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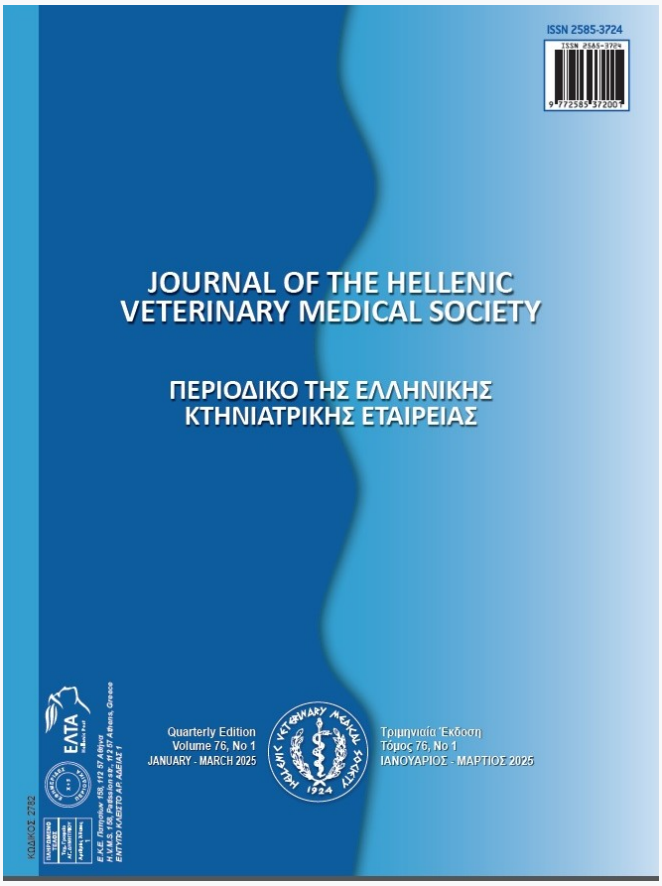

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Elisa And Mat Tests For Leptospira Interogans Detection From Cattle In Eastern Algeria

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ABSTRACT: *Leptospira interrogans* is one of the most common infectious organisms worldwide that causes several losses in cattle. As far as we know, few previous studies have focused on *Leptospira interrogans* infection in cattle from Algeria. This study aimed to assess the prevalence of bovine leptospirosis, identify selected risk factors, and compare two different detection tests. 611 blood samples from 67 cattle farms were collected in six Algerian provinces; Algiers, Boumerdès, Bordj Bou Arreridj, Sétif, Batna, and Souk Ahres. Sera samples were analyzed for the presence of antibodies against five serovars of *Leptospira interrogans* using a microscopic agglutination test (MAT), using 50% agglutination, at a dilution ≥1: 100 as a cut-off point. A commercial indirect enzyme-linked immunosorbent assay (ELISA) test was used to determine the seroprevalence against *L. interrogans* serovar Hardjo. Moreover, a survey through breeders' questionnaires was conducted to identify the potential risk factors of Leptospira interrogans infection. The seroprevalence of *L. interrogans* infection using MAT in the cows was 17.02% (95% confidence interval [CI]: 14.12-20.24) and in the farms was 83.58% (95% CI: 59.31-81.99). The most commonly detected serovar was Hardjo 6.71% (95% CI: 4.86-8.99) followed by Icterohaemorrhagiae 5.07% (95% CI: 3.47-7.12). Finally, the last serovar present was the Grippotyphosa 2.78 (95% CI: 1.63-4.42). The comparison between the two serological methods, considering the MAT as the reference test, shows that the PrioCHeck ELISA kit had a sensitivity of 63.4% (95% CI: 48.7-78.2), a specificity of 98.9% (95% CI: 98.1-99.8), and a reliability of 96.6% (95% CI: 95.1-98.0). The kappa coefficient was 0.62, and the McNemar test showed a P = 0.23. Multivariable logistic regression analysis showed that the semi-intensive system was a protective factor against leptospirosis, with an odds ratio of 0.35 (95% CI: 0.16-0.78). The study findings indicate that leptospirosis is a serious issue in farms located in selected provinces in Algeria, with a high incidence rate noted there. The semi-intensive system's significance as a leptospirosis protective factor is to create control strategies that decrease the probability of infection in both humans and cattle.

***Keywords*:** Algeria; *Leptospira interrogans;* cattle; MAT, ELISA; seroprevalence.

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INTRODUCTION

A wide range of domestic and wild animal species have the potential to serve as accidental or natural hosts, leading to the recurrence and extensive distribution of leptospirosis (Coppola et al., 2020). Until now, 38 species of pathogenic Leptospira have been identified, which include subclades 1 and 2, which were formerly known as intermediate and pathogenic Leptospira, respectively (Vincent et al., 2019). More than 300 serovars of the pathogenic Leptospira taxa currently identified have been divided into serogroups based on their antigenic similarities (Levett, 2015).

