

# Serodiagnosis of leptospirosis in cattle in north of Iran, Gilan

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**Abstract:** In order to investigate the seroprevalence of leptospirosis in traditional dairy farms, a serological study was conducted in Gilan province. For this study,, a total of 205 serum samples were randomly collected from nine districts of this province. All serum samples were serologically tested by the standard MAT using live antigens representing the following *Leptospira interrogans* serovars: Grippityphosa, Icterohaemorrhagiae, Hardjo, Canicola, Ballum and Pomona. The lowest dilution that each serum was considered positive was 1:100. The results of this study showed that 53 (25.8%) animals had a positive reaction against one or more serovars. The most prevalent *Leptospira* serovar was Canicola representing representing 24 (11.7%) samples, and the least prevalent *Leptospira* serovars were Icterohaemorrhagiae representing 1 (0.5%) sample and Hardjo, 2 (1%) samples. The most prevalent reciprocal titer was 100 and the highest titer was 400. The results of this study may support that cows may have a role in maintaining Canicola serovar. As mostany of the visited herds had at least one unvaccinated dog for guarding the herds, it has beenis concluded that the high prevalence of Canicola serogroup can be associated with close contact between dogs and cows. In addition, infected cows are a potential zoonotic risk to farmers, milkers, abattoir workers and meat inspectors, which previously has not been seriously considered.

**Key words:** leptospirosis, seroprevalence, Gilan, cattle, Iran.

## Introduction

Leptospirosis affects most mammals throughout the world and is a common zoonotic disease. It causes financial loss to the cattle industry from decreased milk production, abortion, stillbirth, infertility and mortality. Leptospirosis has been recognized in Gilan province in human and cattle in last 10 years. Some aspects of bovine leptospirosis are still inadequately defined, particularly in the cattle population of North of Iran, where published reports indicate widespread serological evidence of infection. The most prevalent leptospire serovars reported in Iran include Hardjo, Pomona, Grippotyphosa and Icterohaemorrhagia (Hooshmand Rad and Magami 1976, Rafyi and Magami 1968, Ebrahimi *et al.*, 2003; Magami *et al.*, 1977; Haji Hajikolaei *et al.*, 2005 and 2007). More recent published data in Iran indicated that serovar

Canicola is widely prevalent in the cattle population in different provinces (Ebrahimi *et al.*, 2004; Khaki *et al.*, 2005; Haji Hajikolaei *et al.*, 2006).

Gilan province is located in the north of Iran and South of the Caspian sea and is characterized by high rainfall and tropical temperature. These environmental characteristics make Gilan province different from the rest of Iran. The association of wild carnivores, rodents and rice field workers was observed in last three years' investigations. In order to investigate the seroprevalence of leptospirosis in the cattle population, a serological study was conducted in Gilan province.

## Materials and Methods

For this study, a total of 205 serum samples were randomly collected from cows of traditional dairy farms in nine districts of Gilan province where there is no vaccination program against Leptospirosis in

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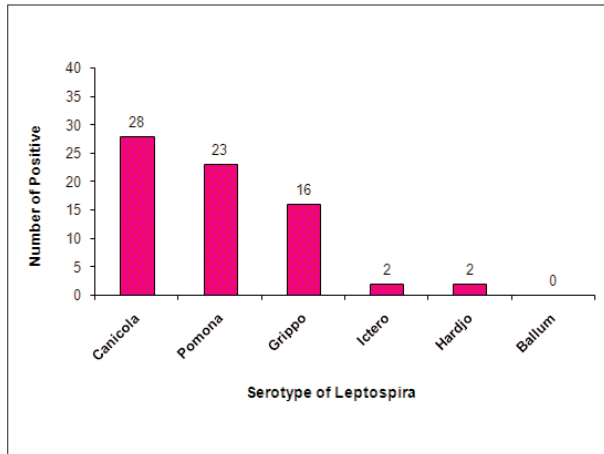


Fig. 1: Seroprevalence of leptospira in cattle by serotypes in Gilan Province.

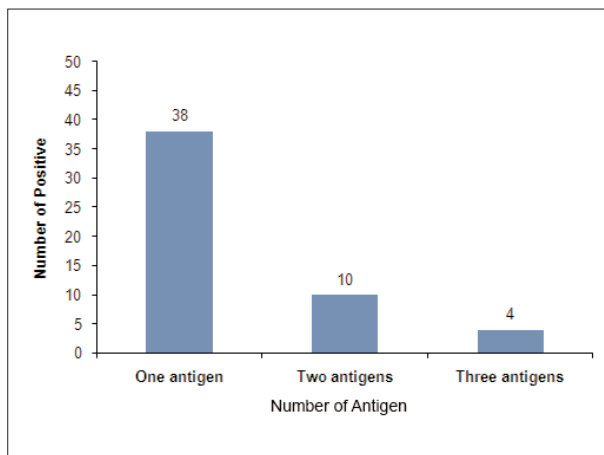


Fig. 2: Incidence of MAT reaction with one or more antigens in 53 Positive reactors.

