

Serological study of Leptospirosis in cattle, sheep and goats in Baghdad Province.

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Abstract

A total of 565 serum samples from cattle (260) sheep (171) and goats (134) were screened for the presence of Leptospiral antibodies by macroscopic plate agglutination test and microscopic agglutination test. The prevalence was found to be 57.3% in cattle, 24.6% in sheep and 22.4% in goats. *L. hardjo*, *L. mini*, and *L. hebdomadis* were found to be more common by using microscopic agglutination test.

دراسة سيروولوجية لداء البريميات في الأبقار والأغنام والماعز في محافظة بغداد

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الخلاصة

لقد تم فحص (565) عينة مصلية شكلت الأبقار (260) عينة والأغنام (171) والماعز (134) وذلك للكشف عن وجود الأجسام المضادة باستعمال اختبار الصفيحة العياني واختبار التلازن المجهرى حيث وجدت بنسبة 57.3% في الأبقار و 24.6% في الأغنام و 22.4% في الماعز. ووجدت الأنماط المصلية *L.hardjo* و *L.mini* و *L.hebdomadis* الأكثر شيوعا باستعمال اختبار التلازن المجهرى.

Introduction

Leptospirosis is an infectious disease of man and animals occurs in all farm animals species and is an important zoonotic diseases caused by the members of genus leptospira. The disease is universal in distribution and causes sever losses in domestic animals, particularly in cattle (1) (2) (3). Human, animals or environment that may be contaminated by infected animals (4) (5). Convalescent and chronic cases act as carriers by the leptospira in urine for a considerable length of time (6). It costs farmers considerable amounts of money every year due to losses resulting from abortion, fetal death, stillbirth, repeat breeding , production failure and reduction of milk (7) (8).

Review of literature revealed that there is no published report about its prevalence in Iraq. Therefore the purpose of this study was to conduct a serological survey on serum samples from cattle, sheep and goats from different areas in and around Baghdad province to determine the prevalence of leptospirosis in sera of them.

Materials and Methods

A total of 565 serum samples were collected from cattle (260), sheep (171) and goats (134) of different ages, breeds, and localities in and around Baghdad. Majority of these animals were clinically normal, while some had a history of mastitis, abortion, retained placenta or infertility.

- **Serological technique:**

Macroscopic plate agglutination test was used in this study. This test has been developed by Galton (1) in which formalin killed organism was used as antigen.

Microscopic agglutination test, this test has been employed in this study as shown in (9) (10) (11).

- **Antigens:**

Pool and individual leptospira antigens were obtained from Difco laboratories*.

Serum samples from 74 cow, 28 sheep and 19 goats were send to WHO/ FAO collaborating center for reference and research on leptospirosis, Royal tropical institute in Amsterdam employing microscopic agglutination test with live antigen using 24 serotype for further screen. In addition, serum samples from 75 cow, 29 sheep and 19 goats were sent to WHO/FAO reference laboratories for leptospirosis at public health laboratory service, county hospital, Herford United Kingdom for microscopic agglutination test with formalized antigens which they have been used 19 serogroups.

Results

A total of 565 serum samples were collected from cattle, sheep and goats as shown in Table (1). The results of total positive samples by using macroscopic plate agglutination test and prevalence for each species are summarized in Table (2), while in Table (3), showed the results of positive serum samples with individual leptospira antigens.

Table (1) Source of serum samples

Source	Cattle	Sheep	Goats
Baghdad Slaughter house	55	140	103
Different Farms	37	17	16
Al-Faudailia	74	9	0
Al-thahab Al-Aabyad village	70	2	8
Clinic in Baghdad	24	3	7
Total serum samples	260	171	134

Table (2) Results of Macroscopic Plate Agglutination Test of serum samples with pool* and individual Leptospira antigens

Animal species	Total examined	Total positive	Prevalence%
Cattle	260	149	57.3
Sheep	171	42	24.6
Goats	134	30	22.4

Table (3) Results of Macroscopic Plate Agglutination Test of serum samples with individual Leptospira antigens

Leptospira antigens	Cattle		Sheep		Goats	
	Total N0. exam./+	+ve %	Total N0. exam./+	+ve %	Total N0. exam./+	+ve %
icterohaemorrhagiae	260/28	10.8	171/7	4.1	134/6	4.5
grippotyphosa	260/13	5.0	171/0	0	134/5	3.07
pomona	124/9	7.3	39/1	2.6	32/2	6.3
hardjo	260/78	30	171/12	7.0	134/11	8.2

Out of 74 cattle serum samples examined with live antigens, 63 samples reacted positively with one or more leptospira antigens when tested against 24 strains representing different serotypes. Twelve out of 28 sheep and 11 out of 19 goats showed

* DIFCO Laboratories, West Molesey Surrey U.K.

positive reaction with similar test as observed in table (4). All sheep and goats sera reacted at low dilution 1:20, 1:40. They failed to react at dilution 1:80 or higher.