Due to medical professionals' unawareness and the wide range of clinical symptoms that often imitate other infectious diseases, the diagnosis is frequently overlooked, especially in mild cases. Since a diagnosis based solely on clinical grounds may be difficult, a laboratory-based diagnosis is therefore required (Biggs et al., 2011; Levett, 2001).

Maintenance hosts are natural sources of pathogens that have a significant impact on the epidemiology of Leptospira spp. even though they typically lack clinical symptoms of the disease (Cerri et al., 2003). Several serogroups that were previously unknown in domestic and wild animals have surfaced recently, indicating that the epidemiology of leptospirosis may vary over time (Tagliabue et al., 2016). As an illustration, in wild boars, the assessed seropositivity for pathogenic *Leptospira* was 96.10%, and for intermediate *Leptospira*, 3.90%. Similarly, Cilia et al. (2020) reported no sex preference in the pathogenic *Leptospira* infection ratio among wild boars, with infection rates of 11.50% in males and 12.75% in females (Cilia et al., 2020).

In particular, Hardjo serovars and the Sejroe serogroup depend on cattle as maintenance hosts. These are made up of two genetically different but serologically identical strains: *Leptospira interrogans* serovar Hardjo (Hardjo-prajitno), which is frequent in various parts of the world, and Leptospira borgpetersenii serovar Hardjo (Hardjo-bovis), which is the common strain of this serovar in cattle (Aliberti et al., 2022).

In Italy, the Pomona serogroup is the second most common serotype among cattle, even though serious infections in cattle caused by the Pomona serogroup are uncommon and primarily affect young animals (Ellis, 2015). The frequency of this disease has increased in North-Central Italy due to contact with wild animals, especially wild boars, since extensive farming is the widespread practice (Bertelloni et al., 2019).

According to epidemiological data retrieved from recent investigations conducted at MAT laboratories in Italy, Germany, and France, 19% to 26% of tested animals were serppositive (André-Fontaine, 2016; Bertelloni et al., 2019; Strutzberg-Minder et al., 2018), Meanwhile, the most common serovars are Australis and Icterohaemorrhagiae, with frequencies of 48.5% and 38.2%, respectively (Coppola et al., 2020; Naudet et al., 2022).

Algeria's veterinary community has gradually come to terms with the losses caused by leptospirosis at the regional and national levels. In this regard, we started collaborating with other groups to establish a more precise epidemiological map of the leptospirosis situation in the country (Benseghir, 2021; Benseghir et al., 2020; Derdour et al., 2017; Zaidi et al., 2018).

This study is the first epidemiological approach to bovine leptospirosis in the Eastern region of Algeria using MAT (Microscopic agglutination test) which is the reference method for detection as a screening test. The main objective of this study is to proceed with risk factors analysis, serological tests assessment (MAT and ELISA), and provide the epidemiological situation of leptospirosis in this region.

MATERIALS AND METHODS

The study area

The study was carried out from January 2014 to September 2019. The study was conducted in six provinces: Algiers, Boumerdes, Bordj Bou Arreridj, Setif, Batna, and Souk Ahres. These Provinces are located in North-Central and the Eastern Algeria. This region lies between longitudes 2°48'E-8°20' and northern latitudes 36°55'-35°03'. The geographical locations of all districts selected in this study are indicated in Figure 1. The Mediterranean climate (semi-arid, cold, rainy winters and hot, dry summers) is characteristic of this region.

Sampling approach

This study targets small and medium-sized cattle farms located in some provinces of Algeria. We calculated the sample size using this formula $n = (1.96)^2 P(1-Pexp) / d^2$ Where: n = The required sample size; Pexp = Expected prevalence; d = desired absolute precision (Thrusfield, 2007). Using an expected individual prevalence of 50%, an absolute precision of 5%,

