DE GRUYTER OPEN

Bull Vet Inst Pulawy 59, 29-35, 2015
DOI:10.1515/bvip-2015-0005

Leptospirosis in cattle from markets of Almaty province, Kazakhstan

Zhumagul Kirkimbayeva[1], Bozena Lozowicka[2], Kadyr Biyashev[1], Nurzhan Sarsembaeva[3], Gulnur Kuzembekova[1], Assel Paritova[3]

[1]Department of Biological Safety, Kazakh National Agrarian University, 050010 Almaty, Kazakhstan
[2]Institute of Plant Protection - National Research Institute, 15-195 Bialystok, Poland
[3]Department of Veterinary Sanitary Examination and Hygiene,
Kazakh National Agrarian University, 050010 Almaty, Kazakhstan
b.lozowicka@iorpib.poznan.pl

Received: June 2, 2014 *Accepted: January 14, 2015*

Abstract

This paper is the first study of the prevalence of leptospirosis in the cattle at slaughter from a rural area of Kazakhstan. Five hundred and seventy three samples of serum, urine, and kidneys from cattle of Alatau, Kazakh white and Auliyekol breed, aged from 2 to 5 years (unknown vaccination status), from the province of Almaty in the South-Eastern region were collected during four years (March 2010 to October 2013). The serological, bacteriological, and molecular analyses were performed. Serum samples were tested with 14 reference *Leptospira* serovars by microscopic agglutination test (MAT). MAT results showed that 89 (15.53%) serum samples had detectable antibodies against seven serovars of *L. interrogans* at a dilution of ≥1:100. Serovars: Pomona (38.2%), Tarassovi (27.2%), and Kabula (18.8%) were the most prevalent and their titres ranged from 100 to 1200. The spirochetes were detected in 11 samples of urine and nine samples of kidneys under dark-field microscope observation. The pure cultures were obtained from three samples. PCR technique confirmed leptospirosis in 23 out of 89 urine samples from cows, which showed the presence of leptospiral antibodies in microagglutination test. The high disease prevalence in cows indicates the high *Leptospira* contamination in this area. It was concluded that the bovine leptospirosis is an endemic and locally widespread disease in Kazakhstan, and that it may play a role in zoonotic transmission to humans.

Keywords: cattle, leptospirosis, Kazakhstan.

Introduction

Vast grazing lands and favourable climatic conditions create a good basis for the industry development in Kazakhstan. Cattle breeding in Kazakhstan is one of the most important agricultural sectors of the economy, producing meat and milk. During 11 years, between 1997 and 2008, the volume of milk production increased by an annual average of 4.5%, almost returning to its 1990 level (of 5.6 million tones) in 2008 (7). A significant part of this growth is connected with the increase in cattle population from 2.3 million in 2004 to 2.7 million in 2008, according to the data from the Kazakhstan Statistics Agency (26).

The Kazakh government has made the development of the livestock sector one of the top agricultural priorities (10). While Kazakhstan had a very large livestock herd during connection with the Soviet Union, and the reduction of subsidies and the breakup of large government farms at its end, the number of cattle in Kazakhstan decreased significantly, from nearly 10 million animals in 1990 to 4 million in 1998. The dairy farming in Kazakhstan can be divided into three sectors: the household farming sector, holding more than 85% of the cattle inventory (85% of meat and 90% of milk production in the country); the peasant/small- and medium-scale farming sector, holding approximately 10% of the cattle inventory; and the enterprise farming sector, holding the remaining 5% of the cattle inventory. As in most other countries, it is extremely difficult to find reliable information on the exact number of livestock in Kazakhstan.

On February 2013, the Kazakh government published a new "Program for the Development of the Agricultural Sector in Kazakhstan from 2013 to 2020, Agribusiness - 2020" (30). This document contains the

© 2015 Z. Kirkimbayeva et al. This is an open access article distributed under the Creative Commons Atribution-NonCommercial-NoDerivs license (http://creativecommons.org/licenses/by-nc-nd/3.0/)

government's strategy and goals for the agricultural sector for the next seven years. It requires a continuation of the development of the livestock sector, including providing support to the expansion of overall cattle (and other livestock) numbers, and improving the genetic makeup of the livestock herds. The document sets a plan for large-scale subsidized imports of cattle to be continued for the next few years, and ending in 2015 for beef cattle and in 2016 for dairy cattle. The project implementation will allow to create conditions for production of about 60 thousand tons of meat for export by 2016 and to increase this amount to 180 000 tons per year during the next five years, which will make beef cattle breeding the leading branch of agriculture (23).

