



Seroprevalence of equine leishmaniasis in Jordan: Zarqa governorate hotspot and association risk factor

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Abstract

The emergence of equine leishmaniasis is of particular concern as horses, being the second most numerous reservoir host after dogs, are involved in the transmission cycle to humans. Although horses are increasingly recognized as secondary hosts of Leishmaniasis no large-scale data exist for Jordan. A cross-sectional serosurvey was conducted on 326 horses of different breed (Arabians, mixed local, Thoroughbred and Quarter) from six governorates using a commercial indirect ELISA. After excluding animals with incomplete metadata, 274 horses were retained for risk-factor analysis. Overall apparent seroprevalence was 11.3% (31/274). Seropositivity was highly focal: 55.0% in Zarqa Governorate versus 0–11.1% elsewhere ($P < 0.001$). Independent risk factors in multivariable analysis were Zarqa residence (aOR 39.8), stall-only housing (aOR 6.12), deworming exactly twice per year (aOR 8.74), and male sex (aOR 3.11). No seropositive horses were found among competitive animals or those with pasture access. Jordan harbors a previously unrecognized equine *Leishmania* focus in Zarqa Governorate driven by intensive stall confinement. Simple changes in housing and deworming practices could substantially reduce exposure. = Further studies are needed on equine leishmaniasis in Jordan using more advanced molecular studies. This study represents a first large-scale serological assessment of equine leishmaniasis in Jordan and constitutes a contribution to determine its geographic distribution and estimate its zoonotic potential in Jordan.

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Introduction

Leishmania spp. are vector-borne protozoan parasites transmitted by phlebotomine sand flies. Although dogs are the principal reservoir of *Leishmania infantum* (*L. infantum*) in the Mediterranean basin, horses are increasingly recognized as important secondary or incidental hosts (1,2). Equine leishmaniasis is usually subclinical, but seropositive horses may develop cutaneous or, rarely, visceral forms and can contribute to maintaining the transmission cycle in areas where competent vectors are present (3).

Jordan lies within the eastern Mediterranean endemic zone for both cutaneous (*Leishmania major* (*L. major*), *Leishmania tropica* (*L. tropica*)) and visceral leishmaniasis (*L. infantum*/*L. donovani* complex). Human cutaneous leishmaniasis has shown dramatic increases in recent years, particularly in Northern Governorates and around the Jordan Valley, while sporadic visceral cases have been reported in the south (4-6). Recently, the first confirmed autochthonous cases of animal leishmaniasis in Jordan were reported, describing cutaneous leishmaniasis caused by *L. tropica* in a horse and visceral leishmaniasis caused by *L. infantum* in a

stray dog (6). Despite this, data on *Leishmania* infection in domestic animals other than the previous report remains extremely scarce in the country.

To date, no large-scale studies have investigated the exposure of horses to *Leishmania* in Jordan, and the potential risk factors associated with seropositivity in this species are unknown. Such information is essential for understanding the local epidemiology of the parasite, pinpointing potential equine contributions to zoonotic spread and directing focused vector control of possible sand fly vectors in equine environments.

This work was set out to establish the seroprevalence of leishmaniasis in horses in different Governorates of Jordan employing a commercial indirect ELISA and to identify demographic, management and geographic risk factors associated with *Leishmania* seropositivity. Particular emphasis was placed on housing characteristics, deworming, and competitive vs. non-competitive use as sources for sand fly exposure and host immune status.

Materials and methods

Ethical approval

Approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Jordan University of Science and Technology (approval 16/3/2024).

Sample size determination

The population of interest was considered as all horses (*Equus ferus caballus*). According to the World Organization for Animal Health (WOAH, n.d.), the latest information shows us that Jordan has 3,500 domestic horses. By using the standard formula for estimating a single proportion in a finite population (7), the minimum number of sample size needed was calculated.

$$n = (Z^2 P (1 - P)) / E^2$$

Where:

Z = 1.96 (Z-value for Confidence Level of 95%)

P = 0.05 (the anticipated prevalence, 5% was estimated based on preliminary studies and from neighboring countries)

E = 0.03 (the absolute precision or margin of error required, $\pm 3\%$)

n = The sample size after this first calculation was 203. We used the finite population correction since the initial sample was a substantial proportion of the entire population (7):

$$n_{\text{adjusted}} = (n * N) / (n + N - 1)$$

Where N is the entire population (3,500). This was rectified bringing the effective minimum sample size to 192.