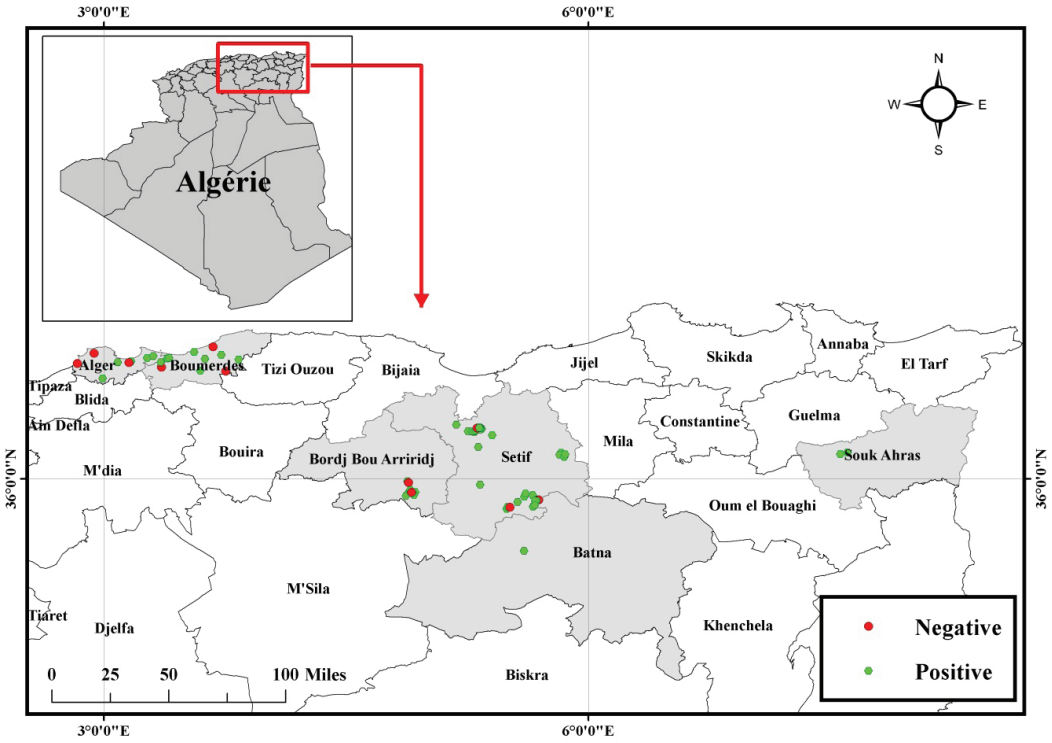

Figure 1. Map of studied provinces located in North-Central and the Eastern Algeria showing the coordinates of the sample locations and spatial distribution of seropositive cattle herds for *Leptospira interrogans* infection using ArcGIS software.

and a confidence level of 95%, we obtained a minimum sample size of 384 cows. However, to increase the accuracy of the results, we took a random sample of 611 blood samples from cows aged between 3 and 13 years from six provinces. In total, 67 cattle farms were involved in our study (Table 1). On each farm, at least 10% of the cows were randomly selected (having aborted or not, sick or not, pregnant or not, of different ages and breeds) (Cannon, R. M. & Roe, 1982).

The study was conducted over six years, from 2014 to 2019. Blood samples (5 to 10 ml) were taken in dry tubes (vacutainer vacuum system) from the caudal vein, and serums were obtained after centrifugation for 5-10 minutes at 3000 rpm. The serums were stored at -20°C until serological tests were carried out.

Preset questionnaire and data collection

An epidemiological questionnaire was used and filled out by breeders during farm visits to analyze the potential risk factors linked to infection by *Leptospira interrogans*. The questionnaire includes elements related to **(i)** the visited farms (breeding system, type of farm, and size of herd) and **(ii)** the breeding cows (breed, age, pregnancy, body condition), emphasizing whether the farm had experienced episodes of abortion to analyze its risk factors.

Serological analyses

611 sera were collected and analyzed at the National Centre for Research in Biotechnology of Constantine for the ELISA test and at the Pasteur Institute of Algeria for the test MAT (Leptospira Unit).

For the detection of antibodies directed against *Leptospira interrogans* serovar Hardjo, we used an indirect ELISA test of the PrioCHECK™ L. Hardjo Ab Strip Kit (Thermo Fisher Scientific, Holland) according to the manufacturer's recommendations.

The Microscopic Agglutination Test (MAT) was used for all serum samples, as described by OIE standards applied to diagnostic tests for terrestrial animals 2008 and Wasinski and Pejsak, 2010. This test served us not only to confirm the results obtained by the ELISA tests but also to identify different serovars of the species *Leptospira* spp. Icterohaemorrhagiae, Hardjo, Pomona, Grippotyphosa, and Canicola.

The MAT test was carried out on live strains belonging to the serogroups *L. interrogans* maintained and cultivated in the Pasteur Institute laboratory (Leptospira's unit, Hamma, Algiers).

Statistical and risk factors analysis

The potential association between risk factors and

seropositivity towards *Leptospira interrogans* was analysed in two stages: multivariable and multivariable analyses. In the univariable analysis, the Chi-square test (Zar, 1999) was used to verify the independence of each variable concerning the seropositivity of *Leptospira* spp. (5 tested serovars for MAT). Variables with $P \leq 0.20$ were subjected to multivariable logistic regression analysis (Hosmer and Lemeshow, 2000). The model of the multivariable analysis is expressed by a significance level (P) of 5%, odds ratio (OR), standard error (SE), and 95% confidence interval (CI). Statistical analysis was performed using SPSS IDEM 20.0 software for Windows.

