogeneity observed in this meta-analysis was substantial ($I^2 \approx 97\%$). This high level of variability indicates that the differences between studies exceed what can be explained by random error alone, necessitating an examination of multiple potential factors (such as host, sample type, diagnostic method, study design, and geographical or management conditions) to explain this variance. Our subgroup analyses and meta-regression successfully clarified the sources of heterogeneity, highlighting key methodological and biological moderators (Migliavaca et al., 2022; Mokhtari, 2024).

The subgroup analysis showed that the prevalence of *C. perfringens* was higher in certain hosts, particularly quail (14%–50%, $p = 0.00$) and sheep (16%–42%, $p = 0.00$), compared with other species. A high prevalence of this bacterium in sheep (Hakeem et al., 2024; Mohiuddin et al., 2020) and quail (Shivaprasad et al., 2008) has also been reported in other regions. Several factors may explain this pattern. Physiological and immunological differences between species (Sumners et al., 2012), variations in intestinal microbial composition (Jost et al., 2006; Shi et al., 2019; Zaytsoff et al., 2022), and differences in susceptibility to nutritional disorders (Goo et al., 2025; Guo et al., 2023; Huang et al., 2024) may all influence the occurrence and proliferation of *C. perfringens*.

In addition, sheep farming systems and feeding management practices in Iran are often associated with high flock density, inadequate ventilation, fluctuations in feed quality, and the use of commercial or traditional feeds with limited control of mycotoxins and secondary contamination. These conditions may disrupt intestinal microbial balance and create a favorable environment for the growth and proliferation of anaerobic bacteria, such as *C. perfringens* (Ahsani et al., 2011; Antonissen et al., 2014; Finnie & Uzal, 2024).

In addition to host species, sample type was strongly associated with the reported prevalence. Studies using intestinal and gastrointestinal content samples reported a higher prevalence (19% – 37%) than studies based on carcass surfaces, environmental feces, or finished products. This finding is consistent with the biological characteristics of *C. perfringens*, as the bacterium is a common inhabitant of the

gastrointestinal tract (Li et al., 2016) and is therefore more likely to be detected in intestinal tissues or contents (both in healthy animals and clinical cases) than during later stages of slaughter, processing, and storage (Craven et al., 2001). Conversely, the lower prevalence observed in carcasses and finished products may reflect the effects of slaughtering procedures, cold storage, washing, and hygiene interventions (Talukdar et al., 2017). However, this reduction does not necessarily indicate complete elimination of the risk, since resistant spores may remain viable and become reactivated during subsequent stages, such as transportation, storage, or food preparation.

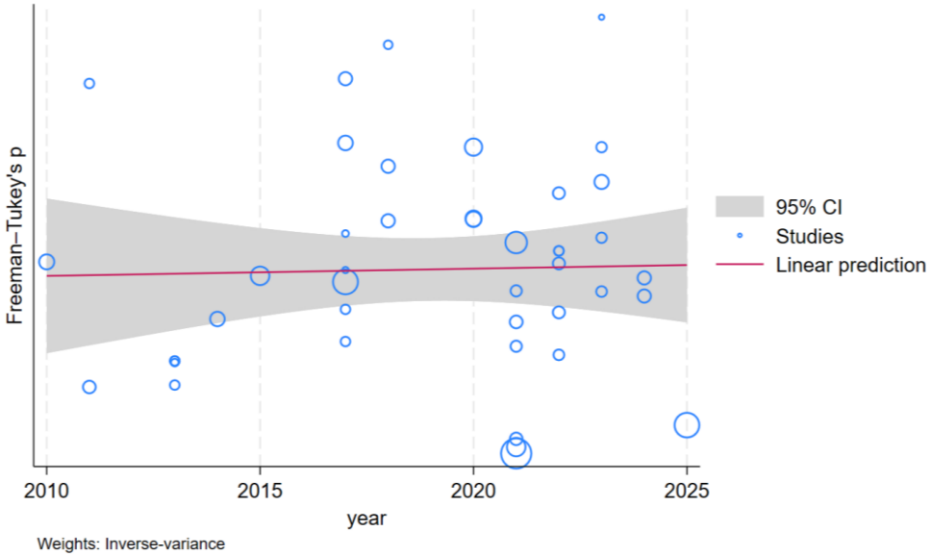

Figure 6. Meta-regression bubble plot showing the association between publication year and *C. perfringens* prevalence. The horizontal and vertical axes represent publication year and prevalence, respectively, with prevalence expressed on the Freeman–Tukey transformed scale. Bubble sizes are proportional to study weights (inverse-variance weighting). The red line indicates the fitted random-effects meta-regression model, and the shaded gray area denotes the 95% confidence interval. The nearly horizontal slope suggests that publication year was not significantly associated with changes in prevalence over time.

Among Iran's neighboring countries, comparable epidemiological evidence is limited. In Punjab, Pakistan, *C. perfringens* was detected in 184 of 399 samples collected from sheep and goats, corresponding to a prevalence of 46.1% (Mohiuddin et al., 2020). In a separate study from Pakistan, 33 of 160 cattle and buffaloes tested positive, corresponding to an overall prevalence of 20.6% (18.8% in cattle and 22.5% in buffaloes) (Khan et al., 2022). Thus, the pooled estimate for Iran was lower than that reported for Pakistani sheep and goats but was broadly comparable to the estimate reported for Pakistani cattle and buffaloes.