Leptospirosis is a bacterial spirochaetal zoonotic disease caused by several immunologically distinct serovars of the genus *Leptospira*, affecting many animal species and humans (2). Leptospirosis occurs worldwide and is acquired through a direct or indirect contact with the urine of infected animals (2). According to many researchers (12, 19), leptospirosis of cattle is widespread in the world and causes a considerable economic damage due to high lethality (25%-45% and more), drop in milk yields (by 23% -37%), body weight loss (by 18%-28%), young growth retardation, offspring death (up to 90%), abortion (15%-20%), deterioration in commercial quality of leather of animals affected, culling of livestock products in slaughter houses and meat processing plants, disturbance of reproductive functions, as well as spending significant means for diagnostic, preventive, medical, and quarantine-restrictive measures.

Therefore, control of epizootic situation of this dangerous zooanthroponosis is an important task of veterinary services of Kazakhstan as far as infectious diseases such as leptospirosis may cause heavy economic losses in the country. A successful animal leptospirosis control requires broad knowledge of the disease epizootiology, serotypes and serovariants of the agent circulating in this region, natural and climatic features, and methods of cattle breeding. The purpose of our study was to find out the prevalence of leptospirosis infection in meat-producing animals and to determine aetiological structure of cattle leptospirosis in the South-Eastern region of the Kazakhstan.

In order to assess the potential risk for humans, the aim of the study was focused on investigation of the occurrence of *Leptospira* sp. in cattle in a rural area of the South-Eastern Kazakhstan.

Material and Methods

Study area and sample collection. Cattle from different enterprise and private farms of the South-Eastern region of Kazakhstan, Almaty province, were investigated between 2010 and 2013. Five hundred seventy three healthy animals of Alatau (225), Kazakh white (185), and Auliyekol (163) cattle breeds, aged from two to five years were randomly selected during slaughter. Urine, kidney, and blood samples were collected about 10 min right after the slaughter. Approximately 5 mL of urine sample were collected to the three sterile test tubes from each animal by direct bladder puncture on a slaughterhouse eviscerating table. After collection, urine samples were transferred to a local laboratory of Kazakh National Agrarian University in Almaty, where they were examined by dark field microscopy (DFM) and PCR. Positive samples were used for culturing. Blood samples were collected into a sterile, plain 10-mL vacutainer tubes (Becton Dickinson Vacutainer Systems, UK) by jugular venipuncture, labelled and transported in a refrigerated cool box to the laboratories of Kazakh National Agrarian University in Almaty. Kidney samples without any visible macroscopic lesion were collected on a slaughterhouse eviscerating table. These samples were then cut into two fragments of around 1 cm^3 each. One of them was placed in transport medium containing 1% bovine seroalbumin in phosphate buffer saline pH 7.4 (23). The other fragment was fixed in 10% buffered formalin. The samples were sent to the Kazakh National Agrarian University in Almaty and processed about two hours after collection.

Culture media. The 0.5 mL aliquots of urine samples were inoculated into 5 mL of Fletcher's medium, supplemented with 5-fluorouracil (200 μg/mL) as a selective inhibitor of contaminating microorganisms. Cultures were incubated at ambient temperatures (25–30°C) for 24 h. Then, 0.5 mL portions were subcultured in duplicate in 5 mL of the same medium but without antibiotic. The cultures (including the primary cultures) were examined weekly for 16 weeks under the dark field microscope (Meiji Techno CO., Japan). Fragments of the kidneys were put to sterile vessels and inoculated with Fletcher's medium (Hi Media Laboratories Pvt., India) supplemented with 5-fluorouracil (200 μg/mL) using Pasteur pipette (to the depth of 0.5 cm).

