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Prevalence of internal and external parasites in club horses in western Iran: a comparative cross-sectional study

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Abstract

Gastrointestinal parasites remain a cause of health impairment in horses worldwide. This cross-sectional study (spring to winter 2025) aimed to determine the prevalence and diversity of internal and external parasites in club horses in Sanandaj and Kermanshah, western Iran, and compare management characteristics. One hundred horses (50 per city) were randomly selected from equestrian clubs. Fecal samples were examined using direct smear and Clayton Lane flotation techniques, blood smears were stained with Giemsa for hemoparasite detection, and physical examinations were performed for ectoparasites. Management information was collected by questionnaire. In Kermanshah, 5/50 horses (10%; 95% CI: 3.3–21.8%) were positive for gastrointestinal parasites, including *Parascaris equorum* (2%), Cyathostomins (4%), and *Anoplocephala perfoliata* (4%). In contrast, 40/50 horses (80%; 95% CI: 66.3–89.9%) from Sanandaj were infected with gastrointestinal parasites. *P. equorum* was predominant (56%), followed by *Oxyuris equi* (36%), Cyathostomins (24%), *Anoplocephala magna* (22%), *Trichostrongylus axei* (16%), and *A. perfoliata* (10%). Univariable logistic regression showed higher odds of infection in Sanandaj (crude OR = 36.0, 95% CI: 11.4–113.7, $p < 0.001$), and a cluster-adjusted GEE model supported an association between city and infection after adjustment for age, sex, and breed (adjusted OR = 72.1, 95% CI: 8.9–583.1, $p < 0.001$). No hemoparasites or ectoparasites were detected. These findings indicated a regional difference in parasite prevalence coinciding with distinct management characteristics in the two cities; however, causality cannot be inferred from this cross-sectional design.

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Introduction

Horses hold significant economic, sporting, and cultural value in Iran. Parasitic infections, particularly of the gastrointestinal tract, are among the most important causes of reduced performance, colic, weight loss, and mortality in horses worldwide (Reinemeyer and Nielsen, 2009; Nielsen, 2012). The most common gastrointestinal parasites affecting horses include strongyles (both large and small), ascarids (*Parascaris equorum*), pinworms (*Oxyuris equi*), and cestodes of the genus *Anoplocephala* (Lyons *et al*., 2011; Matthews, 2014).

Gastrointestinal parasites cause direct damage through mucosal irritation, blood loss, malabsorption, and intestinal obstruction. Cyathostomins (small strongyles) are associated with larval cyathostominosis, a severe syndrome characterized by weight loss, diarrhea, and edema (Love *et al*., 1999). *P. equorum* is particularly pathogenic in foals, causing poor growth, unthriftiness, and potentially fatal intestinal impaction or rupture (Reinemeyer and Nielsen, 2009). *A. perfoliata* has been consistently associated with colic and ileocecal intussusception (Proudman and Holdstock, 2000).

Beyond gastrointestinal parasites, hemoparasites including *Theileria equi* and *Babesia caballi* cause equine piroplasmosis, a tick-transmitted disease that can result in fever, anemia, icterus, and death (Rothschild, 2013; Wise *et al*., 2013). Ectoparasites such as lice, mites, and ticks directly cause irritation and serve as vectors for various pathogens.

In Iran, several studies have investigated the prevalence of gastrointestinal parasites in horses. Darabi *et al*. (2023) reported a 32% infection rate in horses around Tehran, with *P. equorum* (30%) and *O. equi* being the most common. Mirian *et a*l. (2019) found a 20.7% infection rate in 294 horses from 15 clubs in Tehran province. Shahbazi *et al*. (2018) reported infections in Ardabil province including strongyles (34%), *P. equorum* (20%), and *Strongyloides westeri* (12%). Ashrafzadeh-Shiraz *et al*. (2023) examined 483 horses in Hamedan, Yazd, and Tabriz, finding a 26.5% overall infection rate and documenting anthelmintic resistance to fenbendazole and ivermectin.

No comprehensive study has previously been conducted on equine parasites in the western Iranian provinces of Kurdistan and Kermanshah. The objectives of this study were to: (1) determine the prevalence and diversity of internal and external parasites in club horses in Sanandaj and Kermanshah; (2) compare management characteristics between the two cities alongside parasite prevalence; and (3) provide baseline information to inform parasite control programs. We hypothesized that parasite prevalence would differ between the two study populations, and that this variation would coincide with differences in management systems.