In Samsun Province, northern Turkey, *C. perfringens* was detected in 45.2% (100/221) intestinal-content samples from sheep and goats that had died suddenly or were suspected of enterotoxemia (Tutuncu et al., 2018). This estimate should be interpreted as detection in a clinically selected population rather than as prevalence in the general small ruminant population. Similarly, the detection of *C. perfringens* in 33 of 38 lambs and goat kids with hemorrhagic abomasitis (86.8%) represents a disease-associated detection rate and should not be interpreted as the national prevalence in Turkey.

At the broader Asian level, Tan et al. (2025) reported a pooled prevalence of 38.8% (95% CI: 30.9–46.9%) among sheep and goats based on 29 studies. This estimate was higher than the pooled prevalence observed in Iran; however, it represents Asian small ruminant populations and should not be considered a pooled estimate for the entire Middle East or the global animal population. Considerable variation has also been reported in Egypt. Among apparently healthy animals, the prevalence was 65.45% in sheep, 58.0% in goats, 55.0% in buffaloes, and 47.1% in cattle (Hamza et al., 2018). In contrast, a more recent Egyptian study identified confirmed *C.*

perfringens in 23 of 110 intestinal-content samples from camels, sheep, and cattle, corresponding to 20.9% (Wahdan & Elhaig, 2024), which was close to the pooled prevalence observed in Iran.

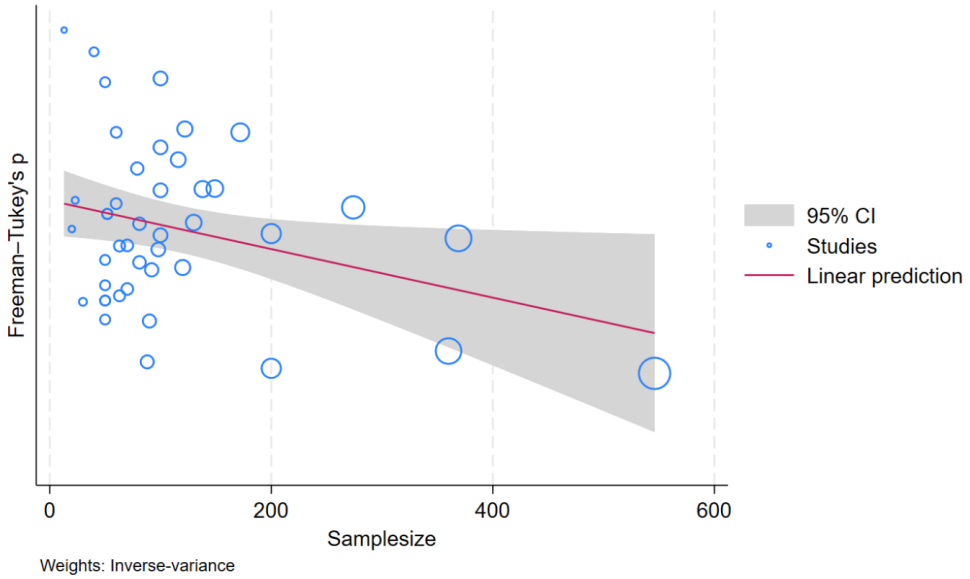

Figure 7. Meta-regression bubble plot showing the relationship between sample size and *C. perfringens* prevalence. The horizontal axis indicates sample size, and the vertical axis represents prevalence (Freeman–Tukey transformed). The red line shows the predicted trend, and the gray area represents the 95% confidence intervals. The increased scatter of data points at lower sample sizes highlights greater heterogeneity among these studies.

Another important pattern identified in this meta-analysis was the higher prevalence reported in studies using molecular methods, particularly PCR (13% – 25%), compared with studies primarily based on conventional culture and phenotypic techniques (1% – 8%). This difference is likely attributable to the higher sensitivity of PCR, its ability to detect slow-growing or damaged strains, and its capacity to identify toxin genes even in the absence of substantial bacterial growth on culture media. This represents a strength of more recent studies, as molecular methods provide a more accurate assessment of the genetic presence and pathogenic potential of the bacterium. On the other hand, differences in diagnostic methodology complicate direct comparisons of prevalence estimates between PCR-based and culture-based studies and may partially explain the observed heterogeneity (Nagpal et al., 2015).

Furthermore, meta-regression analysis based on sample size demonstrated that studies with smaller sample sizes tended to report higher prevalence estimates. This pattern, reflected by the negative slope of the regression line and the clustering of points on the left side of the bubble plot, may indicate the presence of small-study effects. Smaller studies may have preferentially targeted herds or farms with evident clinical conditions, such as necrotic enteritis or diarrhea, potentially inflating prevalence estimates. In addition, smaller studies are generally more susceptible to sampling errors, selection bias, and limited representativeness of the target population (Afonso et al., 2024). Publication bias favoring more striking findings from smaller studies may have also contributed to this pattern. This relationship suggests that sample size is an important source of between-study variability, and greater interpretive weight should therefore be assigned to larger and methodologically robust studies when evaluating pooled prevalence estimates (Nakagawa et al., 2022).