Microscopic agglutination test (MAT). Serum was separated by centrifugation of blood at 3 000 g for 10 min at room temperature, transferred into 1.5 mL sterile micro tubes (Eppendorf), and kept at -20°C until use. MAT was performed in the Research Laboratory of Kazakhs Agrarian National University. A 7-10 d culture of *Leptospira interrogans* in liquid medium (GRA-Sina) was used as antigen. The density of leptospires was assessed using a counting chamber (Petroff-Hauser, USA). It was adjusted to 2×10^8 leptospires/mL. Fourteen reference strains of *Leptospira interrogans* were also used as antigen (Table 1). All serum samples were serially diluted 1:50, 1:100, 1:200, 1:400, 1:800, and 1:1600 in phosphate buffered saline solution (PBS) in a microtitre plate (Greiner). Then, 10 μL of each diluted serum was added to 10 μL of appropriate antigen on a microscopic

slide, placed in a Petri dish with moist paper to avoid evaporation, and incubated at 30°C for 90 min. Finally, the slide was examined under the dark-field microscope. One antigen control and two positive and negative standard serum controls were used each time. Titres 100 or greater were considered positive. The end-point titre was determined as the highest serum dilution showing agglutination of at least 50% of the leptospires.

Table 1. List of investigated *Leptospira* serovars

Serovar	Serogroup	Strain
Autumnalis	Autumnalis,	Akijami A
Canicola	Canicola	Hond Utrecht IV
Copenhageni	Icterohaemorrhagiae,	M-20
Cynopteri	Cynopteri	Vleermuis 3868
Djatzi	Bataviae	HS-26
Eriacei eurapaei	Australis	Jez-1
Gryppotyphosa	Grippotyphosa	Moskva
Kabura	Hebdomonas	Kabura
Javanica	Javanica	Veldrat Bataviae 46
Pomona	Pomona	Pomona
Polonica	Serjoe	493 Poland
Pyrogenes	Pyrogenes	Salinem
Szwajizak,	serogroup Mini	Szwajizak
Tarassovi,	Tarassovi	Perepelicin

Dark-field microscopy (DFM). Five to six millilitres of urine samples collected with equal quantity of sterile PBS was immediately centrifuged at 10 000 × g for 30 min. The supernatant was collected and its one drop was placed on a clear grease free glass slide, mounted under a coverslip (18 mm^2), and examined under dark field microscope. A minimum of 50 high power fields were examined.

Two to three grams of kidney samples were grounded in approximately 5 mL of phosphate-buffered saline to obtain homogenous suspension. The suspension was transferred to sterile PBS and immediately centrifuged at 3000 × g for 10 min. A layer of clear fluid (2-3 drops) was placed on a clear grease free glass slide and mounted under a coverslip (18 mm^2). The preparation was examined under the dark field microscope. Control samples were obtained from healthy cattle (n = 5) negative by MAT. Reciprocal agglutination titres equal to 100 or higher were considered as positive.

PCR examination. To detect *Leptospira*, test-system (Narvak Ngo CJSC, Russia) was used. Urine samples (5 to 10 mL) were centrifuged at 5000 × g for 10 min and the cells were washed with a sterile 0.01 M PBS (pH 7.2). Polymerase chain reaction based on the LipL32 gene was performed using the primers 5'ATCTCCGTTGCACTCTTTGC3'and 5'ACCATCA TCATCATCGTCCA3' as previously described by Tansuphasiri *et al.* (28), resulting in a 264 bp amplicon between positions 270 and 692 of the *lipL32* coding region. This set of primers was designed to distinguish between pathogenic and saprophytic *Leptospira* species, because the LipL32 gene is amplified only in pathogenic species (28). Polymerase chain reaction amplification was performed using the following programme: an initial cycle of denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 1.5 min, annealing at 60°C for 90 s, extension at 72°C for 20 min, final extension at 72°C for 10 min and holding at 4°C. Control amplification templates included water as a negative control and *L. interrogans* genomic DNA as positive controls. Amplified products were separated on 1% agarose gels, stained with ethidium bromide, and viewed using a UV light source.

Infection of laboratory animals. Twenty golden hamsters (*Mesocricetus auratus*), weighing 80-90 g, were infected with urine (eleven) or (nine) kidney suspension, in which *Leptospira* organisms were found microscopically. The material was introduced abdominally in the dose of 0.5-1 mL. The animals were subjected to euthanasia on day 21 after infection. Samples of the kidneys were collected and introduced into Fletcher medium (Hi Media Laboratories Pvt. Limited, India). Blood samples from the heart were collected and sera were tested in MAT.