This number was increased by 15% to adjust for possible non-response or inadequate sample quality, yielding an overall goal of collecting samples from 221 animals.

During field sampling, increased cooperation from veterinary clinics and horse owners resulted in a final samples size of 309 horses. The precision of the estimate of prevalence has increased significantly with this larger sample size. A post-hoc sample size calculation shows that under $n = 309$ and $P = 0.05$, the effective margin of error is around $\pm 2.2\%$, which enhances the power of our results.

Study design and sampling

A cross-sectional serosurvey was conducted between March 2024 and August 2025 on 326 horses from six Governorates of Jordan: Irbid, Amman, Zarqa, Jarash, Wadi Mousa (Petra region), and the Dead Sea area. Farms and stables were selected by convenience sampling to represent a wide range of management systems. Only horses with complete owner consent and a minimum age of 6 months were included.

Sample collection and serological testing

Jugular venous blood was collected and serum separated within 6 h. Each serum sample was tested for anti-*Leishmania* antibodies using a commercial ELISA kit (*Leishmania* vet ELISA; MedTec, Germany; REF: de0310) according to manufacturer's recommendations. Samples were examined in duplicate. Samples with an optical density higher than the cut-off value were considered to be positive. Known positive and negative control sera were included in each test run for quality control.

Epidemiological data and statistical analysis

A standardized questionnaire recorded signalment, reproductive status, usage, housing, and deworming history. Horses with incomplete critical data ($n = 52$) were excluded, leaving 274 for risk-factor analysis. Univariate analysis used χ^2 or Fisher's exact test; multivariable logistic regression was performed by backward stepwise selection. Analyses were conducted using IBM SPSS Statistics (version 29).

Results

Study population

After excluding 52 horses with incomplete critical data, 274 horses from six Governorates were retained for analysis. The median age was 6 years (range 7 months–24 years). The population comprised 143 females (52.2%) and 131 males (47.8%). Most horses were used for non-competitive purposes (breeding or working; $n = 215$, 78.5%), while 59 (21.5%) were competitive (racing, polo, showing, or jumping). The majority were kept in stalls (88/274, 32.1%) or stall + paddock systems (92/274, 33.6%). The breeds consisted of Arabians (116/274, 42.3), mixed local breeds

(84/274, 30.7%), Thoroughbred (28/274, 10.2% and Quarter horse (46/274, 16.8%).

Overall seroprevalence

After exclusion, 274 horses were analyzed. Thirty-one were seropositive, yielding an apparent seroprevalence of 11.3% (95% CI 7.8–15.7%).

Geographic distribution

Table 1 shows the distribution of the seropositive *Leishmania* cases in different Governorates in Jordan. The seropositivity was highly clustered in Zarqa Governorate (55.0%, 22/40) and, to a lesser extent, Wadi Mousa (11.1%, 9/81). No positives were found in Amman, Dead Sea, Irbid, or Jerash ($P < 0.001$).

Risk factors

Univariate and multivariable results are summarized in Tables 2 and 3. Independent risk factors were residence in Zarqa (OR 39.8), stall-only housing (OR 6.12), deworming exactly twice per year (OR 8.74), and male sex (OR 3.11).

Competitive usage and any pasture access were perfectly protective.

Multivariable logistic regression

All variables with $P < 0.20$ in univariate analysis were entered. The final model retained four independent risk factors as shown in Table 3.

Table 1: Seroprevalence of leishmaniasis in regions of Jordan using ELISA test

Governorate	No. tested	No. seropositive	Percentages (95% CI)
Zarqa	40	22	55.0% (38.5–70.7%)
Wadi Mousa	81	9	11.1% (5.2–19.9%)
Amman	67	0	0.0%
Dead Sea	22	0	0.0%
Irbid	51	0	0.0%
Jerash	13	0	0.0%

Table 2: Results of univariate logistic regression analysis evaluating associations between risk variables and seropositivity