Method comparisons with the calculation of specificity, sensitivity, accuracy, and Cohen's Kappa coefficient were carried out using WinEpiscope 2.0 (available online: http://www.winepi.net/ uk/index.htm).

The Cohen's kappa (k) is a coefficient intended to measure the agreement between two qualitative variables having the same modalities. Classically, it is used to measure the degree of concordance between the stages attributed by two judges. It can also be applied to measure intra-observer agreement. It was calculated and evaluated as previously described (Kirkwood and Sterne, 2003). The coefficient k varies between -1 and 1 (1 is the maximum agreement).

The McNemar test was applied to the analytical test results and the P values were calculated using SPSS software. The test was considered to be significantly different from the reference test when $P < 0.05$ (Kirkwood, B. R. & Sterne, 2003).

RESULTS

Overall Leptospira-specific antibodies seroprevalence by the MAT test

A total of 611 sera from 67 different cattle farms were analyzed. An individual seroprevalence of 17.02% (CI 95%, 14.12-20.24%) was obtained, with 104 animals found to be positive for one or more Leptospira serovars at a dilution ≥ 1:100 and 50% agglutination by the MAT test. In addition, a value of 56/67 farms (83.58%, 95% CI, 59.31-81.99%) was positive for Leptospira (Table 1). The number of positive cows per farm ranged from 1 to 4 out of 6-10 tested.

Seroprevalence of cattle Leptospirosis using the MAT technique

Overall individual and combined serovar prevalence

The most prevalent Leptospira serovar was Hardjo with 41 positive samples (6.71%), followed by Icterohaemorrhagiae and Canicola serovars with 31 (5.07%) and 28 (4.48%) respectively. Finally, the last prevalent serovar was Grippotyphosa serovar with 17 positive samples (2.78%) (Table 2).

The results of the MAT protocol showed that 104

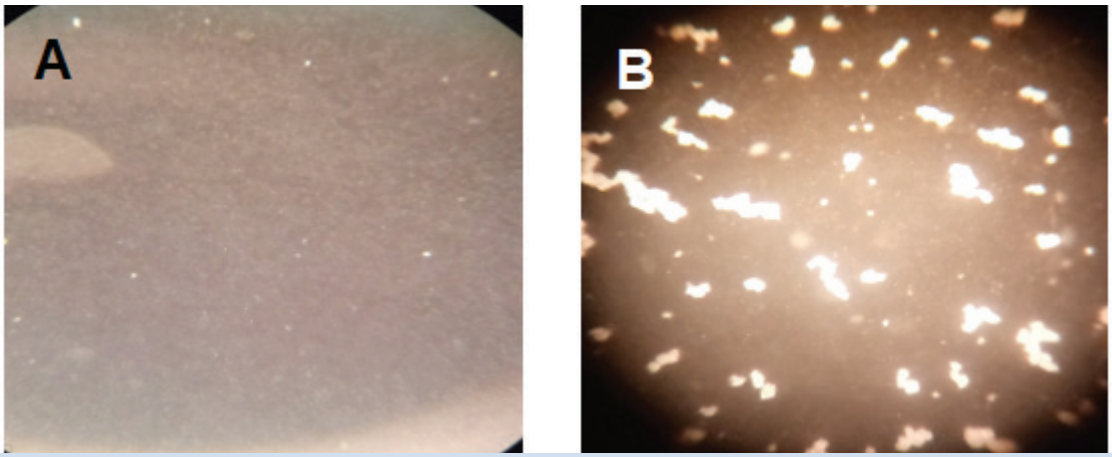

Figure 2. Photomicrograph of Microagglutination Test (MAT) using dark-field microscopy (A) Live leptospiral organism with no agglutination (Negative control), (B) Agglutination (Positive control).

Table 1. Results of the serological analyses obtained by MAT against five different serovars of Leptospira in the studied area

Cow /farms	Number of samples	Number of positive samples	Seroprevalence	Confidence interval 95%	
				Lower	Upper
Cow	611	104	17.02	14.12	20.24
Farms	67	56	83.58	74.70	92.50

serum samples (17.02%) had detectable antibodies against at least one serovar of *L. interrogans* at a dilution of ≥ 1:100. Positive titers against more than one serovar were detected in 26 sera of the positive samples (Table 3). Therefore, there were 141 positive reactions against different serovars of *L. interrogans.*

Antibody titer obtained by the MAT test for 5 serovars of Leptospira

Animals were considered positive when titers were ≥ 1:100. We have noticed that the most frequent antibody titer is the 1:100 dilution (48.94%) with 69 animals, followed by the dilution of 1:200 (21.99%) and finally, the titer of 1:3200 (0.71%). For the serovar Icterohaemorrhagiae, different dilution degrees were not enough to reach a level of sample clearance (Table 4). At a dilution over 1:400, Canicola antibodies were not detected at all, while serovar Hardjo, at a dilution 1:200, needed a higher titer.