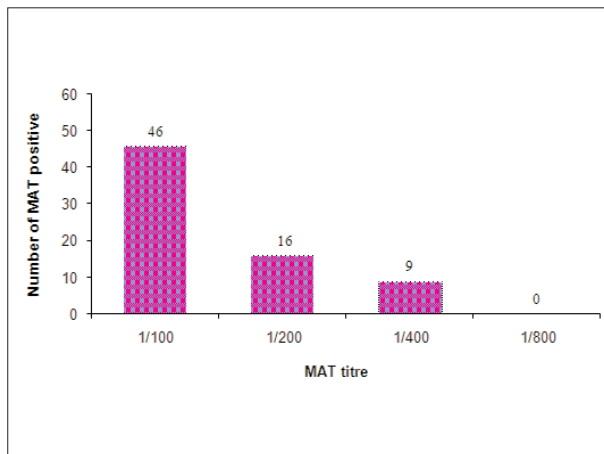


Fig. 3: Seroprevalence of Leptospira by MAT titres in Gilan Province.

cattle. Blood samples were collected from the jugular vein in and collected to 10 ml evacuated glass tubes and then were then transferred to the Leptospira Research Laboratory of the Faculty of Veterinary

Table 1: Distribution of the MAT positive samples in 4 age groups of cows in Gilan province.

Age groups	Positive (%)	Negative (%)	Total
6-12 months	11 (18)	50 (82)	61
1-2 years	12 (23.5)	39 (76.5)	51
2-4 years	12 (38.7)	19 (61.3)	31
>4 years	18 (29)	44 (71)	62
Total	53 (25.8)	152 (74.2)	205

Table 2: Distribution of the seropositive leptospira reactors in 9 districts of Gilan province in cows.

District	Positive (%)	Negative (%)	Total
Fouman	5 (50)	5 (50)	10
Somea Sara	8 (34.8)	15 (65.2)	23
Rasht	20 (32.8)	41 (67.2)	61
Siakal	3 (27.3)	8 (72.7)	11
Masal	2 (22.2)	7 (77)	9
Anzali	6 (20)	24 (80)	30
Shaft	6 (20)	24 (80)	30
Roudsar	1 (16.7)	5 (83.3)	6
Lahijan	2 (8)	23 (92)	25
Total	53 (25.8)	152 (74.2)	205

Medicine, University of Tehran. Serum was separated by centrifugation and stored in 2 mL aliquots on cryotubes at  $-20^{\circ}\text{C}$  until testing.

Microscopic agglutination test (MAT) was performed on all of the serum samples, as described earlier (Sakhaei *et al.*, 2007). MAT was implemented using live antigens representing the following *Leptospira interrogans* serovars: Grippyphosa, Icterohaemorrhagiae, Hardjo, Canicola, Ballum and Pomona. All strains were grown in liquid Ellinghausen McCullough Johnson Harris (EMJH) medium for 7-10 days. The serum to be tested was serially diluted in a microtitre plate, starting from 1 in 50 dilution, using 2-fold dilution (1 in 100, 200, 400, up to 3200). The lowest dilution that each serum was considered significant was 1:100. The end point titre was the highest titre in which 50% agglutination occurred. The antigen that gave the highest titre was considered to be the infective serovar.



## Results

The results of this study showed that 53 (25.8%) cows had a positive reaction against one or more serovars. The most prevalent *Leptospira* serovars were Canicola, Pomona and Grippotyphosa respectively. The less prevalent *Leptospira* serovars were Hardjo and Icterohaemorrhagiae, respectively (Fig 1). Fourteen samples (6.8%) showed serological reaction with more than one serovar (Fig 2). The most prevalent serological titer was 1/100 and the highest titer was 400 (Fig 3).

The results of the present study also showed that cows in age group between 2-4 years had the highest rate of seropositive (38.7%) as compared to the other age groups (Table 1). The highest seropositive reactors were belonged to the Fouman, Somea Sara and Rasht districts, respectively (Table 2).