Statistical analysis. Statistical analysis was performed using the SPSS statistical programme. Normal distribution of the data was determined using Anderson-Darling Normality test. Duncan-ANOVA test to compare the parameters between the groups was used. Significant level at $P < 0.05$ was set.

Ethical considerations. The study was approved by the Local Ethical Committee of the Kazakh National Agrarian University, in accordance with the ethical standards of Principles of Laboratory Animal Care.

Results

The results of the MAT showed that 89 (15.5%) serum samples had detectable antibodies against at least one serovar of L. *interrogans* at a dilution of ≥1:100 (Table 2). Positive titres against more than one serovar were detected in 27 of the positive samples. The prevalence of L. *interrogans* in cattle of Alatau, Kazakh white, and Auliyekol breeds was 5.5%, 3.8%, and 5.2% respectively. According to Table 2, the most prevalent positive serovar was Pomona (31 samples) and then Tarassovi (23 samples), Kabura (20 samples), Polonica (seven samples), Eriacei eurapaei (four samples), Copenhageni (three samples), and Gryppotyphosa (one sample).

Fig. 1 presents the data on correlation between the prevalence of leptospirosis and the age and cattle breeds. The cattle of five years old showed the highest percentage of sera positivity (30.4% in group) and were significantly different ($P < 0.05$) from other age groups. The cattle of Alatau breed (20.0%) displayed the highest level of positivity and differed significantly

($P < 0.05$) from other breeds. The leptospiral antibody titres, ranging from 100 to 200, were detected in serum of 56 (9.8%) cows. Nineteen sera had antibody titre 400, 11 sera - 800, and three sera - 1200. The diagnostic tests for leptospirosis, including dark field microscopy, gave positive results in eleven urine and nine kidney samples. The number of the microorganisms in urine was different. During examination of over 50 of vision fields, in many samples single *Leptospira* organisms were detected, others contained from one to ten *Leptospira* in each field. Not all isolates showed typical morphology and characteristic motility for the genus *Leptospira* in direct examination under the dark-field microscope (data not shown) of urine samples. Contrary to isolates from urine, *Leptospira* organisms from kidney samples were detected in 10-30 vision fields, and in one case they were present in each field. The pure cultures of *Leptospira* were separated from samples of three cows and visible growth of *Leptospira* was seen on days 15-20.

At necropsy of infected hamsters, haemorrhages in all internal organs were observed. In two cases, a pure culture of *Leptospira* was isolated. The results of comparative evaluation using DFM and bacteriological tests are shown in Table 3.

Table 2. The distribution of sera positive for *L. interrrogans* serovar antibodies in cattle of Alatau, Kazakh white, and Auliyekol breeds

Serovar*	Antibody titres					Total	Positive (%)	Total positive of all samples (%)
	100	200	400	800	1200			
Pomona	11	8	6	5	1	31	34.8	5.4
Tarassovi	8	7	4	4	-	23	25.8	4.0
Kabura	6	5	5	2	2	20	22.5	3.5
Polonica	2	2	3	-	-	7	7.9	1.2
Eriacei eurapaei	2	1	1	-	-	4	4.5	0.7
Copenhageni	2	1	-	-	-	3	3.4	0.5
Gryppotyphosa	1		-	-	-	1	1.1	0.2
Positive	32	24	19	11	3	89	100	15.5
Negative						484		84.5
∑						573		100

* Other *Leptospira* serovars (autumnalis, canicola, cynopteri, djatzi, javanica, pyrogenes, szwajizak) were negative with MAT