Comparison between the serological methods applied for the detection of *L. interrogans* serovar Hardjo-specific antibodies

The performance of the ELISA kit (PrioCHeck) for demonstrating antibodies directed against *L. interrogans* serovar Hardjo was evaluated using MAT as a

Table 2. Individual seroprevalence of five Leptospira serovars demonstrated by MAT in cattle in the studied regions

Serovars	Positive Number	Frequency % (CI 95%)
Hardjo	41	6.71 (4.86-8.99)
Icterohaemorrhagiae	31	5.07 (3.47-7.12)
Canicola	28	4.58 (3.07-6.56)
Pomona	24	3.93 (2.53-5.79)
Grippotyphosa	17	2.78 (1.63-4.42)

Table 3. Frequency (%) and number of positive serum samples by MAT at a dilution of 1:100 in terms of number of serovars among 611 samples

Number of serovars	Number of positive sera	Frequency %
One serovar	78	75
Two serovars	17	16.34
Thress serovars	8	7.69
Four serovars	/	/
Five serovars	1	0.96
Total	104	17.02

Table 4. Distribution of antibody titers according to Leptospira serovar

Serovar	Antibodies titers (n: %)						Total
	1:100	1:200	1:400	1:800	1:1600	1:3200	
Hardjo	14 (34)	17 (41)	3 (7)	5 (12)	2 (5)	0 (0)	41(29.08)
Icterohaemorrhagiae	12 (38)	4(13)	7(23)	4 (13)	3 (10)	1 (3)	31 (21.99)
Canicola	18(64)	6 (21)	0 (0)	2 (7)	2 (7)	0 (0)	28 (19.86)
Pomona	14 (58)	2 (8)	3 (13)	3 (13)	2 (8)	0 (0)	24 (17.02)
Grippotyphosa	11 (65)	2 (12)	1 (6)	2 (12)	1 (6)	0 (0)	17 (12.06)
Total	69 (48.94)	31 (21.99)	14 (9.93)	16 (11.35)	10 (7.09)	1 (0.71)	141 (100)

Table 5. Comparison between serological tests (MAT and ELISA) in the detection of specific antibodies to *L. interrogans* serovar Hardjo

Comparison between Hardjo cross table * ELISA Prevalence					
			Prevalence by MAT		**Total**
			Positive	Negative	
ELISA	Positive	Effective	26	6	32
	Negative	Effective	15	564	579
	Total	Effective	41	570	611
Sensitivity: 63.4% (48.7%, 78.2%)					
Specificity: 98.9% (98.1%, 99.8%)					
Fiability: 96.6% (95.1%, 98.0%)					

reference test (Table 5). We calculated the sensitivity, specificity, and reliability of the test, as well as Cohen's kappa coefficient and the McNemar test.

In the study condition, the obtained results demonstrated that the ELISA test sensitivity was 63.4% (95% CI 48.7-78.2%), specificity 98.9% (95% CI 98.1-99.8%), and the reliability 96.6% (95% CI, %95.1-98.0%). The concordance calculation between the two methods (ELISA and MAT) using Cohen's Kappa coefficient gave a value of k=0.62 corresponding to a satisfactory agreement of concordance between the two methods. The result of the McNemar test showed a P = 0.23 (P>0.05) which means that the two methods gave significantly non-different values.

Risk factors analysis

Based on the results of this seroprevalence study, we were able to identify the risk factors that seem to increase the chances of seropositivity to Leptospira by the MAT test.

We analyzed two types of factors: those related to the farms (breeding system, type of farm, herd size, close contact with other animals, presence of dogs, and the source of water) and those related to the animals sampled (gestation, age of cows, parity, breed and history of abortions).

The univariable statistical study using the Chi-square test showed a significant association (P < 0.05) depending on the type of breeding system. All factors with a P < 0.2 were retested by multivariable analysis with binary logistic regression (Table 6).