Meta-regression analysis by year of publication (2010–2025) yielded a nearly horizontal regression slope, suggesting no clinically significant trends of increasing or decreasing prevalence over time. While this relative stability might superficially be interpreted as a lack of worsening conditions, it indicates that over the past 15 years, control

measures, hygiene interventions, and management improvements across the production, storage, and processing continuum have failed to significantly reduce *C. perfringens* contamination levels. In essence, this issue appears to be both chronic and persistent (Elkenany et al., 2025). The increased dispersion of findings in recent years, particularly post-2020, may be attributed to several factors. First, the widespread adoption of sensitive molecular diagnostic techniques, such as PCR and real-time PCR, alongside an increased focus on toxin-encoding genes, has enabled the detection of a broader spectrum of contaminants than conventional culture-based methods (Ghourchian et al., 2021; Mohiuddin et al., 2020; Radomirović et al., 2025; Shakerian et al., 2017). Second, contemporary studies appear to encompass a more heterogeneous mix of host species, sample matrices, and study designs, which likely drives increased variability between studies (Azimirad et al., 2021; Chowdhury et al., 2026; Ghourchian et al., 2021; Hassani, Mahmoudi, et al., 2022; Hassani, Pakbin, et al., 2022; Seman & Milkowski, 2026). Finally, a larger body of recent research may have rendered underlying regional and management-related differences more apparent, thereby amplifying the observed heterogeneity.

The high prevalence of *C. perfringens* in Iranian livestock and poultry is critical not only for animal welfare and production efficiency but also has direct implications for food safety and public health. *C. perfringens* is a recognized agent of foodborne illness (Hosseinzadeh et al., 2018; Li et al., 2016; Shakerian et al., 2017; Takanosu et al., 2024) and human necrotic enteritis, and its ubiquitous presence in farms, slaughterhouses, and the cold chain serves as a persistent reservoir for final-product contamination (Alimolaei, 2023; Katalani et al., 2024; Shivaprasad et al., 2008). Given the inherent resistance of *C. perfringens* spores to heat and adverse environmental conditions (Chowdhury et al., 2026; Li et al., 2016; Talukdar et al., 2017), this risk cannot be mitigated through superficial interventions alone. Instead, it necessitates a multi-layered holistic approach:

Given the relatively high prevalence of *Clostridium perfringens* in livestock and food samples, control strategies in Iran should follow an integrated farm-to-fork and One Health framework. (Destoumieux-Garzón et al., 2018; Grenda et al., 2023). At the farm level, optimizing nutritional management, ensuring feed quality, maintaining litter hygiene, regulating stocking density, and applying preventive measures, such as vaccination, where indicated, may help limit intestinal colonization and reduce the environmental *C. perfringens* spore burden (Elkenany et al., 2025; Tan et al., 2025). Furthermore, Rigorous sanitation, cross-contamination prevention, and stringent monitoring of time-temperature conditions remain paramount (Chowdhury et al., 2026). At the policy level, implementing national surveillance programs based on standardized diagnostic and reporting frameworks would enhance oversight across the food chain, particularly for high-consumption livestock products (Tast Lahti et al., 2023).

Limitations

Several limitations should be considered when interpreting the findings of this meta-analysis. First, although 4,821 specimens were included, this number remains modest relative to Iran's extensive livestock population, geographical coverage, and diverse range of animal-derived food products. Moreover, the available studies did not provide uniform coverage of all provinces, production systems, animal populations, or food categories studied. Therefore, the pooled estimate should be interpreted as a summary of the currently available published evidence rather than a definitive nationally representative prevalence estimate for Iran.

Second, substantial heterogeneity was observed among the included studies in this meta-analysis. This variability may reflect differences in study design, sampling strategies, target populations, sample types, sample handling, laboratory procedures, culture conditions, PCR protocols, primer sets, and criteria used to define positive cases. Although subgroup analyses and meta-regression were used to explore potential sources of heterogeneity, these analyses could not fully account for the observed variability between studies. In addition, some subgroup analyses, particularly those based on geographical region and host type, included only a limited number of studies in certain categories. Consequently, the corresponding pooled

estimates may be unstable and should be regarded as exploratory and hypothesis-generating rather than conclusive evidence of regional or host-related differences.

Third, funnel-plot asymmetry and statistically significant Egger's ($p < 0.0001$) and Begg's ($p = 0.0002$) tests suggested possible small-study effects. Smaller studies tend to report higher prevalence estimates, which may reflect targeted sampling in high-risk populations, methodological differences, or selective publication of studies with higher detection rates. However, because conventional tests for funnel-plot asymmetry can be unreliable in prevalence meta-analyses with substantial heterogeneity, these findings should not be interpreted as definitive evidence of publication bias. Future large-scale, geographically distributed, and methodologically standardized surveillance studies are needed to provide more representative national estimates of *C. perfringens* prevalence across the livestock–food chain in Iran.