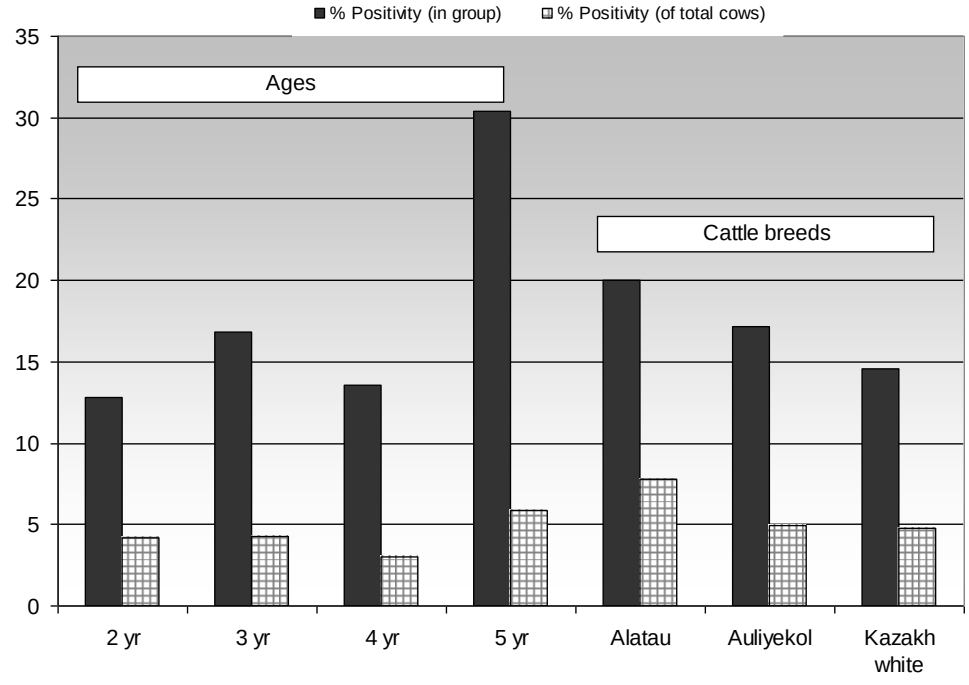


Fig. 1. Leptospirosis in slaughtered cattle from the South-Eastern Kazakhstan: Alatau (225), Kazakh white (185) and Auliyekol (163) breeds

Table 3. Results of DFM and bacteriological tests of urine and kidney samples of seropositive slaughtered cows and infected hamsters

No. of cows	DFM				Bacteriological tests	Infection of hamsters	
	Urine samples		Kidney samples			Number of infected hamsters	Bacteriological culture
	living	dead	living	dead			
15		positive	-	positive	-	2	-
112	positive	-	positive	-	positive	2	-
163	-	positive	positive	-	-	-	-
203	positive	-	positive	-	positive	2	1
220	-	positive	-	positive	-	2	-
312	-	positive	-	-	-	1	-
318	-	positive	positive	-	positive	2	-
332	-	positive	-	positive	-	2	1
383	-	positive	-	-	-	1	-
486	positive	-	positive	-	positive	2	1
523	-	positive	-	positive	-	2	-
SUM	11		9		4	20	3

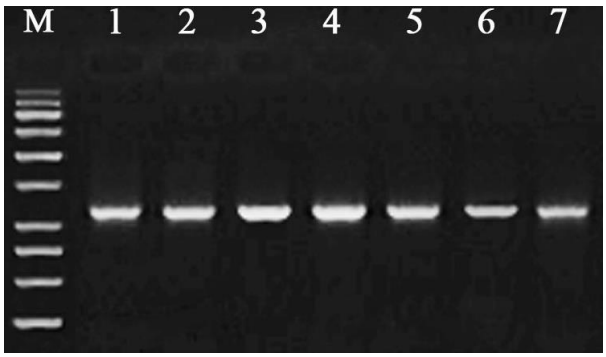


Fig. 2. Detection of *Leptospira* DNA in urine samples by polymerase chain reaction based on LipL32 gene (M, 100 bp molecular weight markers; lane 1-7, positive amplification of 474 bp product)

Urine samples from animals in which antibodies were earlier detected by microagglutination test, were additionally investigated using PCR technique. Positive results were obtained in 23 (25.8%) out of 89 urine samples. In the case of animals, which blood serum had antibody titre above 100, positive and negative results in urine samples were obtained by PCR. Out of 24 urine samples of these cows, five (20.8%) of them showed positive results by PCR. In the case where antibody titre was ≤200 (MAT), seven out of 19 (36.8%) tested urine samples gave positive results. Out of 11 sera positive animals, which had antibody titre ≤400, eight (72.7%) urine PCR samples were positive. In all animals with high concentration of antibodies determined by MAT (titre ≤800), results of PCR in urine samples were positive. MAT protocol showed that positive and doubtful reactions were obtained in 15.5% samples, whereas PCR gave positive results in 25.8% of 89 animals.