Materials and Methods

Study areas

The study was conducted in two cities in western Iran: Sanandaj (capital of Kurdistan province, 35°18′N 46°59′E, elevation 1,480 m) and Kermanshah (capital of Kermanshah province, 34°19′N 47°07′E, elevation 1,350 m). Both cities have cold, mountainous climates with cold winters and mild summers. The study period extended from spring to winter 2025.

Animals and sampling

A total of 100 horses were randomly selected using computer-generated random numbers from equestrian clubs in Sanandaj (n = 50) and Kermanshah (n = 50). Five clubs were included in each city (Sanandaj: 5 of 8 active clubs; Kermanshah: 5 of 7 active clubs), selected by computer-generated random numbers from the list of all active clubs. All selected clubs agreed to participate; no replacement was required. Within each club, horses were selected using the same random number procedure from the club's horse inventory. The number of horses per club ranged from 8 to 12 (mean = 10.0 ± 1.4). Inclusion criteria were age ≥ 1 year and apparently healthy at the time of sampling. Age was determined by dental examination or owner interview. Breed was categorized as Arab, Turkoman, or Other, and dichotomized as Arab versus non-Arab for statistical analysis given the limited sample size.

Fresh fecal samples were collected directly from the rectum or from freshly voided material within two hours, placed in sterile labeled containers, and transported on ice to the

Parasitology Laboratory of Lorestan University. Five ml of blood were collected from the jugular vein into EDTA-containing tubes for hemoparasite examination. The entire body surface of each horse was examined macroscopically for ectoparasites, with particular attention to the tail, perianal region, mane, and axillae.

Questionnaire survey

A structured questionnaire was administered to club owners and managers to collect information on anthelmintic treatment protocols (frequency, drugs used, and rotation practices); access to free-range grazing or outdoor paddocks; stable management practices (bedding type and cleaning frequency); quarantine practices; and history of clinical signs (colic, diarrhea, weight loss, and tail rubbing). One questionnaire was completed per club by the club manager. The questionnaire comprised 15 closed-ended questions and was developed based on published studies and expert opinion. Content validity was established through independent review by two faculty members with expertise in veterinary parasitology. The questionnaire was pilot-tested in one equestrian club before data collection; pilot data were excluded from the final analysis. Because the questionnaire consisted of categorical management variables rather than a composite score, no internal consistency analysis was applicable.

Coprological examination

Fecal samples were examined using two techniques. Direct smear: a small amount of feces was mixed with a drop of saline on a glass slide, covered with a coverslip, and examined under a light microscope at 10× and 40× magnifications. Clayton Lane flotation technique: two grams of feces were mixed with 30 ml of Sheather's sugar solution (specific gravity 1.27), strained through cheesecloth into a 15-ml centrifuge tube, and centrifuged at 1,500 rpm for 5 minutes. A coverslip was placed on the tube, removed after 10 minutes, and transferred to a glass slide for microscopic examination. Eggs were identified based on their morphological characteristics using standard diagnostic keys (Thienpont *et al*., 1986; Zajac and Conboy, 2012) by an experienced veterinary parasitologist, with independent confirmation by a second examiner in ambiguous cases. These techniques identify patent infections but may fail to detect low-intensity or prepatent infections. Because only a single fecal sample was collected per horse, intermittent egg shedding may have resulted in underestimation of prevalence. It should also be noted that Sheather's sugar flotation has suboptimal sensitivity for *Anoplocephala* spp. eggs compared to zinc sulfate centrifugation; therefore, cestode prevalence may be underestimated. Cyathostomin eggs were identified morphologically and reported collectively, because species-level identification is not possible by coprological examination alone.

Blood smear examination

Thin blood smears were prepared immediately after collection, air-dried, fixed with absolute methanol for 2 minutes, and stained with 10% Giemsa solution for 30 minutes. Smears were examined under oil immersion (1000×) for *T. equi* and *B. caballi*. Microscopic examination of blood smears has considerably lower sensitivity than PCR-based methods for detecting subclinical or low-level parasitemia; results should therefore be interpreted as non-detection rather than confirmed absence.

Statistical analysis

Data were analyzed using SPSS version 25 (IBM Corp., Armonk, NY, USA) and Python (statsmodels v0.14). Prevalence was expressed as percentages with 95% confidence intervals (Wilson score method). Differences between categorical variables were evaluated using Pearson's chi-square test or Fisher's exact test, as appropriate. Univariable logistic regression was used to calculate crude odds ratios (ORs) with 95% confidence intervals (CIs). Because management variables (grazing, deworming, and stable hygiene) were completely clustered by city, they could not be included in multivariable models as independent predictors.

