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Original Article **Open Access**

Seroprevalence of and associated risk factors for *Leptospira interrogans* serovar Hardjo infection of cattle in Setif, Algeria

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Abstract:

Background: Leptospirosis is a cosmopolitan zoonosis caused by *Leptospira interrogans* responsible for heavy loss both economically and in health, in humans and animals. This study was conducted to determine the seroprevalence and risk factors associated with *Leptospira interrogans* serovar Hardjo infection in cattle in the state of Setif, northeastern Algeria.
Methodology: Between the period 2015 and 2019, a total of 48 randomly selected herds of cattle were investigated, and 406 sera from apparently healthy cattle were analyzed using an indirect enzyme-linked immuno-sorbent assay (ELISA). In order to determine possible risk factors related to leptospirosis, a pre-validated questionnaire was administered to herd owners.
Results: The herd prevalence of *Leptospira interrogans* serovar Hardjo was 31.25% (15/48, 95% CI 19.95–45.33) while the cattle prevalence was 5.42% (22/406, 95% CI 3.61–8.07). Multivariable logistic regression analysis showed that the age of cattle between 3 and 6 years (OR = 9.25; $p< 0.03$), breeding herd size > 20 cows (OR = 13.65; $p< 0.01$), and semi-intensive management system (OR = 0.21; $p< 0,02$) were significantly associated with seropositivity to *Leptospira interrogans* serovar Hardjo.
Conclusion: We concluded from this study that *Leptospira interrogans* serovar Hardjo is circulating among cattle farms in the state of Setif, Algeria. Furthermore, we recommend more studies to be carried out to prove the infectivity and implementation good hygienic practices among cattle farms and people at risk.

Keywords: ELISA, herds, questionnaire, leptospirosis, prevalence, Algeria

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Séroprévalence et facteurs de risque associés de l'infection à *Leptospira interrogans* serovar Hardjo des bovins à Sétif, Algérie

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Abstrait:

Contexte: La leptospirose est une zoonose cosmopolite causée par Leptospira interrogans, responsable de lourdes pertes économiques et sanitaires, chez l'homme et l'animal. Cette étude a été menée pour déterminer la séroprévalence et les facteurs de risque associés à l'infection à *Leptospira interrogans* serovar Hardjo chez les bovins de l'État de Sétif, dans le nord-est de l'Algérie.
Méthodologie: Entre 2015 et 2019, un total de 48 troupeaux de bovins sélectionnés au hasard ont été étudiés, et 406 sérums de bovins apparemment sains ont été analysés à l'aide d'un test d'immunosorbant indirect lié à une enzyme (ELISA). Afin de déterminer les facteurs de risque possibles liés à la leptospirose, un questionnaire pré-validé a été administré aux propriétaires de troupeaux.

Résultats: La prévalence du troupeau de *Leptospira interrogans* serovar Hardjo était de 31,25% (15/48, IC 95% 19,95–45,33) tandis que la prévalence bovine était de 5,42% (22/406, IC 95% 3,61–8,07). Une analyse de régression logistique multivariable a montré que l'âge des bovins entre 3 et 6 ans (OR=9,25; p<0,03), la taille du troupeau reproducteur >20 vaches (OR=13,65; p<0,01) et le système de gestion semi-intensive (OR=0,21; p<0,02) étaient significativement associés à la séropositivité pour le sérovar Hardjo de *Leptospira interrogans*. **Conclusion:** Nous avons conclu de cette étude que *Leptospira interrogans* serovar Hardjo circule parmi les élevages bovins de l'Etat de Sétif, en Algérie. De plus, nous recommandons de mener plus d'études pour prouver l'infectivité et la mise en œuvre de bonnes pratiques d'hygiène dans les élevages bovins et les personnes à risque.