Conclusion

Overall, the findings of the present meta-analysis demonstrate that *C. perfringens* remains widely prevalent among livestock and poultry populations in Iran, with no marked reduction in prevalence over the last 15 years. A notable proportion of the variability observed among studies can be attributed to differences in host species, types of samples analyzed, diagnostic approaches, and study sample sizes. The leave-one-out sensitivity analysis further indicated that sequential removal of individual studies did not meaningfully change the pooled prevalence estimate, suggesting that the overall results were not driven by any single study. Nevertheless, the very high heterogeneity across studies, together with the possibility of publication bias, should be taken into consideration when interpreting the pooled prevalence reported in this meta-analysis. Looking ahead, future research would benefit from coordinated multicenter epidemiological studies carried out across different regions of the country. Studies that apply consistent sampling strategies and standardized diagnostic procedures (ideally combining conventional culture methods with molecular detection techniques) would provide a clearer and more representative understanding of the epidemiology of *C. perfringens* in Iran.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

Disclaimer

The authors used OpenAI's ChatGPT (GPT-5.6) solely as an AI-assisted language tool to improve the clarity, grammar, and tone of the manuscript. The use of this tool was limited to language editing only, and all scientific content, analyses, interpretations, and conclusions are the sole responsibility of the authors.

References

Afonso, J., Ramirez-Campillo, R., Clemente, F. M., Büttner, F. C., & Andrade, R. (2024). The perils of misinterpreting and misusing "Publication Bias" in Meta-analyses: An education review on funnel plot-based methods. *Sports Medicine*, *54*(2), 257-269. https://doi.org/10.1007/s40279-023-01927-9

Afshari, A., Jamshidi, A., Razmyar, J., & Rad, M. (2015). Genotyping of *Clostridium perfringens* isolated from broiler meat in northeastern of Iran. *Veterinary Research Forum*, *6*(4), 279–284.

Ahmadi, M., Alikord, M., Pirhadi, M., Masoumi, S., & Molaee, A. E. (2021). Screening and investigation of microbial and chemical properties of meat products in Hamadan Province, Iran during 2012-2015. *Infection Epidemiology and Microbiology*, *7*(4), 327-335. https://doi.org/10292252/iem.7.4.327

Ahsani, M., Bafti, M. S., Esmailizadeh, A., & Mohammadabadi, M. (2011). Genotyping of isolates of *Clostridium perfringens* from vaccinated and unvaccinated sheep. *Small Ruminant Research*, *95*(1), 65-69. https://doi.org/10.1016/j.smallrumres.2010.09.001

Ahsani, M. R., Mohammad, A. M., Shamsodini, B. M., Ezatkhah, M., Hasani, D. M., & Esmailzadeh, K. A. (2010). Application of triplex PCR technique in identification of *Clostridium perfringens* B, C and D types. *Iranian Journal of Animal Science Research*, *2*(2), 185-190. https://sid.ir/paper/218339/en

Alimolaei, M. (2023). Isolation and molecular characterization of *Clostridium perfringens* toxinotypes F & G in diarrhoeic sheep (*Ovis aries*) flocks in Southeast of Iran. *Archives of Razi Institute*, *78*(3), 1159-1168. https://doi.org/10.22092/ARI.2023.360450.2582

Alimolaei, M., & Ezatkhah, M. (2022). Prevalence and genotypic characterization of *Clostridium perfringens* associated with goat (*Capra hircus*) enterotoxemia in Southeast Iran. *Small Ruminant*

Research, *216*, 106805. https://doi.org/10.1016/j.smallrumres.2022.106805

Alimolaei, M., Ezatkhah, M., & Soltani, S. (2023). Toxin genotypes of *Clostridium perfringens* isolates from common quail (*Coturnix coturnix*) with or without acute necrotic enteritis. *Toxicon*, *221*, 106984. https://doi.org/10.1016/j.toxicon.2022.106984

Antonissen, G., Martel, A., Pasmans, F., Ducatelle, R., Verbrugghe, E., Vandenbroucke, V., Li, S., Haesebrouck, F., Van Immerseel, F., & Croubels, S. (2014). The impact of *Fusarium* mycotoxins on human and animal host susceptibility to infectious diseases. *Toxins*, *6*(2), 430-452. https://doi.org/10.3390/toxins6020430

Azimi, T., Nasrollahian, S., Sabour, S., Mosadegh, M., Hadi, N., Azimi, L., Fallah, F., & Pourmand, M. R. (2024). Intestinal colonization by *Campylobacter jejuni, Clostridium difficile*, and *Clostridium perfringens* among commensal *Rattus norvegicus* in the urban areas of Tehran, Iran. *BioMed Research International*, *2024*(1), 2929315. https://doi.org/10.1155/2024/2929315

Azimirad, M., Nadalian, B., Alavifard, H., Panirani, S. N., Bonab, S. M. V., Azimirad, F., Gholami, F., Jabbari, P., Yadegar, A., & Busani, L. (2021). Microbiological survey and occurrence of bacterial foodborne pathogens in raw and ready-to-eat green leafy vegetables marketed in Tehran, Iran. *International Journal of Hygiene and Environmental Health*, *237*, 113824. https://doi.org/10.1016/j.ijheh.2021.113824