Variable	Category	Prevalence (%)	Odds Ratio (95% CI)	P-value
Governorate	Zarqa vs others	55.0 vs 3.4	34.8 (14.8–81.9)	<0.001
Sex	Male vs Female	15.3 vs 7.7	2.17 (1.01–4.68)	0.044
Age group	Juvenile / Adult / Old	10.1 / 12.4 / 10.4	–	0.884
Breed	Quarter	100	∞	<0.001*
	Mix Arabians + local	20.0	3.58 (1.06–12.1)	0.034
	Others	7.4–12.2	Reference	
Usage	Non-competitive vs Competitive	14.4 vs 0.0	∞	0.002
Housing	Stall only vs any pasture/paddock	18.2 vs 4.3	4.95 (2.27–10.8)	<0.001
Deworming frequency per year	Exactly 2 times vs others	27.6 vs 3.5	10.5 (4.51–24.5)	<0.001
	≥ 3 times	0.0	0.0 (0.0–0.6)	0.013
Pregnancy stage (females only)	Second trimester vs non-pregnant	17.6 vs 8.5	2.31 (0.58–9.25)	0.227

Table 3: Results of multivariable logistic regression analysis showing factors independently associated with seropositivity

Risk Factor	Adjusted OR (95% CI)	P-value
Zarqa Governorate (vs all others)	39.8 (15.6–101.6)	<0.001
Stall-only housing (vs any pasture exposure)	6.12 (2.41–15.5)	<0.001
Deworming exactly twice per year	8.74 (3.45–22.1)	<0.001
Male sex	3.11 (1.27–7.62)	0.013

Discussion

This study documents the first equine *Leishmania* focus in Jordan, with an unprecedented 55% seroprevalence in

Zarqa Governorate. The extreme geographic clustering and strong association with stall-only housing reflects patterns observed in canine leishmaniasis foci where sand flies thrive in poorly ventilated buildings (8).

This is the first large-scale study to document *Leishmania* seroprevalence in horses across Jordan and the first to identify a dramatic geographic hotspot in Zarqa Governorate (55.0%). The overall apparent seroprevalence of 11.3% in horses with complete data is less than the reported prevalence of *L. infantum* infection in healthy horses (*Equus caballus*) from Eastern Spain (27.5%) using ELISA (1). Furthermore, our results were within the range reported from other serosurvey of *L. infantum* in equids in different European countries (9.8%) but showed extreme spatial heterogeneity that has not been previously described at this scale (9). The absence of seropositive horses in Irbid, Amman, Jerash, and the Dead Sea area, despite adequate

sampling, strongly suggests that competent *Phlebotomus* vectors are either absent or present at very low density outside Zarqa and, to a lesser extent, the southern desert region of Wadi Mousa.

Zarqa Governorate combines several conditions that are ideal for sand fly proliferation: warm climate, abundant organic waste from dense human and animal populations, poorly maintained drainage systems, and numerous small, poorly ventilated stables—exactly the type of peri-urban environment repeatedly associated with intense *Leishmania* transmission in the Mediterranean basin (10). The fact that stall-only housing was an independent risk factor (aOR 6.12) supports the hypothesis that horses kept permanently indoors in these poorly ventilated buildings are exposed to higher biting rates from endophilic vectors such as *Ph. papatasi*, *Ph. perfiliewi*, or *Ph. tobbi*, all of which have been recorded in Jordan (11).

The strong protective effect of any pasture or paddock access (even partial) is striking. Horses with daily turnout had a >80% lower odds of seropositivity, probably because they spend the critical dusk-to-dawn period outdoors when sand flies are far less active in open areas compared with dark, humid stables.

The association between exactly twice-yearly deworming and the highest seroprevalence (aOR 8.74) is counterintuitive at first glance but has a plausible explanation. In Jordan, the most common protocol is ivermectin + praziquantel in spring and autumn—exactly two treatments per year. Farms that follow this routine are typically the better-managed private stables, which are also the ones that keep valuable horses permanently indoors to protect them from injury or theft. In contrast, horses dewormed ≥ 3 times per year are almost exclusively high-level competition animals that receive intensive veterinary care and spend significant time outdoors for training, explaining their near-zero prevalence. The “never dewormed” group consists mainly of working horses in remote areas or village settings where sand fly density is low, again resulting in low exposure. Thus, twice-yearly deworming is likely a proxy marker for the combination of indoor housing and moderate (but insufficient) parasite control rather than a direct biological effect.

Male sex emerged as an independent risk factor (aOR 3.11), consistent with findings in Spain and Portugal (12). Stallions and geldings used for breeding or tourist rides are more often kept in individual boxes with limited turnout, whereas many mares (especially pregnant ones) are managed in groups with paddock access, reducing their exposure.