Discussion

This is the first survey of leptospiral infection in dairy cattle in the Republic of Kazakhstan. Previous surveys were conducted by questionnaire method without confirmatory laboratory tests. Serological survey of antibodies to infectious agents in beef cattle in the North - Southern Australia (6) showed a seroprevalence of 7.3% against serovar Hardjo and 16.1% against serovar Pomona in industrial dairy farms, while in traditional dairy farms, the seroprevalence was 16.13% against serovar Hardjo and 8.1% against serovar Pomona. Reports for bovine leptospirosis in Africa and other tropical regions revealed varied prevalence, as exemplified by 19.4% in South Africa (24), 46.9% in Brazil (18), and 62.8% in Mexico (25). The most common cause of leptospirosis among cattle throughout the world is the infection with leptospires belonging to serovar Hardjo (8), type hardjoprajitno, which was isolated primarily from cattle in the United Kingdom (3). The prevalence of infection with other serovars of *Leptospira* in cattle varies between different husbandry conditions; serovars Pomona and Grippotyphosa are other relatively common causes of bovine leptospirosis in the USA (4). The studies conducted in Tanzania showed that the seroprevalence of leptospirosis in traditional cattle was 7% in Morogoro (22), 10.3% in graded cattle in Tanga (27), and 21.3% in graded cattle in eastern Usambara highland (18). The seroprevalence against six reference strains of *L. interrogans* serovar Hardjo, Pomona, Icterohaemorrhagiae, Grippotyphosa, Canicola, and Ballum in Tehran suburb dairy farms by MAT (14.5%)

was reported (11). Authors suggested that there is high correlation between hygiene level and prevalence of leptospirosis in dairy farms. Cattle, goats, sheep, and dogs seem to be the most important animal sources of human infection. Serovar Hardjo has been found to be infecting sheep (1)). The reactions with eight serovars of *Leptospira* (Bataviae, Bratislava, Canicola, Hardjo, Hebdomadis, Pomona, Cynopteri, Grippotyphosa), belonging to two species (*L. interrogans, L. kirschneri*) were found in 20.1% of Polish cows (29).

The results of our serological studies have shown that *Leptospira* Pomona (34.8%), Tarassovi (25.8%), Hebdomadis (22.5%), Sejroe (7.9%), Australis (4.5%), Icterohaemorrhagiae (3.4%), Grippotyphosa (1.1%) are causative agents of leptospirosis in slaughtered cattle in South-Eastern part of Kazakhstan. These data coincides with the data obtained by Ilyasov (15), who developed the epizootic map of incidence of leptospirosis in animals within the territory of Kazakhstan between 1970 and 1990. He reported that five serovars occurred predominantly in the eastern zone were: Hebdomadis - 56.8%, Pomona - 25.7%, Tarassovi - 15.5%, Grippotyphosa - 0.3%, and Icterohaemorrhagiae - 1.8%. In the last years, these proportions have changed. At present, there are three dominant species: Pomona, Tarassovi, and Hebdomadis. However, during our studies we also found positive response by MAT to new serogroups Sejroe and Australis.

The highest percentage of seropositive reactions was recorded in the Alatau cattle (also Ala Tau). The breed of Kazakhstan cattle was named after the Alatau Mountains (from Turkic languages "Motley Mountain"). Ala Tau cattle are used for beef and dairy production. The Kazakh white-headed cattle had the lowest percentage of positive reactions (14.6%). The Kazakh white-headed cattle are a beef cattle bred in Kazakhstan and Russia. The breed was developed between 1930 and 1950 on state farms in the Kazakh Republic and the Lower Volga by crossing Hereford cattle with local Kazakh and Kalmyk cattle (14).