Chowdhury, M. A. H., Reem, C. S. A., Ashrafudoulla, M., Yoon, H. J., & Ha, S. D. (2026). Biofilm formation and spore-mediated persistence of *Clostridium perfringens* in meat and poultry processing environments and their implications for control strategies. *Journal of Food Science*, *91*(6), e71193. https://doi.org/10.1111/1750-3841.71193

Craven, S. E., Stern, N. J., Bailey, J. S., & Cox, N. A. (2001). Incidence of *Clostridium perfringens* in broiler chickens and their environment during production and processing. *Avian Diseases*, *45*(4), 887-896. https://doi.org/10.2307/1592868

Destoumieux-Garzón, D., Mavingui, P., Boetsch, G., Boissier, J., Darriet, F., Duboz, P., Fritsch, C., Giraudoux, P., Le Roux, F., & Morand, S. (2018). The one health concept: 10 years old and a long road ahead. *Frontiers in Veterinary Science*, *5*, (14), 1-13. https://doi.org/10.3389/fvets.2018.00014

Doosti, A., Pasand, M., Mokhtari-Farsani, A., Ahmadi, R., & Chehelgerdi, M. (2017). Prevalence of *Clostridium perfringens* type A isolates in different tissues of broiler chickens. *Bulgarian Journal of Veterinary Medicine*, *18(4), 294–303*. https://doi.org/10.15547/bjvm.919

Elkenany, R., Elsayed, M., Zakaria, A., Elnagar, R., Salem, M., Auob, A., & Awad, A. (2025). Epidemiology of potential source, risk attribution of *Clostridium perfringens* from Egyptian broiler farms and genetic diversity of multidrug resistance strains. *Scientific Reports*, *15*(1), 28638. https://doi.org/10.1038/s41598-025-12519-0

Fahimeh, Y., Peyman, N., Gholamreza, H., Gholamali, K., Mohammad, R., & Jamshid, R. (2018). Major and minor toxins of *Clostridium perfringens* isolated from healthy and diseased sheep. *Small Ruminant Research*, *168*, 1-5. https://doi.org/10.1016/j.smallrumres.2018.09.008

Finnie, J. W., & Uzal, F. A. (2024). Neurotoxicity of *Clostridium perfringens* type D enterotoxemia. In Oliveira, M. R. (Ed.), *Natural Molecules in Neuroprotection and Neurotoxicity* (pp. 701-717). London: Academic Press. https://doi.org/10.1016/B978-0-443-23763-8.00066-X

Firmesse, O., Maladen, V., Bourelle, W., Federighi, M., Tran, C., & Mtimet, N. (2025). sporulation abilities and heat resistance of *Clostridium perfringens* strains isolated from French food borne outbreaks. *Foods*, *14*(21), 3735. https://doi.org/10.3390/foods14213735

Fohler, S., Klein, G., Hoedemaker, M., Scheu, T., Seyboldt, C., Campe, A., Jensen, K. C., & Abdulmawjood, A. (2016). Diversity of *Clostridium perfringens* toxin-genotypes from dairy farms. *BMC Microbiology*, *16*(1), 199. https://doi.org/10.1186/s12866-016-0812-6

Fujikawa, H., & Araki, M. (2020). *Clostridium perfringens* septicemia with massive intravascular hemolysis. *Internal Medicine*, *59*(4), 591. https://doi.org/10.2169/internalmedicine.3793-19

Gharibi, D., Ghorbanpoor, M., Haji Hajikolaei, M. R., & Mahmoudi Koohi, Z. (2017). Typing of *Clostridium perfringens* isolates from sheep in Ahvaz. *Iranian Veterinary Journal*, *13*(3), 75-85. https://doi.org/10.22055/ivj.2017.53716.1733

Ghezel, A., & Arfaee, C. S. (2025). Comments on "Case report on *Clostridium perfringens*-induced gas gangrene following an intramuscular injection: A stark reminder of injection-associated risks". *International Journal of Surgery Case Reports*, *130* (111317), 1-5. https://doi.org/10.1016/j.ijscr.2025.111317

Ghourchian, S., Douraghi, M., Baghani, A., & Soltan, D. M. M. (2021). Detection and frequency of enterotoxin (*cpa, cpe*) genes of *Clostridium perfringens* isolated from dehydrated vegetables by PCR. *Journal of Advances in Medical and Biomedical Research*, *29*(133), 63-67. https://doi.org/10.30699/jambs.29.133.63

Giannitti, F., García, J. P., Adams, V., Armendano, J. I., Beingesser, J., Rood, J. I., & Uzal, F. A. (2023). Experimental acute *Clostridium perfringens* type D enterotoxemia in sheep is not characterized by specific renal lesions. *Veterinary Pathology*, *60*(4), 412-419. https://doi.org/10.1177/03009858231171669