The multivariable analysis confirmed that the seroprevalence significantly varied depending on the breeding system. Specifically, cattle in semi-intensive systems had a lower risk of seropositivity to *Lepto-*

Table 6. Seroprevalence and univariate analysis of risk factors associated with seropositivity to Leptospira in cows analyzed by the MAT test in this region

Independent variables	Categories	Number of animals sampled	Number of positive animals	Prevalence %	P-Value
Gestation	Yes	369	60	16.26	0.54
	No	242	44	18.18	
Parity	Primiparous	124	19	15.32	0.57
	Multiparous	487	85	17.45	
Age	(3-6)	471	78	16.56	0.57
	>6	140	26	18.57	
Body condition	Good	242	37	15.29	0.65
	Average	313	57	18.21	
	Bad	56	10	17.86	
Type of farms	Dairy	494	78	15.79	0.09
	Mixed	117	26	22.22	
Type of breeding system	Intensive	113	26	23.01	0.003
	Semi-intensive	465	67	14.41	
	Extensive	33	11	33.33	
Herd size	(5-10)	93	18	19.35	0.31
	(10-20)	288	42	14.58	
	>20	230	44	19.13	
Breed	Cross	84	19	22.62	0.33
	Imported	498	80	16.06	
	Local	29	5	17.24	
Abortion history	Yes	38	8	21.05	0.49
	No	573	96	16.75	
Contact with other animals	Yes	551	97	17.60	0.24
	No	60	7	11.67	
Presence of dogs	Yes	414	73	17.63	0.56
	No	197	31	15.74	
Water source	Wells	268	44	16.42	0.93
	Rivers	296	52	17.57	
	Ponds	47	8	17.02	

Table 7. Multivariable logistic regression analysis of risk factors associated with seropositivity to Leptospira tested by MAT in cows in the studied regions.

Risk factors	Standard error (SE)	P Value	Odds ratio (OR)	Confidence interval 95%	
				Lower	Upper
Intensive System		0,006			
Extensive system	0,43	0,25	0,61	0,26	1,40
Semi-intensive system	0,39	0,01	0,39	0,16	0,78

spira interrogans compared to those in intensive or extensive systems (OR = 0.21; 95% CI: 0.16-0.78]; P < 0.05) (Table 7). This finding suggests that semi-intensive systems might employ practices or environmental conditions that reduce the exposure or transmission of *Leptospira*."

DISCUSSION

The diagnosis of bovine leptospirosis is frequently sensitive and difficult, applying serological techniques often represents the alternative to detect infected animals *in vivo*. The ELISA method has been used in this investigation to accelerate the screening of the entire tested population. The reason behind using MAT is to confirm the obtained positive results by ELISA and to remove the ambiguity of samples deemed doubtful during the first screening testing (Benseghir, 2021).

A total of 611 sera from 67 different cattle farms were analyzed. An individual seroprevalence of 17.02% (CI 95%. 14.12-20.24%) was obtained, with 104 animals found to be positive for one or more Leptospira serovars at a dilution ≥ 1:100 and 50% of agglutination by the MAT test. In addition, a value of 56/67 farms 83.58% (95% CI: 59.31-81.99) was positive for Leptospira. Within the 56 farms that tested positive for *Leptospira*, the within-farm seroprevalence ranged from a minimum of 10% (1 positive cow out of 10 tested) to a maximum of 40%. This variability highlights the heterogeneity of infection levels across the farms studied.

The variation observed in seroprevalence within farms compared to that obtained in the current work is likely due to differences in the sensitivity and specificity of the tests and methods used, annual fluctuations in the prevalence of bovine leptospirosis, geographical location, the health status of the cows at the time of sampling, breeding conditions, and other risk factors illustrated in Tables 5 and 6.

Leptospirosis epidemiological analysis over the world has confirmed a high hierarchy of value compared to our results from 83.3 % in Brazil (Bomfim et al., 2005) 1% in Sweden (Lindahl et al., 2011), and 4.64% in Quebec (Vincent, C.T., Munger, C., Sylvestre, F. & Levesque, 2007)

If we refer to published studies on the seroprevalence of leptospirosis in cattle using the MAT as the reference test, the seroprevalence of Leptospira obtained in this study (17.02%) is quite similar to those reported in some regions around the world; in Morocco 15% (Benkirane et al., 2016), in Iran 15.79% and 17.36% (Khalili et al., 2014; Sakhaee and pour, 2011), in India (21.18%)(Mariya et al., 2006), in Trinidad (21.5%) (Suepaul et al., 2011), in South Africa (19.4%) (Hesterberg et al., 2009), in Malaysia (27.7%) (El Jalii, 2008), and finally, in Mexico (28.4%) and (10.33%) (Leon et al., 2008; Segura-Correa et al., 2010). In our previous study, in Setif province (Algeria), the seroprevalence reached a value of 14.44% (IC 95% 37.07-46.67%) (Benseghir, 2021).