To account for clustering of horses within clubs, a multivariable model was fitted using Generalized Estimating Equations (GEE) with a binomial family, logit link, and exchangeable working correlation structure, with club as the clustering unit. The model included city, age, sex, and breed as covariates. Robust (sandwich) standard errors were used for confidence intervals and *p*-values. These estimates should be interpreted as population-averaged associations. The intraclass correlation coefficient (ICC) was estimated separately using a one-way random-

effects ANOVA. Because only five infected horses were observed in Kermanshah, quasi-complete separation occurred for the city variable; however, maximum likelihood estimates remained finite and penalized likelihood methods were not applied. Because only ten clusters were available, robust standard errors may remain biased despite sandwich variance estimation; therefore, GEE results should be interpreted with caution. A two-sided p-value < 0.05 was considered statistically significant.

Sample size justification

The sample size of 100 horses (50 per city) was calculated using the formula $n = (Z^2 \times p \times (1-p)) / d^2$, assuming a prevalence of 50% ($p = 0.5$), a desired precision of 10% ($d = 0.1$), and a 95% confidence level ($Z = 1.96$), yielding a calculated sample size of 96, rounded to 100. A conservative prevalence estimate of 50% was used because no prior data were available for the study region. The calculation did not account for the clustered sampling design because no prior ICC estimate was available; the estimated effective sample size based on the observed ICC (0.51) and design effect (5.63) was approximately 18. This reduced effective sample size should be considered when interpreting the precision of the estimated associations.

Ethical considerations

This study was conducted in accordance with the principles of the 3Rs and institutional regulations of Lorestan University. All procedures were minimally invasive: fecal sampling and jugular venipuncture performed by trained veterinary personnel using standard aseptic techniques. Written informed consent was obtained from all club owners and managers. The study was approved by the Animal Ethics Committee of Lorestan University (Approval No. LU.ECRA.2025.3).

Results

Overall parasite prevalence and population characteristics

A total of 100 horses were examined (50 in Kermanshah, 50 in Sanandaj). Demographic and management characteristics were presented in Table 1. The two populations were balanced by sex (52% male in each city) and breed distribution.

In Kermanshah, 5/50 horses (10%; 95% CI: 3.3–21.8%) were positive for at least one gastrointestinal parasite; the remaining 45 (90%) had no detectable infection. In Sanandaj, 40/50 horses (80%; 95% CI: 66.3–89.9%) were infected with at least one gastrointestinal parasite. Co-infection, defined as the detection of two or more parasite species in the same horse, was observed in 15 horses, all in the Sanandaj population (37.5% of infected Sanandaj horses; 30% of the total Sanandaj sample). The most common co-infections were *P. equorum* + *O. equi* (n = 6) and *P. equorum* + Cyathostomins (n = 4). No hemoparasites or ectoparasites were detected using the diagnostic methods employed in this study.

Species-specific prevalence

The prevalence and diversity of parasite species identified in both cities were presented in Table 2.

Statistical analysis

Chi-square analysis demonstrated a highly significant association between city and infection status ($\chi^2 = 48.8$, df = 1, $p < 0.001$).

Univariable analysis. Horses from Sanandaj had substantially higher odds of infection than those from Kermanshah (crude OR = 36.0, 95% CI: 11.4–113.7, $p < 0.001$). Age (OR = 0.79 per year, 95% CI: 0.60–1.04, $p = 0.08$), sex (OR = 1.10, 95% CI: 0.50–2.41, $p = 0.81$), and breed (OR = 1.25, 95% CI: 0.82–1.90, $p = 0.29$) were not significantly associated with infection (Table 3).

Cluster-adjusted multivariable analysis (GEE). The intraclass correlation coefficient (ICC) for infection within clubs was 0.51, corresponding to a design effect of 5.63 and an effective sample size of approximately 18. The GEE model supported a strong and statistically significant association between city of origin and infection status (adjusted OR = 72.1, 95% CI: 8.9–583.1, $p < 0.001$) after adjustment for age, sex, and breed. The wide confidence interval reflects the small number of clusters and the high within-club clustering. Sex showed a statistically significant association in the GEE model (adjusted OR = 2.28, 95% CI: 1.08–4.84, $p = 0.031$); however, this finding should be interpreted cautiously given the limited number of

clusters (five per city), which may render the result unstable. Age (adjusted OR = 1.01, 95% CI: 0.64–1.60, $p = 0.952$) and breed (adjusted OR = 0.51, 95% CI: 0.13–1.94, $p = 0.321$) were not significantly associated with infection (Table 3).

Table 1. Demographic characteristics of the study horses and club-level management characteristics (100 horses from 10 equestrian clubs).