Goo, D., Ko, H., Choi, J., Lee, J., White, D. L., Sharma, M. K., & Kim, W. K. (2025). Valine and isoleucine deficiency in necrotic enteritis challenge impact growth performance, intestinal health, and muscle growth in broilers. *Poultry Science*, *104*(6), 105143. https://doi.org/10.1016/j.psj.2025.105143

Grenda, T., Jarosz, A., Sapała, M., Grenda, A., Patyra, E., & Kwiatek, K. (2023). *Clostridium perfringens*—opportunistic foodborne pathogen, its diversity and epidemiological significance. *Pathogens*, *12*(6), 768. https://doi.org/10.3390/pathogens12060768

Guo, W., Han, D., Zhang, F., Zhan, Q., Liu, Y., Peng, Q., Huang, S., Xue, Z., & Yang, X. (2023). Effects of dietary β-1,3-glucan addition on the growth performance, mRNA expression in jejunal barrier, and cecal microflora of broilers challenged with *Clostridium perfringens*. *Poultry Science*, *102*(2), Article 102349. https://doi.org/10.1016/j.psj.2022.102349

Hakeem, A., Kamboh, A. A., Matar, A. M., Ahmad, S., Ahmad, S., Kumar, V., & Taj, I. (2024). Prevalence and antibiogram of *Clostridium perfringens* in small ruminants of district Kalat, Balochistan. *Indian Journal of Animal Research*, *58*(4), 660-665. https://doi.org/10.18805/IJAR.BF-1483

Hamza, D., Dorgham, S. M., Elhariri, M., Elhelw, R., & Ismael, E. (2018). New insight of apparently healthy animals as a potential reservoir for *Clostridium perfringens*: A public health implication. *Journal of Veterinary Research*, *62*(4), 457-462. https://doi.org/10.2478/jvetres-2018-0073

Hashemi, M., Erfani, A., Touranlou, F. A., Doustinouri, M., Shahraki, A., & Afshari, A. (2025). Identification of *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Bacillus cereus, Bacillus subtilis*, and *Clostridium perfringens* in hospital food. *Revista Argentina de Microbiología*, *57*(1), 78-85. https://doi.org/10.1016/j.ram.2024.11.005

Hassani, S., Mahmoudi, R., Kaboudari, A., Ghajarbeygi, P., Peymani, A., Mousavi, S., & Shasavari, S. (2022). The prevalence of *Clostridium difficile* and *Clostridium perfringens* in minced and ground beef in Iran. *Journal of Chemical Health Risks*, 12(4), 575-580. https://doi.org/10.22034/jchr.2021.1909225.1177

Hassani, S., Pakbin, B., Brück, W. M., Mahmoudi, R., & Mousavi, S. (2022). Prevalence, antibiotic resistance, toxin-typing and genotyping of *Clostridium perfringens* in raw beef meats obtained from Qazvin city, Iran. *Antibiotics*, 11(3), 340. https://doi.org/10.3390/antibiotics11030340

Hayati, M., Shamseddini, M., Tahamtan, Y., Sadeghzadeh, S., Manavian, M., & Nikoo, D. (2020). Isolation and toxin typing of *Clostridium Perfringens* from sheep, goats, and cattle in Fars Province, Iran. *International Journal of Enteric Pathogens,* 8(3), 89-93. https://doi.org/10.34172/ijep.2020.20

Hosseinzadeh, S., Bahadori, M., Poormontaseri, M., Dehghani, M., Fazeli, M., & Nazifi, S. (2018). Molecular characterization of *Clostridium perfringens* isolated from cattle and sheep carcasses and its antibiotic resistance patterns in Shiraz slaughterhouse, southern Iran. *Veterinarski Arhiv*, *88*(5), 581-591. https://doi.org/10.24099/vet.arhiv.0009

Holtby, I., Tebbutt, G. M., Grant, K. A., McLauchlin, J., Kett, J., & Pinkney, S. (2008). A *Clostridium perfringens* food poisoning outbreak associated with consumption of chicken curry supplied by a home caterer. *Public Health*, *122*(12), 1311–1314. https://doi.org/10.1016/j.puhe.2008.05.013

Huang, J., Hou, Q., & Yang, Y. (2024). Replacing hydrolyzed soybean meal with recombinant β-glucosidase enhances resistance to *Clostridium perfringens* in broilers through immune modulation. *International Journal of Molecular Sciences*, *25*(21), 11700. https://doi.org/10.3390/ijms252111700

Jang, Y.-S., Kim, D.-H., Bae, D., Kim, S.-H., Kim, H., Moon, J.-S., Song, K.-Y., Chon, J.-W., & Seo, K.-H. (2020). Prevalence, toxin-typing, and antimicrobial susceptibility of *Clostridium perfringens* from retail meats in Seoul, Korea. *Anaerobe*, *64*, 102235. https://doi.org/10.1016/j.anaerobe.2020.102235

Jost, B. H., Trinh, H. T., & Songer, J. G. (2006). Clonal relationships among *Clostridium perfringens* of porcine origin as determined by multilocus sequence typing. *Veterinary Microbiology*, *116*(1-3), 158-165. https://doi.org/10.1016/j.vetmic.2006.03.025