High Leptospira seroprevalences have been reported in other regions of the world, such as 83.3% in Brazil (Bomfim et al., 2005), 81.7% in Malaysia, 70.4% in Mexico (Fuente et al., 2012), 70.51% in India (Balamurugan et al., 2018), and 56.21% in Ecuador (Ruano et al., 2020). This may be explained by the fact that these countries are located in tropical regions of the world, which provide excellent conditions for the survival and spread of leptospires, due to the climate and particularly high precipitation throughout the year. On the other hand, very low seroprevalences have been recorded for Leptospira in cows, such as 8% in Spain (Alonso-Andicoberry et al., 2001), 4.64% in Quebec in 2005, and 7.6% in 2006 (Vincent, C.T., Munger, C., Sylvestre, F. & Levesque, 2007), 3.4% in Turkey (Kocabiyik, 2004), 1% in Sweden (Lindahl et al., 2011), and 6.44% in the Santa Catarina region of Brazil (Fávero et al., 2017).

The prevalent serovar among pathogenic *Leptospira interrogans* species was found to be Hardjo (6.71%), followed by Icterohaemorrhagiae (5.07%), Canicola (4.48%), and Grippotyphosa (2.78%). The

high prevalence of Hardjo serovar in cattle could be explained by the fact that cattle are the reservoirs of this serovar (Fávero et al., 2017). Similarly, the presence of Icterohaemorrhagiae serovar in cattle is related to their contact with different animal species, which act as their reservoirs (Suepaul et al., 2011). Several studies have been conducted worldwide to determine the dominant serovar responsible for bovine leptospirosis, and the results have shown that Hardjo serovar is mainly found in some European countries such as Ireland (Egan, 1986), the United Kingdom (Pritchard, 1986), Portugal (Rocha, 1998), as well as in Africa, such as Nigeria (Ezeh et al., 1990), Zimbabwe (Feresu, 1987), Tanzania (Machang'u et al., 1997), and in Asia, such as Malaysia (Bahaman et al., 1987), serovar has been reported in Holland (Hill et Weenink, 1976), Trinidad and Tobago (Suepaul et al., 2011), Pomona serovar in northern Spain (Espi et al., 2000) and Canada (Prescott, J.F., Miller, R.B., Nicholson, V.M., Martin, 1988), and Grippotyphosa serovar in northern Jordan (El-Sukhon et al., 1992). In the United States, Hardjo is the most commonly isolated and serologically detected serovar in cattle (Ellis, W.A. & Thiermann, 1986; Miller et al., 1991).

OIE (2018) confirms that animal leptospirosis antibody titers of 1:100 and 1:400, respectively, are regarded as positive in endemic and non-endemic areas. On the other hand, a reduced titer of an antibody may indicate prior exposure to Leptospira spp. because of the high specificity of MAT. The overall occurrence of leptospirosis was found to be 17.02% by MAT in serum samples collected from cattle with an antibody titer ranging from 1:100 to 1:3200.

The seroprevalence was high with 48.94% of the animals with titers 1:100. According to the literature in the MAT, the dilution 1:50 indicates animal exposure to the etiological agent, but titers of 1:100 or higher are an indicator of disease (Fávero et al., 2017). However, the observation of 1:200 and 1:400 dilutions show a serious form of leptospirosis with 21.99% and 9.93 % respectively.

Samples with a titer of 1:3200 representing the Icterohaemorrhagiae serovar are probably from animals recovering from exposure to the agent. However, in this study, seropositive cows could not be further evaluated. For this reason, cows with titers up to 1:3200 might have been recently infected, probably releasing lots of microorganisms into the environment, increasing the risk of infection to the susceptible cattle.

In the second and third positions, we notice the high agglutination frequencies against *L. icterohaemorrhagiae* (21.99%) and *L. canicola* (19.86%) which highlights the higher presence of this serogroup in the studied population. Titers of Icterohaemorrhagiae serovar indicate the likely transmission by contact of cows with mice and rats, which are the main reservoir hosts of this serogroup, while the canicola serovar suggests the canine transmission mainly by herding dogs in the farms (Jimenez-Coello et al., 2008).

To evaluate the performance of the ELISA test in the detection of antibodies directed against *L. interrogans* serovar Hardjo, we calculated the sensitivity, specificity, and fiability of the test using MAT as a reference test, as well as Cohen's kappa coefficient and the McNemar test. The sensitivity reached 63.4% (95% CI: 48.7-78.2%), while the specificity got up to 98.9% (95% CI: 98.1-99.8%) and the fiability was 96.6% (95% CI: 95.1-98.0%). Furthermore, the results of Cohen's Kappa coefficient (k=0.62) and McNemar ($p = 0.23$, $P>0.05$) showed that the two methods gave significantly non-different values in detecting seropositive animals against *L. interrogans*. Given this satisfactory agreement between the two tests, it is concluded that ELISA can behave like MAT and remains a good means of screening for bovine leptospirosis caused by serovar Hardjo in the absence of the MAT test. The lack of sensitivity may be because the ELISA used is based on leptospires sonicate that can lead to the loss of some epitopes, or else, it is based on only purified LPS, whereas MAT detects antibodies to both LPS and other surface antigens (MAT uses whole leptospires). In the same vein, the MAT test can detect IgM and IgG antibodies whereas the ELISA test only detects IgG, hence its low sensitivity compared to the MAT test.