The apparent higher seroprevalence in second-trimester pregnant mares did not reach statistical significance in the multivariable model, probably due to small numbers, but is biologically plausible: pregnancy-induced immunomodulation may increase susceptibility or lead to

recrudescence of latent infections, as documented in canine leishmaniasis (13).

From a One Health perspective, the 55% seroprevalence in Zarqa horses represents a previously unrecognized amplification focus for *L. infantum* (or closely related species) in a governorate that already reports human cutaneous cases (14).

Jordanian authorities should implement enhanced surveillance of equine leishmaniasis and other neglected zoonotic diseases, particularly in horses, where the transmission cycle may involve zoonotic pathogens having significant public health relevance. Targeted studies to examine epidemiological factors and GIS-based predictive models combined with mosquito collection and molecular species identification are also required to further characterize the equine Leishmaniasis epidemiological landscape and improve risk-mapping accuracy.

Conclusion

Leishmania infection in Jordanian horses is geographically focal, with Zarqa Governorate acting as a major hotspot driven by intensive stall confinement of non-competitive animals. Twice-yearly deworming protocols mark the highest-risk management system. Shifting toward more frequent deworming combined with daily pasture access and basic vector-proofing of stables offers a realistic strategy to reduce *Leishmania* circulation in equine populations and potential zoonotic transmission.

Conflict of interest

None

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انتشار الأجسام المضادة لداء الليشمانيات الخيلي في الأردن: بؤرة تفشي المرض في محافظة الزرقاء وعوامل الخطر المرتبطة بها

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الخلاصة

يمثل ظهور داء الليشمانيات في الخيول مصدر قلق خاص، إذ تُعدّ الخيول ثاني أكثر العوائل الخازنة عدداً بعد الكلاب، ولها دور في دورة انتقال العدوى إلى الإنسان. وعلى الرغم من تزايد الاعتراف بالخيول كعوائل ثانوية لداء الليشمانيات، لا تتوفر حتى الآن بيانات واسعة النطاق عن هذا المرض في الأردن. أجريت دراسة مسحية مصلية مقطعية على ٣٢٦ حصاناً من سبع محافظات باستخدام اختبار الإليزا غير المباشر التجاري. وبعد استبعاد الحيوانات التي تفتقر إلى بيانات وصفية مكتملة، تم الإبلاغ على ٢٧٤ حصاناً لتحليل عوامل الخطورة. بلغت نسبة الانتشار المصلية الظاهرية الكلية ١١,٣% (٢٧٤/٣١). وكان التوزيع المكاني للإيجابية المصلية بؤرياً بشكل واضح؛ إذ بلغت ٥٥,٠% في محافظة الزرقاء مقابل ١١,١-٠% في المحافظات الأخرى ($P < 0.001$). وأظهرت التحليلات متعددة المتغيرات أن عوامل الخطورة المستقلة تمثلت في الإقامة في محافظة الزرقاء (نسبة الأرجحية المعدلة = ٣٩,٨)، والإيواء داخل الإسطبل فقط (نسبة الأرجحية المعدلة = ٦,١٢)، وإجراء التجريع ضد الديدان مرتين سنوياً بالضبط (نسبة الأرجحية المعدلة = ٨,٧٤)، وكون الحيوان ذكراً (نسبة الأرجحية المعدلة = ٣,١١). ولم تُسجل أي حالات إيجابية مصلية بين الخيول المشاركة في الأنشطة التنافسية أو تلك التي لديها إمكانية الوصول إلى المراعي. وتشير النتائج إلى أن الأردن يضم بؤرة غير معروفة سابقاً لداء الليشمانيات في الخيول في محافظة الزرقاء، تقودها ممارسات الإيواء المكثف داخل الإسطبلات. ويمكن لإجراء تغييرات بسيطة في نظم الإيواء وبرامج التجريع أن تسهم بشكل كبير في تقليل التعرض للعدوى. كما أن هناك حاجة إلى إجراء المزيد من الدراسات حول داء الليشمانيات في الخيول في الأردن باستخدام تقنيات جزيئية أكثر تقدماً. وتمثل هذه الدراسة أول تقييم مصلي واسع النطاق لداء الليشمانيات في الخيول في الأردن، وتشكل إسهاماً مهماً في تحديد توزيعه الجغرافي وتقدير إمكاناته في البلاد.