Katalani, C., Ahmadian, G., Nematzadeh, G., Amani, J., Ehsani, P., Razmyar, J., Mirabzadeh Ardakani, E., & Ghazizadeh, L. (2024). Toxinotyping of *Clostridium perfringens* strains isolated from broiler flocks with necrotic enteritis and evaluation of the effect of toxins on Leghorn Male Hepatoma cells. *Iranian Journal of Veterinary Research*, *25*(2), 135-142. https://doi.org/10.22099/ijvr.2024.47200.6808

Keerqin, C., McGlashan, K., Van, T. T. H., Chinivasagam, H. N., Moore, R. J., Choct, M., & Wu, S.-B. (2022). A lytic bacteriophage isolate reduced *Clostridium perfringens* induced lesions in necrotic enteritis challenged broilers. *Frontiers in Veterinary Science*, *9*, 1058115. https://doi.org/10.3389/fvets.2022.1058115

Khan, M. U. Z., Khalid, S., Humza, M., Yang, S., Alvi, M. A., Munir, T., Ahmad, W., Iqbal, M. Z., Tahir, M. F., & Liu, Y. (2022). Infection dynamics of *Clostridium perfringens* fingerprinting in buffalo and cattle of Punjab province, Pakistan. *Frontiers in Veterinary Science*, *9*, 762449. https://doi.org/10.3389/fvets.2022.762449

Khiav, L. A., Emadi, A., & Dadar, M. (2025). Conventional and molecular identification of Iranian Clostridia species associated with animal infection. *Veterinary Research Forum, 16(6), 345–352.* https://doi.org/10.30466/vrf.2024.2035437.4361

Li, J., Paredes-Sabja, D., Sarker, M. R., & McClane, B. A. (2016). *Clostridium perfringens* sporulation and sporulation-associated toxin production. *Microbiology Spectrum*, *4(3), 10.1128* https://doi.org/10.1128/microbiolspec.TBS-0022-2015

McClane, B.A., Robertson, S.L., Li, J., et al., (2012). *Clostridium perfringens.* In M.P. Doyle and R.L. Buchanan (Eds.), Food Microbiology: Fundamentals and frontiers, 4th edition (pp. 465–489). American Society for Microbiology, Washington, DC, USA.

Migliavaca, C. B., Stein, C., Colpani, V., Barker, T. H., Ziegelmann, P. K., Munn, Z., & Falavigna, M. (2022). Meta-analysis of prevalence: I^2 statistic and how to deal with heterogeneity. *Research Synthesis Methods*, *13*(3), 363-367. https://doi.org/10.1002/jrsm.1547

Mohiuddin, M., Iqbal, Z., Siddique, A., Liao, S., Salamat, M. K. F., Qi, N., Din, A. M., & Sun, M. (2020). Prevalence, genotypic and phenotypic characterization and antibiotic resistance profile of *Clostridium perfringens* type A and D isolated from feces of sheep (*Ovis aries*) and goats (*Capra hircus*) in Punjab, Pakistan. *Toxins*, *12*(10), 657. https://doi.org/10.3390/toxins12100657

Mokhtari, M. (2024). Heterogeneity among included studies. In M. Ghajarzadeh, S. Hanaei, N. Rezaei (Eds.), *Systematic Review and Meta-Analysis: Stepwise Approach for Medical and Biomedical Researchers* (pp. 169-179). London: Academic Press. https://doi.org/10.1016/B978-0-443-13428-9.00013-6

Nagpal, R., Ogata, K., Tsuji, H., Matsuda, K., Takahashi, T., Nomoto, K., Suzuki, Y., Kawashima, K., Nagata, S., & Yamashiro, Y. (2015). Sensitive quantification of *Clostridium perfringens* in human feces by quantitative real-time PCR targeting alpha-toxin and enterotoxin genes. *BMC Microbiology*, *15*, 219. https://doi.org/10.1186/s12866-015-0561-y

Nakagawa, S., Lagisz, M., Jennions, M. D., Koricheva, J., Noble, D. W., Parker, T. H., Sánchez-Tójar, A., Yang, Y., & O'Dea, R. E. (2022). Methods for testing publication bias in ecological and evolutionary meta-analyses. *Methods in Ecology and Evolution*, *13*(1), 4-21. https://doi.org/10.1111/2041-210X.13724

Poursoltani, M., Razmyar, J., Mohsenzadeh, M., & Peighambari, M. (2013). Isolation and antibiotic susceptibility testing of *Clostridium Perfringens* isolated from packaged wing, neck, liver and gizzard of broilers in retail stores of northeastern of Iran. *Iranian Journal of Medical Microbiology*, *7*(1), 35-39.

Radomirović, M., Gligorijević, N., & Rajković, A. (2025). Immuno-PCR in the analysis of food contaminants. *International Journal of Molecular Sciences*, *26*(7), 3091. https://doi.org/10.3390/ijms26073091

Seman, D. L., & Milkowski, A. L. (2026). Estimation of the prevalence of *Clostridium perfringens* in raw, heat-treated, and fully cooked processed meat products in the USA. *Journal of Food Protection*, *89*(6), 100778. https://doi.org/10.1016/j.jfp.2026.100778

Shahdadnejad, N., Mohammadabadi, M., & Shamsadini, M. (2016). Typing of *Clostridium perfringens* isolated from broiler chickens using multiplex PCR. *Genetics in the third millennium*, *14*(4), 4368-4374.