## Discussion

Results of this study showed that of the serovars included in the MAT panel, *Leptospira interrogans* serovar Canicola is the most prevalent serovar in dairy herds of the Gilan province. Although it has been stated that this serotype is a common serotype in dogs, and this animal is considered to be the main reservoir for the mentioned serotype, however, the results of the present study indicated that cows may have a role in the epidemiology of the Canicola serotype. The high prevalence of Canicola serovar in this study is in accordance with the results of other serological study conducted in recent years in other parts of Iran (Abdollahpour *et al.*, 2004; Ebrahimi *et al.*, 2004; Khaki *et al.*, 2005; Haji Hajikolaie *et al.*, 2006), which have been conducted in 3 other provinces. These findings will possibly support that cows may have a role in maintaining this serovar. The possibility that cows may be a maintenance host for Canicola serovar suggests that cross-infection between cattle and dogs may occur in the farm. Maintenance of infection by cows will complicate disease control since infected cows may constitute a threat to other ruminants free of *L. Canicola*. In addition, infected dogs and cows are a potential zoonotic risk to farmers, milkers, abattoir workers and meat inspectors, which previously has not been

seriously considered.

In general, the present study support the idea that leptospirosis is still one of the important factors of economic losses in the cattle industry of Iran and is hazardous to the public health. As many of the visited herds had at least one unvaccinated dog for guarding the herds, it is concluded that the high prevalence of Canicola serogroup can be associated with close contact between dogs and cows. As dogs are considered to be the maintenance host for Canicola serogroup, more attention to herd's dogs is needed and vaccination of the dogs together with the prevention of close contact between these species would reduce the transmission of this serotype to the cattle populations. As Canicola serovar is not included in the present multivalent vaccine used in bovine in Iran, it is suggested that at least in those regions where that Canicola serovar is dominant, this serovar should be added to the multivalent vaccine. However, in order to investigate the role of dog in bovine leptospirosis in this area, and the importance of cows in maintaining Canicola serovar, an extensive sero-bacteriological study of leptospirosis in cows and dogs in this region and other parts of Iran is suggested.

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## مطالعه سرولوژیک لپتوسپیروز در گاوهای استان گیلان

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استان گیلان که در شمال ایران و جنوب دریای مازندران واقع شده از نظر شرایط آب و هوایی و میزان بارندگی با سایر نقاط ایران تفاوت دارد و این امر شرایط ویژه‌ای را برای شیوع بیشتر برخی از بیماریها مانند لپتوسپیروز فراهم می‌کند. به منظور مطالعه سرولوژیک لپتوسپیروز در گاودارهای سنتی استان گیلان که واکنشی علیه لپتوسپیروز دریافت نمی‌کنند، تعداد ۲۰۵ نمونه سرم خون از گاوهای ۹ شهرستان این استان بطور تصادفی اخذ گردید. همه نمونه‌های سرمی جهت انجام آزمایش میکروآگلوتیناسیون (MAT) به آزمایشگاه تحقیقاتی لپتوسپیروز واقع در بیمارستان آموزشی و پژوهشی دانشکده دامپزشکی دانشگاه تهران منتقل و تا زمان آزمایش در فریزر ۲۰- درجه نگهداری شدند. آزمایش MAT طبق پروتکل استاندارد و با استفاده از شش سروتیپ گریپوتایفوزا، ایکتر و هموراژیه، هارجو، کنیکولا و بالوم انجام و تیتراهای سرمی مساوی و یا بیشتر از ۱:۱۰۰ مثبت محسوب گردید. نتایج این مطالعه نشان داد که تعداد ۵۳ نمونه (۲۵/۸ درصد) دارای واکنش مثبت سرمی علیه حداقل یکی از سروتیپها می‌باشد. بیشترین تعداد موارد مثبت مربوط به سروتیپ کنیکولا با ۲۴ مورد (۱۱/۷ درصد) و کمترین آن مربوط به سروتیپهای ایکتر و هموراژیه ۱ مورد (۰/۵ درصد) و هارجو ۲ مورد (۱ درصد) بود. بیشترین موارد مثبت مربوط به تیترا سرمی ۱:۱۰۰ بوده و بالاترین تیترا سرمی بدست آمده ۱:۴۰۰ بود. نتایج این مطالعه این فرضیه را تا حدودی تایید می‌کند که گاوها نیز ممکن است نقشی در نگهداری سروتیپ کنیکولا در جمعیت خود داشته باشند. با توجه به اینکه اغلب دامپرووریهای ویزیت شده دارای حداقل یک قلاده سگ غیر واکنش بر علیه لپتوسپیروز بودند، لذا شیوع بالای سروتیپ کنیکولا در گاوها احتمالاً ناشی از تماس نزدیک این دو گونه حیوان در محیط دامپرووریها می‌باشد. علاوه بر آن به این نکته باید توجه داشت که گاوها و سگهای آلوده به لپتوسپیروز می‌توانند خطرات زیادی برای آلودگی انسان بویژه دامداران، کشاورزان، کارگران شیردوشی و کشتارگاهها بوجود آورند.

واژه‌های کلیدی: لپتوسپیروز، شیوع سرمی، گیلان، گاو، ایران.