Comparison of results of serological, bacteriological studies, and PCR (Fig. 2) demonstrated that out of 89 seropositive cows, 23 responded positively in PCR, whereas *Leptospira* were found only in 11 urine samples. Similar studies were conducted by many researchers (20), who observed negative reaction in bacteriological studies, but positive in 50% cases using PCR (13). In the studies of these authors, PCR demonstrated very good results as compared to traditional methods, which was also confirmed in our study. This method is considered to be fast and sensitive (17), having a great value for early leptospirosis diagnosis. Moreover, bacteriological techniques are laborious and time-consuming (3, 9) and under our conditions this diagnosis of leptospirosis stays out of scheme of bacteriological analysis of meat and meat products accepted in slaughtering units.

The low antibody titres or absence of reaction in some samples by the MAT do not exclude the possibility of infection with others serovars, which were not included in the used antigen panel. Crossagglutinations were commonly observed in this study, which might be also interpreted as an indicator for multiple infections. Generally, if serum reacts with strains of different serovars, which do not cross-react, it is assumed that infection was caused by *Leptospira* of that crossreacting serovar (5).

Taking into consideration the fact that in recent period course of leptospirosis is predominantly without clinical manifestations, but is accompanied by *Leptospira*-carriage, epidemiological and epizootiological danger of this infection can be assumed. Moreover, the existence of natural and zoonotic foci of leptospirosis within the territories of different countries, including Kazakhstan, requires a detailed study of slaughtered animals and development of controlling methods.

Knowledge of occurrence and aetiology of leptospirosis, *i.e*. knowledge of *Leptospira* serovars affecting animals in Kazakhstan, is necessary for successful specific prophylaxis, and creates an opportunity to develop a complex of measures aimed at discontinuity of epizootic and epidemiological chain. Leptospirosis has been recognized as an important emerging global public health problem because of its epidemic proportions and increasing incidence in both developing and developed countries (21). Based on obtained results, it can be concluded that cattle coming into the market without any clinical signs of the disease could be *Leptospira*-carriers and thus they represent a risk to human health. We recommend farming enterprises having seropositive animals even without expressed clinical signs of the disease to conduct PCR analysis for leptospirosis and to exclude slaughter of animals in accordance with the obligatory procedure.

The results of this study can be used to provide recommendations for veterinarians and workers in slaughterhouses to be more aware of the transmission of leptospirosis and its risks for humans. The findings of this study suggest that there is a need for strict application of hygienic practice during the handling of animals and their products. It can be also recommended that large panels of representative strains should be included in order to improve the test sensitivity. More research is needed to identify different leptospiral serovars in cows and other ruminants. Additionally, it is necessary to compare different molecular and other techniques to find a reliable, inexpensive, and accessible method for serovars identification.

In conclusion, overall animal and herd seroprevalence was high, suggesting that leptospirosis is an endemic and locally widespread disease. Leptospirosis is an important disease in cattle-keeping communities in Kazakhstan, and further studies should be performed to understand the epidemiology of leptospirosis in farm animals and its association with human leptospirosis.

Conflict of Interests Statement: The authors declare that there are no conflicts of interest in this research.

Financial Disclosure Statement: This study was funded by the Kazakh National Agrarian University.

Animal Rights Statement: The experiments on animals were conducted in accordance with Local Ethics Committee laws and regulations as regards care and use of laboratory animals.

