Acta Veterinaria (Beograd), Vol. 47. No. 1, 15–22, 1997.

UDK 619:616.986.7:636.2

SEROPREVALENCE OF LEPTOSPIRA INTERROGANS SEROVAR HARDJO IN COWS AND ISOLATION FROM URINE

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(Received, 6 January 1997)

Utilising the microagglutination test (MA) 13364 cow sera originating from public farms and 6601 samples of cow sera originating from the private sector were investigated for leptospirosis. The following nine types of leptospira were used: pomona, icterohaemorrhagiae, grippotyphosa, sejroe, bataviae, australis, canicola, tarassovi and hardjo. Positive results were obtained for 822 of 4,12% of the total number of investigated blood sera. No significant differences in the degree of infection between cows from the public and private sectors were discovered. L. hardjo was the most frequent serotype found in cows from the public sector (84,79%), and L. pomona was detected mostly in cows from the private sector, accounting for 52,53%. Antibodies against L. hardjo were found in 6 out of the total of 18 investigated herds. The number of seropositive animals inside herd ranged from 0,40 to 62,61%. The titer of specific antibodies ranged from 1:100 to 1.32000. For the first time in our country. L. hardjo was isolated from the urine of 6 seropositive animals. L. hardjo was isolated in cows both with low and with high antibody titers in the serum. Compared to investigations conducted 4 years ago the number of seropositive cows is doubled.

Key words: L. serovar hardjo, cows, urine, isolation

INTRODUCTION

Leptospirosis in cattle is widely spread all over the world and its health and economical effects are not negligible. The first reports, with a very difficult clinical picture, were connected to infection by L. grippotyphosa, whereas L. pomona was identified as the causative agent of haematuria in calves and abortions in cows. Some other serovars of leptospira considered to be causative agents in cows are: icterohaemorrhagiae, tarassovi, hardjo, balcanica, canicola, ballum, bataviae, autumnalis, saxkoebing, representing only 24 serovars from 14 serogroups isolated in cows (Blobel and Schliesser, 1985). A drastic increase of infection with L. hardjo in milking cows has been discovered by serological investigations, and its cause is not known so far (Ellis et al., 1976a; Thiermann, 1982; Ellis and Thiermann, 1982; Ellis and Thiermann, 1986; Kaszanyitzky et al.,

1991). Two genotypes of L. hardjo, Hardjopraitno and Hardjobovis, were identified in cattle (Smith et al., 1994). Serological investigations in England and Northem Ireland indicated that subclinical infection by L. hardjo was frequent in their cow herds (Ellis et al., 1976a; Ellis and Mischna, 1976b). Further research confirmed that L. hardjo is adapted to cattle as the primary host (Higgins et al., 1980), and according to some researchers, its pathogenicity for cattle is relatively low (Chappel et al., 1989). Sheep are considered to be an accidental host of L. hardjo, being infected after contact with infected cattle on grazing lands. However, some later research uncovered a high prevalence of seropositive sheep, which were not in contact with cattle (Ellis et al., 1994), which points to sheep as another host of this serotype of leptospira (Cousins et al., 1989; Pekelder et al., 1993). Moreover, abortions, parturition of avital lambs and agalactia in sheep have also been reported (Cousins et al., 1989).

The first results on isolation of L. hardjo from milk and blood of cows showing clinical mastitis were reported by Ellis et al. (1976b). An unexpected low milk production in a seropositive cow herd was described by Higgins et al. (1980). This clinical state may be considered as agalactia or "milk drop syndrome" rather than a true mastitis. Infection with L. hardjo in cows is manifested mostly in a subclinical course, and a decrease in milk production is evident. The clinical picture of infection with L. hardjo is expressed as mastitis (Ellis et al., 1976a; Higgins et al., 1980) or abortion (Akkermans and Kreeft 1992; Murray, 1990).

Data on the importance of L. hardjo infections in milking cows in the world initiated an investigation of this zoonosis in Vojvodina, especially because infection with L. hardjo was confirmed in earlier serological research in 3 cow herds (Vidić et al., 1994).

MATERIAL AND METHODS

Animals. In a period of two years 1995 cow blood sera were serologically investigated. A total of 13364 samples originated from cows from 18 public farms, and 6601 blood sera were taken from cattle from the private sector. Leptospira was isolated from the urine of 9 cows originating from a herd in which 38,30% seropositive animals were demonstrated by serological investigation.

Serological test. A microagglutination test (MA) with 9 serotypes of leptospira (pomona, icterohaemorrhagiae, grippotyphosa, sejroe, bataviae, australis, canicola, tarassovi and hardjo) was used to detect the presence of specific antibodies. The finding of specific antibodies in sera dilutins 1:100 and higher was considered a positive result.