Mots-clés: ELISA, troupeaux, questionnaire, leptospirose, prévalence, Algérie

Introduction:

Leptospirosis is a worldwide zoonotic disease, representing a global public health problem, with high morbidity and mortality (1). It is an emerging infectious disease caused by a pathogenic bacterium, *Leptospira interrogans*, which infects humans as well as wild and domestic animals (2). The dissemination of this pathogen occurs mainly by exposition to urine of the major reservoirs (rodents) and other animals directly or indirectly via urine-contaminated water (1). *Leptospira* species have about 300 serovars clustered into 28 serogroups (3). Among all serovars, *Leptospira interrogans* serovar Hardjo is considered as the main serovar responsible for leptospirosis in cattle (4). Bovine leptospirosis is most often a chronic subclinical disease (5), mainly characterized by reproductive losses including abortions, stillbirth and miscarriages as well as reduced weight gain, mastitis, and decreased milk yield (6,7,8). However, laboratory tests, mainly serological methods, are used to confirm diagnosis (9).

The most widely reported risk factors for leptospirosis in bovine herds are age of cows, large herd size, presence of dogs in rural properties co-grazing with other infected animals, contaminated water sources, use of natural breeding, purchase of replacement heifers from infected herds, and dirty drenching equipment (10,11,12). Nevertheless, these risk factors are highly variable among different regions (13,14,15).

Several studies have been conducted in many countries of the world to determine the prevalence of *Leptospira* serovar Hardjo infection in cattle and have reported prevalence rates ranging from 3% to 50% at the animal level (16, 17). In Algeria, only one serological study on bovine leptospirosis has been conducted targeting a small number of cows in Algiers region (18). Therefore, the objectives of this study are to determine the seroprevalence of *Leptospira interrogans* serovar Hardjo infection in apparently healthy cows in the Setif region of Algeria, and to identify risk factors associated with its seropositivity.

Materials and method:

Study area

The study was carried out in the high plains of Setif in the Northeast of Algeria. This region covers approximately 6,550 km^2 and lies between the longitudes 4°73′ - 6°02′ and the northern latitudes of 35°61′-36°59′. The climate is semi-arid Mediterranean, characterized by cold rainy winters and hot dry summers. The average annual rainfall was 350 mm from 1984 to 2014. The study area contains approximately 161,952 cattle, including 79,354 dairy cows distributed in 4,465 dairy herds (Agricultural Services Direction of Setif 2015) (Fig. 1).

Study design and sampling

The study is descriptive cross sectional targeting small to medium sized cattle herds located in the Setif region of Algeria, conducted during the period 2015 to 2019. We calculated the sample size using the formula for simple random samples recommended by Thrusfield (19) which is $n = (1.96)^2 \frac{Pexp(1-Pexp)}{d^2}$where 'n' is the required sample size; 'Pexp' is expected prevalence; 'd' is desired absolute precision; and 1.96 is the Z value for the selected confidence level (95%). According to this formula, the minimum sample size for an infinite population was calculated to be 384 cows using an expected individual prevalence of 50% (as there were no previous studies in this region), a desired absolute precision of 5%, and a confidence level of 95%. The sample size was increased to 406 in order to increase the absolute precision and compensate for 5% attrition.

A total of 406 apparently healthy cows, from which blood samples were collected, were therefore selected by simple random sampling technique among the cattle population aged 3 and 13 years old from a total of 48 cattle herds (Fig. 1). The minimum number of cows to be tested from each herd was established as 10 (20), corresponding to the probability of detecting at least one seropositive animal per herd. For herds with less than 10 cows, all of them were selected for testing.