Characteristic	Kermanshah (n = 50)	Sanandaj (n = 50)	Total (n = 100)
Age (years), mean ± SD	4.5 ± 1.2	4.3 ± 1.1	4.4 ± 1.2
Sex			
Male	26 (52%)	26 (52%)	52 (52%)
Female	24 (48%)	24 (48%)	48 (48%)
Breed			
Arab	30 (60%)	30 (60%)	60 (60%)
Turkoman	10 (20%)	10 (20%)	20 (20%)
Other	10 (20%)	10 (20%)	20 (20%)
Club-level management†			
Pasture grazing: Yes	0/5 clubs (0%)	3/5 clubs (60%)	3/10 clubs (30%)
Pasture grazing: No	5/5 clubs (100%)	2/5 clubs (40%)	7/10 clubs (70%)
Routine stable hygiene	5/5 clubs (100%)	2/5 clubs (40%)	7/10 clubs (70%)
Regular deworming	5/5 clubs (100%)	2/5 clubs (40%)	7/10 clubs (70%)

†Management variables were measured at the club level; proportions reflect the number of clubs with each characteristic out of five clubs in each city.

Table 2. Prevalence of gastrointestinal parasite species in club horses in Kermanshah and Sanandaj (n = 100).

Parasite species	Kermanshah (n = 50) (%)	Sanandaj (n = 50) (%)	Overall (n = 100) (%)
Nematodes			
Parascaris equorum	1 (2%)	28 (56%)	29 (29%)
Oxyuris equi	0 (0%)	18 (36%)	18 (18%)
Cyathostomin eggs*	2 (4%)	12 (24%)	14 (14%)
Trichostrongylus axei	0 (0%)	8 (16%)	8 (8%)
Cestodes			
Anoplocephala magna	0 (0%)	11 (22%)	11 (11%)
Anoplocephala perfoliata	2 (4%)	5 (10%)	7 (7%)
Horses positive for ≥1 parasite	**5 (10%; 95% CI: 3.3–21.8)**	**40 (80%; 95% CI: 66.3–89.9)**	**45 (45%; 95% CI: 35.2–55.1)**

*Cyathostomin eggs were identified morphologically and reported collectively because species-level identification is not possible by coprological examination alone.

Table 3. Univariable and cluster-adjusted (GEE) associations between explanatory variables and gastrointestinal parasite infection.

Variable	Crude OR	95% CI	*p*	Adj. OR (GEE)*	95% CI	*p*
City (Sanandaj vs. Kermanshah)	36.0	11.4–113.7	< 0.001	72.1	8.9–583.1	< 0.001
Age (per year)	0.79	0.60–1.04	0.08	1.01	0.64–1.60	0.952
Sex (Male vs. Female)	1.10	0.50–2.41	0.81	2.28†	1.08–4.84	0.031
Breed (Non-Arab vs. Arab)‡	1.25	0.82–1.90	0.29	0.51	0.13–1.94	0.321

*GEE with exchangeable working correlation, cluster = club; robust sandwich standard errors. ICC = 0.51; design effect = 5.63. Because only ten clusters were available, robust standard errors may remain biased.
†Statistically significant in GEE model; interpret cautiously given only five clusters per city.
‡Breed dichotomized as Arab versus non-Arab (Turkoman and Other combined).

Descriptive questionnaire findings: management characteristics

The questionnaire revealed contrasting management characteristics between the two cities that accompanied the observed difference in parasite prevalence. Because these variables were completely clustered by city, no inference regarding their independent effects can be made.

Kermanshah clubs (10% infection rate): fixed-interval anthelmintic programs (every 3–4 months); no access to pasture grazing; routine stable hygiene with regular manure removal; quarantine of newly arrived horses; no reported history of clinical parasitic disease.

Sanandaj clubs (80% infection rate): intermittent, non-standardized anthelmintic use; regular access to pasture grazing; inconsistent

cleaning practices; no quarantine for new arrivals; reported clinical signs including tail rubbing, poor body condition, and occasional colic.

Discussion

The most striking finding of this study was the marked difference in gastrointestinal parasite prevalence between Kermanshah (10%) and Sanandaj (80%), despite both cities having similar cold mountainous climates. This difference appears to be larger than that reported in most previous equine parasitological studies conducted in Iran.

The role of management systems

The low prevalence in Kermanshah was accompanied by structured management practices-fixed-interval deworming, no pasture grazing, routine manure removal, and quarantine of new horses-whereas the high prevalence in Sanandaj was associated with less structured management, including more widespread access to pasture grazing. Although the complete clustering of management variables with city prevented estimation of their independent effects, this pattern is consistent with the broader literature indicating that integrated parasite management has been associated with reduced transmission (Kaplan and Nielsen, 2010; Nielsen *et al*., 2014).