Table (4) Results of microscopic Agglutination Test– Live Antigen– on Cattle, Sheep, and Goats serum samples at Royal Tropical Institute- Amsterdam

Animal Species	No. exam.	No. positive	Serum Dilution						
			1:20-1:40	1:80	1:160	1:320	1:640	1;1280	1;2560
Cattle	74	63	31	6	7	6	6	6	1
Sheep	28	12	-	-	-	-	-	-	-
Goats	19	11	-	-	-	-	-	-	-

Results of microscopic agglutination test live antigen on cattle serum samples at the Royal Tropical Institute, Amsterdam with different leptospira serogroups are listed in table (5). The results of the work conducted at the public health laboratory service U.K. were examined by microscopic agglutination test using formalized cultures of leptospira are summarized in table (6). Eighteen out of 75 cows serum samples were positive at dilution from 1:100 to 1:800. Twenty nine sheep serum samples and 19goats serum samples were negative to all of the serotypes used in the test.

Table (5) Results of microscopic agglutination Test -live antigen-on cattle serum samples at the Royal Tropical Institute Amsterdam with different Leptospira serogroups

Ser. No,	Lep. serogroup.	Serum dilution						Total
		1:80	1:160	1:320	1:640	1:1280	1:2560	
1	Sejroe	2	5	6	4	5	1	23
2	Mini	5	3	1	2	1	-	12
3	Hebdomadis	-	1	3	2	2	-	8
4	Semaranga	4	1	-	-	-	-	5
5	Javanica	1	2	1	-	-	-	4
6	Grippotyphosa	2	-	-	1	-	-	3
7	Pomana	-	-	1	1	-	-	2
8	Tarrassovi	-	-	-	-	1	-	1
9	Autumnalis	-	-	1	-	-	-	1

Table (6) Results of Microscopic Agglutination Test -Killed Antigen- on Cattle, Sheep, and Goats serum samples at Public Health Laboratory Service U.K

Animal species	No. examined	No. positive	Serum Dilution			
			1:100	1:200	1:400	1:800
Cattle	75	18	6	8	3	1
Sheep	29	-	-	-	-	-
Goats	19	-	-	-	-	-

Table (7) Results of examination of serum samples with Macroscopic Plate Agglutination Test using pool leptospira antigen

Leptospira antigens	Cattle		Sheep		Goats	
	Total N0. exam./+	+ve %	Total N0. exam./+	+ve %	Total N0. exam./+	+ve %
Pool 1	83/0	0	31/0	4.1	44/0	0
Pool 2	260/27	10.4	171/4	2.3	134/5	3.7
Pool 3	260/42	16.2	171/15	8.8	134/12	8.9
Pool 4	260/18	6.9	171/7	4.1	134/5	3.7

*Pool 1 containing: *L.ballam*, *L. canicola*, *L.icterohaemorrhagiae*.

Pool 2 containing: *L.bataviae*, *L.grippotyphosa*, *L.pyrognos*.

Pool 3 containing: *L.autumnalis*, *L.pomona*, *L.wolfi*.

Pool 4 containing: *L.australis*, *L.hyos*, *L.georgia*, *LT 117*.

Discussion

The results of the present study have indicated the prevalence of leptospirosis in cow 5.703% sheep 24.6% and goats 22.4%. The prevalence of leptospirosis in cattle in and around Baghdad was fairly high, though the prevalence was low in sheep and goats in Baghdad. It is quite necessary and important to give more attention and proximity to animals and with an occupational risk of exposure (12). Tan in 1964 found that 29.6% of patients with pyrexia of unknown origin had antibodies indicative of leptospirosis. Results from table (7) indicate that pool 3 was the common antigens in cattle, sheep and goats. Next in order being pool 2 in cattle with 10.4 % and in sheep with 4.1 % positive reaction. *Leptospira Pomona* member of pool 3 has been reported to be common serotypes affecting cattle and sheep (14) (3) (7). *Leptospira grippotyphosa* member of pool 2 has also reported in cattle and goats (15) (16) (17).

Results of table (4) revealed titers from 1:80 to 1:2560 were found only in cattle, where as in sheep and goats titers were below 1:80. This indicated that there was a constant source of infection as a result of which titers were due to anamnesis response. The low titer may be due to the fact that the samples were collected from slaughter house where the age of such animals was less than one year, as such changes of being exposed to repeated infection were much less. The results of table (6) revealed that only cattle sera had given positive reaction when tested with killed antigens. The negative results in respect of sheep and goats serum samples may be due to very low titers which may not have been detected by this method.

However the macroscopic plate agglutination test has been recommended as a screening test of animals and human sera (18) (19) (9) (20) thought it is less sensitive than microscopic agglutination test. It detects any exposure during previous 1-2 years (21).

The present findings have opened a new avenue for further research in this field, since this is the pioneer study in Baghdad province about leptospirosis in domestic animals.