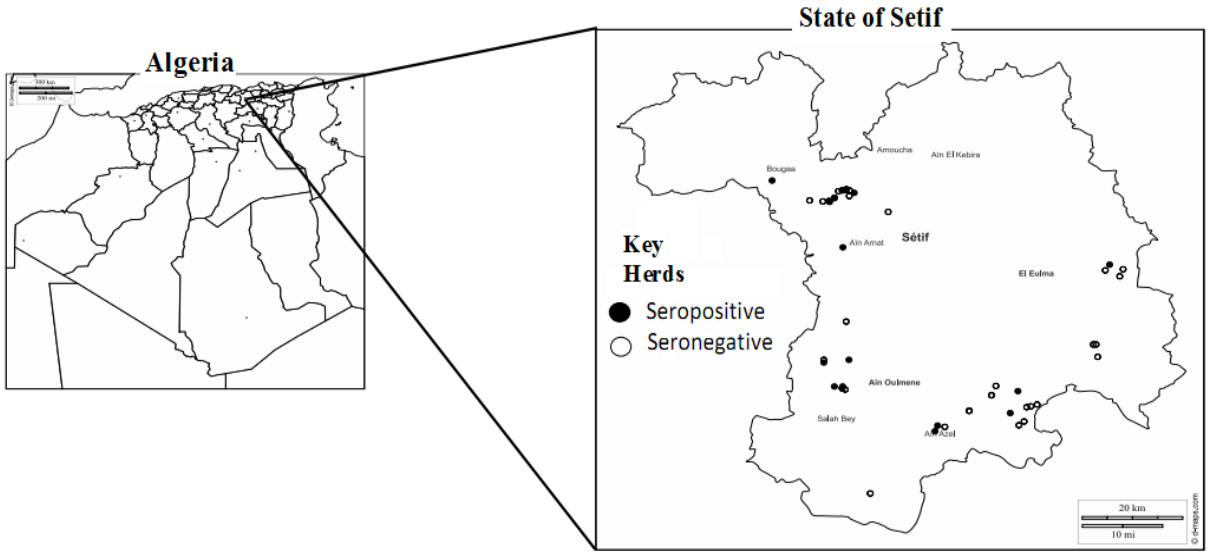

Fig.1: Map of Setif state in the northeastern Algeria, showing the coordinates of the sample locations and spatial distribution of cattle herds that were seropositive for *Leptospira interrogans* serovar Hardjo infection using Arc GIS software

Collection of samples and conservation

After complete physical examination of the enrolled apparently healthy cows, about 10 mL of whole blood was aseptically collected from each cow via the coccygeal vein into plain vacutainer sterile tubes using disposable needles. The tubes were then numbered and immediately transported, on ice, to the laboratory. Serum was obtained by centrifugation of clotted samples at 3000 g for 5 to 10 minutes. The sera were immediately transferred to Eppendorf® tubes and rapidly stored in a freezer at -20°C until laboratory analysis was performed.

Data collection

A pre-validated structured questionnaire was interview-administered to herd owners at the time of blood collection in order to obtain information on potential risk factors associated with *Leptospira interrogans* serovar Hardjo infection. The questionnaire included information related to the herds visited (management system, breeding type and herd size) and the cows enrolled (breed, age, pregnancy, body condition score and history of abortion).

Serological test

The sera were analyzed in the National Center for Biotechnology Research of Constantine (CRBt). For the detection of specific antibodies against *Leptospira interrogans* serovar Hardjo, we used an indirect ELISA test of the PrioCHECK™ *L. hardjo* Ab Strip Kit (Thermo Fischer Scientific, Netherlands) following the protocol prescribed by the manufacturer. The result of each sample was expressed as percent positivity (% PP), which was calculated according to the formula; % PP = (correct OD_{450} test sample/correct OD_{450} reference serum1)x 100. Samples with a % PP greater than 45% were considered positive; % PP between 20 and 45% were considered as inconclusive, and those less than 20% were considered negative. Inconclusive results were considered negative in this study.

Statistical analysis

The relationship of the risk factors potentially associated with *Leptospira interrogans* serovar Hardjo seropositivity was evaluated in two steps; univariable and multivariable analyzes. In the univariable analysis, Chi-square or Fischer tests was used to examine the association of each variable to Hardjo sero-positivity (21). Variables with a $p \leq 0.20$ were subjected to multivariable logistic regression analysis (22), which was expressed by a significance level (p) of 5%, odds ratio (OR), standard error (SE) and 95% confidence interval (CI). The statistical analysis was performed using SPSS IDEM 23.0 software for Windows.