Results obtained by IgG-ELISA showed that 15 serum samples that reacted positively in MAT were negative in ELISA. MAT-positive and ELISA-negative results were also observed. ELISA-negative sera were also found in the acute phase of the disease. In contrast, 6 MAT-negative sera were ELISA-positive, probably due to non-agglutinating antibodies detected by ELISA.

Previous work compared the ELISA technique with the MAT test as a reference. The results obtained were quite similar to ours. Thus, in India, Subathra et al. (Subathra et al., 2011) evaluated and compared the ELISA test with MAT on dog serums and reported a sensitivity of 75.46% and specificity of 93.29%.

Still, in India, assessments and comparisons between the two tests on bovine serums showed 100% sensitivity and 85.3% specificity (Mariya et al., 2006). 100% sensitivity and 97.1% specificity were obtained in another region of India (Deneke et al., 2014). The work on bovine serums in Malaysia and Brazil has achieved 100% sensitivity and specificity (Bomfim et al., 2005; El Jalii, 2008). In Iran, 100% sensitivity and 97.1% specificity were achieved (Sankar et al., 2010). It should be noted that several factors influence the comparison results between the ELISA technique and the MAT test, such as the sample size, the ELISA kit used (same laboratory or not, the type of antigen used, etc.), the packaging of the kit, the operating procedure, the operator, mark of the ELISA reader... etc.

Univariable and multivariable statistical analysis on all the factors tested related to the sampled cow (pregnancy, age, body condition, breed, and abortion history) or the cattle farm (type of farm, type of breeding system, and herd size) showed a significant positive association between type of breeding system and Leptospira seropositivity ($p<0.05$). Indeed, cattle living in the semi-intensive system have a low risk of being infected by Leptospira compared to the intensive and extensive systems (OR 0.21; 95% CI: 0.16-0.78). Recently, a study by Ismail et al. (Ismail et al., 2019) showed, on the contrary, that the semi-intensive system was 11 times more exposed to leptospirosis than the intensive system. This was interpreted as a possible infection in the environment, as the study looked at the factors favoring the occurrence of infection with the Leptospira serovars Hardjo and Pomona. However, it is known that the main reservoir of the Pomona serovar is the wild boar, which means that there is a higher risk of contamination in the animal's environment. The protective effect of the semi-intensive breeding system is observed only in comparison with the intensive and extensive systems, which serve as baseline categories in this study. The breeding system categories recorded were as follows: (i) intensive, characterized by confined management and high animal density, (ii) semi-intensive, involving partial grazing and supplementary feeding, and (iii) extensive, where animals are fully grazed with minimal management intervention. This distinction highlights the varying influence of production systems on the seroprevalence of *Leptospira*.

Most leptospira infections are asymptomatic, and the existence of antibodies in these animals even when there is no infection suggests that they have been exposed to the organism, this was approved by Benseghir et al. (Benseghir, 2021). The observed geographic variation in sero-prevalence may be due to genetic variation in disease resistance among the breeds, variations in the levels of natural immunity, management and husbandry practices utilized, and sensitivities and specificities of the diagnostic methods used by researchers (Parvez, M.A. Prodhan, M.A.M., Rahman M.A. and Faruque, 2015).

However, in other studies reported in the literature, factors such as herd size, cattle breed, age of the animal, access to contaminated water sources, use of infected bulls, inadequate husbandry practices are statistically significantly associated with Leptospira infection (Dreyfus et al., 2018; Fuente et al., 2012; Salgado et al., 2014; Schoonman and Swai, 2010; Suepaul et al., 2011)

CONCLUSION

The findings of this investigation indicate that bovine leptospirosis is prevalent in the selected provinces in Algeria, with a high incidence rate noted at the farm level. The determination of the semi-intensive system as a protective factor against leptospirosis is a significant finding that can inform the development of control measures to decrease the risk of infection in cattle and humans. The comparison of diagnostic tests for detecting *Leptospira interrogans* also provides valuable information for selecting appropriate testing methods. Overall, the results of this study contribute to our understanding of the epidemiology of bovine leptospirosis in Algeria and can inform public health policy and strategies for disease prevention and control. To fully comprehend the epidemiology of leptospirosis in farm animals and its connection to human leptospirosis, more research must be done. Additionally, PCR and other molecular techniques could be used to circumvent some of the limitations of serologic testing.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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