Urine samples. Urine samples were taken by catherization. Streaking on nutrient culture medium was carried out immdiately on the farm.

Isolation of leptospira. 0,1 ml urine was used for preparing a tenfold dilution in phosphate - buffered saline (PBS; pH 7,2) and to streak it on prepared culture media. For isolation of leptospira the following culture media were used: EMJH medium to which 5% rabbit serum was added, as well as furasolidone and neomycin 5 μ g/ml and EMJH with a supplement of 5-fluorouracyl 5-FU 200 μ g/ml.

Streaked culture media were incubated at 28^{0}C and controlled weekly up to 60 days. Cultures identified as suspicious or positive were subcultured in fresh media and monitored microscopically.

RESULTS

The results of serological investigations of cow blood sera are shown in Table 1. Positive results were obtained in 822 or 4,12% of the total of 19956 investigated sera. No significant differences in the infection rate in cows from public farms and the private sector were discovered. The most frequent serotype in farm-raised cows was L. hardjo (84,79%), while other serotypes of leptospira appeared in a significantly lower degree. The most frequent serotype in cows raised on private farms was L. pomona (52,53%), while L. hardjo was detected only in 8,2% of the investigated cow sera.

Table 1. Finding of antibodies against leptospira in cows

Origin	No investigated	Positive		Serovar Pomona		Ictero.		Grippo.		Hardjo	
		No	%	No	%	No	%	No	%	No	%
Public sector	13364	605	4.53	93	15.37	38	6.28	4	0.66	513	84.79
Private sector	6601	217	3.29	114	52.53	31	4.28	11	5.07	18	8.29
Total	19965	822	4.12	207	25.18	69	8.39	15	1.82	531	6459

Antibodies against L. hardjo were detected in 6 herds out of the total of 18 (13364 animals (Table 2.) The prevalence of seropositive animals in the herd was 0,40 - 62,61%. The titer of specific antibodies against L. hardjo reached 1: 32000.

Table 2. Finding of antibodies against L. hardjo in cows

Origin	No of investigated herds	No of samples	Positive herds	percent	Titre
Public sector	18	13364	6	0.40-62.61	1:100-1:32000

Table 3. Isolation L. hardjo from urine of seropositive cows

Cow No.	377	1324	116	46	3451	2857	486	526	568	Isolation No	%
Titre 1:	500	2000	8000	1000	500	100	2000	2000	4000		
EMJH (f+n)	–	–	–	–	–	–	–	–	–	0	0
RMJH - 5 FU	–	+	+	+	–	+	–	+	+	6	66.6

EMJH (f+n) – (furasolidone + neomycin)
EMJH 5 FU – 5-fluorouracyl

The results for the isolation of L. hardjo from urine of serological positive cows are shown in Table 3. By using EMJH with 5-FU L hardjo was isolated in 6 animals (66,6%) from the total of 9 seropositive cows. It is also significant that L. hardjo was isolated both in cows with a low and in cows with a high titer of specific antibodies. Using EMJH with a supplement of furazolidone and neomycin for the isolation of leptospira, gave negative results.

DISCUSSION

The results of our investigation point at an increase of the number of seropositive animals and cow herds. Serologically positive animals were discovered in 3 herds during eartier investigations (Vidić et al., 1994). In the same period infection by L. hardjo was not detected in cows from the private sector, while now the incidence of infected cows is 8,29%. The origin of the infection on the territory of Vojvodina is still uncertain. Import of animals is assumed to be a possible reason for it. The cow is the primary host for L. hardjo, and the infection is meaintained and spreads in a herd because of the permanent presence of the infection source. Our research on the appearance of L. hardjo in sheep (not published data) points to the presence of infection, but in a very low degree (0,75%). An increase of infection in sheep would make control of the disease more difficult. Moreover, infected sheep are a potential danger for workers and veterinary staff, which has not been seriously taken in to consideration before. Ellis et al. (1994) found a very high degree of infection in sheep, even though they were not in contact with cows. A significantly lower degree (3,3%) of infection in sheep was observed by Pekelder et al. 1993.

According to reports of the veterinary service, the clinical condition of animals in 4 herds, in which infection with L. hardjo was detected, was satisfactory, and clinical disturbances characterising an infection with L. hardjo did not appear. However, this should not be neglected, considering that subclinical infection is most frequently manifested by decreased milk production, which sometimes could be caused also by other factors. Decreased milk production in a herd can last for months or becomes normalised within two to three weeks (Ellis et al., 1976a; Higgins et al., 1980. Investigating the problem of infection with L. hardjo in Australia, Sullivan (1974) has found that characteristic clinical changes could be detected in only 3% of the total number of investigated cows. Mastitis, characteristising an infection with L. hardjo was discovered in two cow herds. The udder was slack, without any visible signs of inflammation and all udders were taken. The milk thick, similar to colostrum and yellow-orange in colour. A mechanical factor was presumed first, but this assumption was eliminated. Symptomatic therapy with streptomycin has proved efficency. Higgins et al. 1980. described a significant decrease of milk production in cow herds in which L. hardjo was serologically diagnosed, and confirmed by isolation of the causative agent from milk and urine.