Results:

Overall seroprevalence of *Leptospira interrogans* serovar Hardjo

The results showed that of the 406 animals, 22 tested positive for *Leptospira interrogans* serovar Hardjo, with an individual prevalence rate of 5.42% (95% CI 3.61–8.07%), and 15 of the 48 herds tested positive, with herd seroprevalence rate of 31.25% (95% CI 19.95–45.33%) (Table 1). The seroprevalence rate of the cows per herd ranged from 7 to 33.3% (95% CI ± 4.63, mean 17.90%, Q1 10%, median 20%, Q3 25%).

Table 1: Seroprevalence of *Leptospira interrogans* serovar Hardjo infection in individual cows and herds sampled in Setif, Algeria

Cow/herd	Number sampled	Number of positive samples	Seroprevalence rate (%)	95% confidence interval	
				Lower	Upper
Individual cow	406	22	5.42	3.61	8.07
Herd of cattle	48	15	31.25	19.95	45.33

Table 2: Seroprevalence and risk factors associated with *Leptospira interrogans* serovar Hardjo infection among cattle in Setif, Algeria during the period 2015 to 2019

Variables	Categories	Number of animal sampled	Number positive	Prevalence (%)	*p* value
Pregnancy	Yes	244	13	5.33	0.929
	No	162	9	5.65	
Age of cows (years)	3-6	286	21	7.34	0.008
	>6	120	1	0.83	
Body condition score	Good	156	11	7.05	0.244
	Medium	250	11	4.40	
Breeding type	Dairy	335	17	5.07	0.51
	Mixed	71	5	7.04	
Management system	Intensive	33	4	12.12	0.129
	Semi-intensive	353	18	5.10	
	Extensive	20	0	0.00	
Herd size	5-10	74	1	1.35	0.001
	10-20	196	4	2.04	
	>20	136	17	12.40	
Breed	Crossed	74	4	5.41	0.959
	Imported	318	17	5.35	
	Local	14	1	7.14	
History of abortion in previous years	Yes	31	3	9.98	0.28
	No	375	19	5.07	

Risk factors analysis

Table 2 shows the results observed for the risk factors. The univariable analysis showed significant association of Hardjo infection with both herd size (p=0.008) and age of cow (p=0.001). In the multivariable (Table 3) analysis, there was significant association between herd size and *Leptospira interrogans* serovar Hardjo sero-positivity. Herds with more than 20 cows were 13 times more likely to contract leptospirosis than those with 10-20 and 5-10, therefore sero-positivity increases with the herd size. Also, cows under 6 years of age were more likely to be seropositive compared to cows over 6 years old (OR: 9, p=0.03). In addition, herds raised in semi-intensive system have lower risk of being seropositive to *Leptospira interrogans* serovar Hardjo compared with herds in the intensive system (OR 0.21, p=0.02).

Table 3: Multivariate analysis of risk factors for *Leptospira interrogans* serovar Hardjo infection of cattle in the State of Setif, Algeria

Risk factors	Standard Error (SE)	Odds ratio (OR)	P value	95% confidence interval	
				Lower	Upper
Semi-intensive system	0.68	0.21	0.02	0.06	0.82
Age of cows (3-6 years)	1.04	9.25	0.03	1.19	71.47
Herd size >20	1.02	13.65	0.02	1.58	117.7

Discussion:

The main objective of this study was to determine the prevalence and risk factors for *Leptospira interrogans* serovar Hardjo infection among herds of cattle in the state of Setif, northeast of Algeria. To our knowledge, this is the first major study to report the seroprevalence and risk factors of *Leptospira interrogans* serovar Hardjo infection in cattle in Algeria. Often, the diagnosis of bovine leptospirosis is complex and difficult. Apart from abortion, *Leptospira interrogans* infection in cows is often asymptomatic and unnoticed (23). Consequently, the use of serological tests remains the only alternative to screen animal for infection *in vivo*. ELISA is a commonly used method for initial serological screening for the detection of specific antibodies against *Leptospira interrogans* (9), because it is practical, rapid, inexpensive, and has higher throughput (24). Compared to the micro-agglutination test (MAT) (the reference serological method for leptospirosis detection), ELISA is characterized by a high sensitivity and specificity (9). For these reasons, we used indirect ELISA to determine the seroprevalence of specific antibodies against *Leptospira interrogans* serovar Hardjo natural infection in healthy unvaccinated cows, as vaccination against bovine leptospirosis is not a usual practice in Algeria.