The role of climate

Both cities share similar cold mountainous climates, comparable elevations (> 1,350 m), cold winters, and mild summers. The marked difference in parasite prevalence despite similar climatic conditions suggests that factors other than climate, including management differences, may contribute to the observed regional variation; however, this cannot be established from the present study design and environmental or other unmeasured regional factors cannot be excluded. Climate may nonetheless contribute to the absence of detected hemoparasites and ectoparasites by limiting the seasonal activity of ixodid tick vectors-a finding consistent with previous reports of equine piroplasmosis being confined to warmer regions of Iran (Bahrami *et al*., 2014).

Absence of hemoparasites and ectoparasites

No hemoparasites (*T. equi* or *B. caballi*) or ectoparasites were detected in either study population. Two explanations are plausible. First, the cold mountainous climate of both study areas (elevation > 1,350 m) may limit the abundance and activity of ixodid tick vectors, thereby reducing transmission risk. Second, thin blood smear examination has considerably lower sensitivity than PCR-based methods for detecting subclinical or low-level parasitemia (Rothschild, 2013; Wise *et al*., 2013). Consequently, the absence of hemoparasites in this study should be interpreted as non-detection rather than confirmed absence, and PCR-based surveillance is recommended for future studies.

Comparison with previous studies in Iran

The 80% prevalence in Sanandaj was substantially higher than previously reported rates: 32% around Tehran (Darabi *et al*., 2023), 20.7% in Tehran province (Mirian *et al*., 2019), 26.5% in Hamedan, Yazd, and Tabriz (Ashrafzadeh-Shiraz *et al*., 2023), and 34% for strongyles in Ardabil (Shahbazi *et al*., 2018). Conversely, the 10% prevalence in Kermanshah was among the lowest reported in Iran; a systematic review by Sazmand *et al*. (2020) documented that most studies found prevalence rates between 20% and 60%.

Statistical considerations

The cluster-adjusted GEE model revealed an ICC of 0.51, indicating substantial clustering of infection status within clubs. This high ICC reduced the effective sample size from 100 to approximately 18, and resulted in substantially wider confidence intervals in the GEE model (adjusted OR = 72.1, 95% CI: 8.9–583.1) compared to the naïve logistic regression (crude OR = 36.0, 95% CI: 11.4–113.7). The naïve confidence intervals should be regarded as overly precise. These findings underscore the importance of cluster-adjusted analyses in equine parasitological studies based on club sampling designs.

Practical implications and limitations

The findings support consideration of integrated parasite management programs in equestrian clubs, including strategic fecal egg count-guided deworming, grazing management,

stable hygiene, manure removal, and quarantine procedures. However, the independent contribution of each practice could not be determined.

Several limitations should be acknowledged: (1) the cross-sectional design precludes causal inference; (2) management variables were completely confounded with city; (3) the small number of clubs per city (n = 5) limits the reliability of cluster-adjusted analyses; (4) a single fecal sample per horse may underestimate prevalence due to intermittent egg shedding; (5) Sheather's sugar flotation has suboptimal sensitivity for *Anoplocephala* spp. eggs; (6) thin blood smear examination has considerably lower sensitivity than PCR for subclinical hemoparasitemia; (7) no fecal egg count reduction testing was performed; (8) parasite identification was based on coprological morphology without molecular confirmation; (9) the sample was limited to equestrian clubs and may not represent the broader horse population; (10) potential selection bias cannot be excluded, as clubs with better management may have been more willing to participate; (11) recall bias may have affected questionnaire responses; (12) the sample size calculation did not account for clustering, resulting in an effective sample size smaller than planned; and (13) the confidence intervals reported for the naïve logistic regression are likely overly precise and should be interpreted with caution.

This cross-sectional study identified a marked regional difference in gastrointestinal parasite prevalence between club horses in Sanandaj (80%) and Kermanshah (10%). The observed difference coincided with distinct management characteristics between the two cities. A cluster-adjusted GEE model supported a strong association between city of origin and infection status after adjustment for age, sex, and breed (adjusted OR = 72.1, 95% CI: 8.9–583.1), with an ICC of 0.51 indicating substantial clustering of infection status within clubs. These findings are exploratory and hypothesis-generating rather than confirmatory; causality cannot be inferred from this cross-sectional design. Future longitudinal studies incorporating quantitative fecal egg counts, molecular diagnostics, anthelmintic resistance testing, and cluster-adjusted approaches are needed to identify the independent contributions of specific management practices to equine parasite transmission in western Iran.

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Conflict of Interest

The authors declare no conflicts of interest.

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