The complex process of diagnosis could be considered as the cause of relatively late detection of infection with L. hardjo in cows, in comparison to other types of leptospira. There are problems in diagnosing and controlling bovine

leptospirosis (Smith et al., 1994.). Infection is usually subclinical, and serological titers vary greatly in peak and duration. Diagnosis is mostly based on the detection of multiple antibody titer increases in pair samples during the acute period or recovery, followed by a complex and long lasting isolation of leptospira. Numerous researchers have pointed at difficulties in the isolation of leptospira (Ellis and Thiermann 1986; Boline et al., 1989). Discovering a nutrient culture medium for successful isolation of this type of leptospira was initialised by the numerous reguirements for growth (Ellinghtausen et al., 1965; Johnson and Haris, 1967).

The efficacy of EMJH-5FU and its applicability in the direct isolation of leptospira from suspect material was also confirmed by results obtained in our investigation on the isolation of L. hardjo from urine. Infected cows excrete L. hardjo in the urine during a period of several years (Thiermann, 1982). In carrier cows leptospira exist in the kidney canals II group enabling continuous sheding, and they are not exposed to circulating antibodies which do not penetrate the glomerular membrane, while antibodies in urine cannot negatively affect leptospira because of their low concentration. Leonard et al. 1993. demonstrated the presence of leptospira in urine up to 60 weeks after expermental infection with L. hardjo. In spontaneously infected heifers with a titer of serum antibodies more that 1:300 secretion was evident until the 40 th week of the research. Cessation of leptospiruiria arose both in spontaneously and in experimentally infected animals, after registering a high increase of anti leptospira IgG and IgA antibodies in the urine.

L. hardjo is of great importance in respect to human health, especially for people who are in contact with infected animals. Infection by penetration through conjunctiva or skin lesions is the most probable way of infection. Milkers are mostly exposed to that risk. Therefore it is not surprising that infection with L. hardjo is the most frequent leptospirosis diagnosted in humans in many countries Ezeh et al., 1991; Zamora et al., 1990. Use of unpasteurized milk is not considered to be of great importance in human infections due to an antileptospiral effect of milk.

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RAŠIRENOST LEPTOSPIRA INTERROGANS SEROTIP HARDJO U KRAVA I IZOLACIJA UZROČNIKA IZ URINA

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SADRŽAJ

U radu su izneti rezultati istraživanja raširenosti leptospiroza, sa posebnim osvrtom na L. interrogans serotip hardjo kod krava u Vojvodini.

Primenom mikroaglutinacionog testa (MA) ispitano je 13364 seruma krava sa farmi i 6601 uzorak seruma krava sa privatnog sektora na leptospirozu. U radu je korišćeno 9 serotipova leptospira: pomona, icterohaemorrhagiae, grippotyphosa, sejroe, bataviae, australis, canicola, tarassovi i hardjo. Pozitivni rezultati ustanovljeni su kod 822 ili 4,12% ukupno ispitanih krvnih seruma krava. Nismo ustanovili značajne razlike u stepenu inficiranosti krava farmskog uzgoja i privatnog sektora. Najzastupljeniji serotip leptospira kod krava farmskog uzgoja je L.

hardjo 84, 79%, zatim L.pomona 15,37%, L. icterohaemorrhagiae 6,28% i L. grippotyphosa 0,66%. Kod krava sa privatnog sektora dominantan serotip je L. pomona 52,53%, a L. hardjo je utvrđena kod 8,29% krava. Od ukupno ispitanih 18 zapata, antitela za L.hardjo dokazali smo u 6 zapata krava. Procenat seropozitivnih životinja unutar zapata iznosio je 0,40-62,61%. Vrednosti titra specifičnih antitela iznosile su od 1:100 do 1: 32000. Kod krava iz 4 zapata nisu konstatovani klinički znaci leptospiroze, dok su kod krava iz 2 zapata registrovani mastitisi. Prvi put u našoj zemlji izolovana je L. hardjo iz urina, i to kod 6 seropozitivnih krava. L. hardjo je izolovana kako u krava sa niskim tako i visokim titrom antitela u serumu. U odnosu na ispitivanja koja su vršena 4 godine ranije, broj seropozitivnih zapata krava se udvostručio.