In the present study, we report an individual seroprevalence rate of 5.42%, which is quite similar to 3.89% reported by the only study performed in Algiers region in Algeria (18). The rate in our study is also similar to what have been reported by studies in other countries such as Malaysia with 2.4% (25), Nigeria 8.44% (26), Laos 3% (27), Iran 5.61% (28), Trinidad 4.1% (29), Mexico 2.4% (30) and Turkey 2.8% (31). Contrariwise, higher prevalence rates have been reported in studies from Tanzania 14.76% (17), India 27.76% (32), Jordan 26.25% (13), Bangladesh 47.27% (14), New Zealand 45.6% (33), and extremely high rate in Brazil 83.3% (34). However, the rate in our study is still higher than 0.8% reported from Morocco (35) and 1% from Spain (36). Similarly, in this study, the herd prevalence was 31.25%, which is much lower than rates reported in other countries such as Brazil with 100% (37), Jordan 92.3 % (13) and Ireland 82.29% (11). On the other hand, this rate is higher than 11% reported in Spain (36). These discrepancies in individual and herd prevalence rates between regions and countries can be attributed to several factors, such as local ecological factors, type of management and husbandry practices, levels of natural immunity and disease resistance among different breeds (13,14).

Another aspect of our work was to study the risk factors associated with *Leptospira interrogans* serovar Hardjo seropositivity. Indeed, univariable and multivariable analyses showed that herd size, age of cows and management system are the major risk factors that have potential impact on the Hardjo seroprevalence in cattle. We observed that Hardjo sero-positivity increases with herd size (OR of 13 and seroprevalence rate of 12.40% for size > 20). In the literature, many studies have reported the same findings (11,14,38,39). This observation might be attributed to poor quality of sanitation facilities and the great difficulty in monitoring the hygienic measures on large-scale compared to small dairy farms, and the rapid spread of infection in overcrowded farms, which is a potential risk factor for higher prevalence of leptospirosis (14).

In this study, cows aged 3 to 6 years old were more likely to be infected by Hardjo (OR of 9 with seroprevalence 7.34%) compared to the other age category (more than 6 years old with seroprevalence of 0.83%). Indeed, Ijaz et al., (40) reported that age is a potential risk factor for *Leptospira interrogans* serovar Hardjo infection, where young animals were more likely infected than adults. This has been reported by others studies (11,41,42). Moreover, cows raised in semi-intensive system have lesser chance of becoming seropositive to the disease, with a seroprevalence of 5.10% (OR of 0.2), compared to those raised in an intensive

system, where the seroprevalence was 12.12%. This finding might be explained by the fact that farms in intensive system suffer from massive infestation by rodents (major reservoirs of leptospirosis), poor food storage (direct contact of rodents with cattle feed), and long-term direct contact between animals, which was observed by us during this study. Moreover, Dos-Santos et al., (38) attributed this problem to the poor husbandry practices and to the fact that infected animals in overcrowded and confined conditions increase the risk of contaminating the environment. In contrast to our findings, Ismail et al., (13) reported that cattle under semi-intensive system were more likely to be infected by leptospirosis. However, in their study, they investigated both Hardjo and Pomona serovars. For the last one, boars were the main reservoir, and were more in contact with semi-intensive farms and less likely to be in touch with animals in well-isolated herds under intensive system.

Conclusion

In conclusion, results of this study showed that *Leptospira interrogans* serovar Hardjo is present and circulating in apparently healthy cows in Setif state of Northeastern Algeria. Herd size, age of cows and as well as management system were key associated factors for leptospirosis in these herds. Therefore, we recommend very wide surveys in animals and humans all over the country to assess the real prevalence of leptospirosis in Algeria. We also recommend the implementtation of hygiene practices and biosecurity measures in farms to reduce the spread of infection, as well as the use of vaccination in animals and people at risk.

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