Shahryari, A., Niknejad, F., Aali, R., Zarajabad, A. M., Shahini, M., Emadi, F., ... & Shahamat, Y. D. (2024). Fungal and bacterial evaluation in drinking water distribution network and their association with physicochemical parameters. *Desalination and Water Treatment*, *319*. https://doi.org/10.1016/j.dwt.2024.100440

Shakerian, A., Rahimi, E., Mesbah, J., & Mousavi, M. (2017). Molecular detection of *Clostridium perfringens* in some raw animal food origin products in Shahrekord, 2014. Payavard-Salamat, 11(4), 391-399.

Shi, H., Huang, X., Yan, Z., Yang, Q., Wang, P., Li, S., Sun, W., & Gun, S. (2019). Effect of *Clostridium perfringens* type C on TLR4/MyD88/NF-κB signaling pathway in piglet small intestines. *Microbial Pathogenesis*, *135*, 103567. https://doi.org/10.1016/j.micpath.2019.103567

Shivaprasad, H. L., Uzal, F., Kokka, R., Fisher, D. J., McClane, B. A., & Songer, A. G. (2008). Ulcerative enteritis-like disease associated with *Clostridium perfringens* type A in bobwhite quail (*Colinus virginianus*). *Avian Diseases*, *52*(4), 635-640. https://doi.org/10.1637/8341-050108-Reg.1

Sumners, L. H., Cox, C. M., Kim, S., Salevsky, J. E., Siegel, P. B., & Dalloul, R. A. (2012). Immunological responses to *Clostridium perfringens* alpha-toxin in two genetically divergent lines of chickens as influenced by major histocompatibility complex genotype. *Poultry Science*, *91*(3), 592-603. https://doi.org/10.3382/ps.2011-01775

Takanosu, T., Izawa, Y., Yonekawa, C., & Mato, T. (2024). *Clostridium perfringens*-induced liver abscess with severe haemolysis. *BMJ Case Reports*, *17*(5). https://doi.org/10.1136/bcr-2024-260800

Talukdar, P. K., Udompijitkul, P., Hossain, A., & Sarker, M. R. (2017). Inactivation strategies for *Clostridium perfringens* spores and vegetative cells. *Applied and Environmental Microbiology*, *83*(1). https://doi.org/10.1128/aem.02731-16

Tan, W.-X., Ye, T., Zhang, Q.-M., Zhang, M., Chen, X.-T., Tang, L.-Y., Yang, M.-T., Jiang, J., & Zhao, Q. (2025). Prevalence of *Clostridium perfringens* in sheep (*Ovis aries*) and goat (*Capra hircus*) populations across Asia: A systematic review and meta-analysis. *Research in Veterinary Science*, *187*, 105605. https://doi.org/10.1016/j.rvsc.2025.105605

Tast Lahti, E., Karamehmedovic, N., Riedel, H., Blom, L., Boel, J., Delibato, E., ... & Jernberg, C. (2023). One Health surveillance—A cross-sectoral detection, characterization, and notification of foodborne pathogens. *Frontiers in Public Health*, *11(83)*, 1-12. https://doi.org/10.3389/fpubh.2023.1129083

Tutuncu, M., Kilicoglu, Y., Guzel, M., Pekmezci, D., & Gulhan, T. (2018). prevalence and toxinotyping of *Clostridium perfringens* enterotoxins in small ruminants of Samsun province, northern. *Journal of Animal and Plant Sciences*, *28*(4), 1204-1207.

Wahdan, A., & Elhaig, M. M. (2024). Epidemiology and diagnostic accuracy of *Clostridium perfringens* toxins in the intestinal contents of camels, sheep, and cattle: a cross-sectional study in Dakahlia governorate, Egypt. *Tropical Animal Health and Production*, *56*(6), 205. https://doi.org/10.1007/s11250-024-04034-7

Yan, Z., Wang, H., Zhu, Y., Wang, X., Wu, Y., Wang, Y., & Zhang, R. (2025). Molecular epidemiology of type F *Clostridium perfringens* among diarrheal patients and virulence-resistance dynamics-1 provinces, China, 2024. *China CDC Weekly*, *7*(3), 69. https://doi.org/10.46234/ccdcw2025.013

Zandi, E., Mohammadabadi, M.R., Ezzatkhah, M., & Esmailizadeh, A.K. (2014). typing of toxigenic isolates of *Clostridium perfringens* by multiplex PCR in ostrich. *Iranian Journal of Applied Animal Science*, *4*(4), 795-801.

Zaytsoff, S. J. M., Montina, T., Boras, V. F., Brassard, J., Moote, P. E., Uwiera, R. R. E., & Inglis, G. D. (2022). Microbiota transplantation in day-old broiler chickens ameliorates necrotic enteritis via modulation of the intestinal microbiota and host immune responses. *Pathogens*, *11*(9), 972. https://doi.org/10.3390/pathogens11090972