Macroscopic plate agglutination test has been found to be satisfactory, though not highly sensitive, as a screening test in preliminary serological survey to identify the prevalence of the disease. It is not possible to conduct microscopic agglutination test in ordinary laboratories due to obvious difficulties of maintaining live culture of several serotypes and risk of human of exposure. It is therefore necessary to depend on macroscopic plate agglutination test.

In a country like Iraq where dairying is a growing industry it is essential to establish the definite causes of abortion in cow. In every case of abortion there is a tendency to consider brucellosis as the most probable cause, since this study has proven that aborted cattle showed high antibody titers by microscopic agglutination test, it was highly suggestive that leptospirosis may be associated with abortion. It is recommended that similar investigation be extended to survey different parts of the country in order to get on overall picture of the leptospirosis in Iraq. Such survey may also be conducted on other species of domestic animals such as horses and dogs as they are considered to be potential source of infection to man and other animals. Finally it is emphasized that further work will be necessary to include the isolation and typing leptospiral organisms.

References

1. Galton, M. M.; Powers, D. K.; Hall, A. D. & Cornell, R. G. (1958). A rapid macroscopic slid screening test for the serodiagnosis of leptospirosis antibodies. *Appl. Microb.*, 13: 81-85.
2. Michna, S. W. (1970). Leptospirosis. *Vet. Rec.*, 86: 484-496.
3. World Health Organization and International Leptospirosis Society. (2003). *Human Leptospirosis: Guidance for Diagnosis, Surveillance and control*.
4. W.H.O. (1972). Expert group on research needs in leptospirosis. *Bull. Wld. Hlth. Org.*, 47: 113-122.
5. Faine, S.; Adler, B.; Bolin, C. & Perolat, P. (1999). *Leptospira and Leptospirosis*, 2nd ed, Medi. Sci. Ed, Melbourne.
6. Kelley, P. W. (1998). Leptospirosis, P. 1580-1587. In S. L. *Infectious diseases*, 2nd ed. W. B. Saunders, Philadelphia, Pa.
7. Higgns, R. J.; Harbourive, J. F.; Little, T. W. A. & Stevens, A. E. (1980 b). Mastitis and abortion in dairy cattle associated with leptospira of the serotype hardjo. *Vet. Rec.*, 107: 307-310.
8. Jenkins, E. M.; Gbadamosi, R. J. & Oraye, E. T. (1979). Survey of leptospiral agglutination in sera of swine of south eastran Alabama. *Am. J. Vet. Res.*, 40: 1019-1021.
9. Chernesky, S. J. (1970). A serological survey of canine leptospirosis in British Colombia. *Cand. J. Comp. Med.*, 34: 102-104.
10. Turner, L. H. (1970). Leptospirosis III. Maintenance, isolation and demonstration of leptospira. *Trans. Roy. Trop. Med. and Hyg.*, 64: 623-646.
11. Maronpot, R. R. & Barsoum, I. S. (1972). Leptospiral microscopic agglutination antibodies in sera of man and domestic animals in Egypt. *Am. J. Trop. Med. and Hyg.*, 21: 467-472.
12. Turner, L. H. (1968). Leptospirosis II. Serology. *Trans. Roy. Trop. Med. and Hyg.*, 62: 880-899.
13. Tam, D. S. K. (1964). *Med. J. Malaya* 18: 164 (cited by Michna. 1970). *Vet. Rec.*, 88: 484-496.
14. Morse, E. V. (1960). New concept of leptospirosis in animals. *J. A. V. M. A.*, 136: 241-246.
15. Maghami, G. (1971). A brief report on the survey of leptospirosis in Iran. *Archs. Inst. Razi* 22: 203-207. (*Abst. Vet. Bull.* 1971. 41: 629).
16. Ular, H. & Alver, H. (1973). Leptospirosis and isolation of leptospire from cattle in western Anatolia Pindik. *Veteoinaer kontrol Vet.*, 6: 41-49. (*Abst. Vet. Bull.* 1974) 44: 290.
17. Vartar, T. (1976). Leptospirosis of domestic animals in Turkey during 1963-1974. *Etlik Veterinar Bakteriyolojii.*, 4: 147-162. (*Abst. Vet. Bull.* 1978) 48: 206.
18. Galton, M. M. (1962). Methods in the Laboratory diagnosis of leptospirosis. *Ann. N. Y. Acad. Sci.*, 98: 675-685.
19. Crawford, R. P. (1964). Evaluation of plate test for the detection of leptospiral antibodies in bovine serum. *J.A.V.M.A.*, 145: 683-687.
20. Tolari, F.; Andreani, E. & Piazza, D. (1979a). Rapid macroscopic test in the diagnosis of leptospirosis in animals comparison with the microagglutination test. *Annali della Facalta di Medicina Veterinaria.*, 31: 89-99. (*Abst. Vet. Bull.* 1980. 50: 856).
21. WHO-FAO-OIE. (1982). *Animal health year book*. 19: 195.