References

1. Bharti A.R., Nally J.E., Ricaldi J.N., Matthias M.A., Diaz M.M., Lovett M.A., Levett P.N., Gilman R.H., Willig M.R., Gotuzzo E.: Leptospirosis: a zoonotic disease of global importance. Lanc Infect Dis 2003, 3, 757–771.
2. Bolin C.A.: Diagnosis and Control of Bovine Leptospirosis, Proceedings of the 6th Western Dairy Management Conference, Reno, 2003, p. 155.
3. Boqvist S., Montgomery J.M., Hurst M., Thu Ho Thi Viet, Evengall E., Olsson Gunnarsson A., Magnusson U.: *Leptospira* in slaughtered fattening pigs in southern Vietnam: presence of the bacteria in kidneys and association with morphological findings. Vet Microbiol 2003, 93, 361–368.
4. Carole A.B.: Leptospirosis in cattle: disease review and update. Proceeding of the NAVC North American Veterinary Conference, Orlando, Floryda, 2005.
5. Carpenter J.A., Scorgieb A., Josephson G.: *Leptospira interrogans* serovar Pomona infection associated with carcass condemnation of swine at slaughter. J Swine Health Prod 2006, 14, 145–148.
6. Durham P.J.K., Paine G.D.: Serological survey for antibodies to infectious agents in beef cattle in Northern South Australia. Aust Vet J 1997, 75, 134–140.
7. Engelen A.: Dairy development in Kazakhstan. FAO, Rome, 2011.
8. Faine S., Adler B., Bolin C.A., Perolat P.: *Leptospira* and leptospirosis. MediSci Press, Melbourne, Australia, 1999.
9. Fearnley C., Wakley P.R., Gallego-Beltran J., Dalley C., Williamson S., Gaudie C., Woodward M.J.: The development of a real time PCR to detect pathogenic *Leptospira* species in kidney tissue. Res Vet Sci 2008, 85, 8–16.
10. Flake L., Zharmagambetov Z.: Kazakhstan Outlines Continued Strategy and Support for Cattle Sector. USDA Forein Agricultural Service, Global Information Network 2013, 6, 1–46.
11. Haji Hajikolaei M.R., Ghorbanpour Najafabadi M., Abdollahpour G.: Serological study of leptospirosis in cattle in Ahvaz. J Fac Vet Med Univ Tehran 2005, 60, 7–14.
12. Hernandez-Rodriguez P., Diaz C.A., Dalmau E.A., Quintero G.M.: A comparison between polymerase chain reaction (PCR) and traditional techniques for the diagnosis of leptospirosis in bovines. J Microbiol Meth 2011, 84, 1–7.
13. Harkin K.R., Roshto Y.M., Sullivan J.T., Purvis T.J., Cheengappa M.A.: Comparison of polymerase chain reaction assay, bacteriologic culture and serologic testing in assessment of prevalence of urinary shedding of leptospires in dogs. J Am Vet Med Assoc 2003, 222, 1230–1233.
14. http://www.fao.org/docrep/009/ah759e/AH759E08.htm
15. Il'yasov B.K: The etiological structure of Leptospirosis in the Republic of Kazakhstan (the epidemiological map). Copyright certificate number 365 of 17.07.1998.
16. Karimuribo E.D., Swai E.S., Kyakaisho P.K.: Investigation of a syndrome characterised by passage of red urine in smallholder dairy cattle in East Usambara Mountains, Tanzania. J S Afr Vet Assoc 2008, 79, 89–94.
17. Kee S., Kim I., Choi M., Chang W.: Detection of leptospiral DNA by PCR. J Clin Microbiol 1994, 32, 1035–1039.
18. Lilenbaum W., Souza G.N.: Factors associated with bovine leptospirosis in Rio de Janeiro. Brazil Res Vet Sci 2003, 75, 249–521.
19. Malakhov Y.A., Panin A.N., Sobolev G.L.: Leptospirosis in farm animals. Moscow 2000, p. 420.
20. Otaka D.Y., Martins G., Hamond C., Penna B., Medeiros M.A., Lilenbaum W.: Serology and PCR for bovine leptospirosis: herd and individual approaches. Veterinary Record published online 2012, March 16.
21. Meites E., Jay M.T., Deresinski S., Shieh Wun-Ju., Zaki S.R., Tompkins L., Smith D.S.: Reemerging leptospirosis, California. Emerg Infect Dis 2004, 10, 406–412.
22. Mgode G.F., Machang'u R.S., Goris M.G., Engelbert M., Sondij S., Hartskeerl R.A.: New *Leptospira* serovar Sokoine of serogroup Icterohaemorrhagiae from cattle in Tanzania. Int J Syst Evol Micr 2006, 56 (Pt 3), 593–977.
23. Resolution of the Government of the Republic of Kazakhstan, Astana, Ukimet Uyi on July. 29, 2011, No. 877 On approval of the comprehensive action plan for implementing the project "Development of export potential of cattle meat" for 2011–2015 http://kazpravda.kz/_pdf/oct11/221011decision.pdf
24. Roach J.M., van Vuuren M., Picard J.A.: A serological survey of antibodies to *Leptospira* species in dogs in South Africa. J S Afr Vet Assoc 2010, 81, 156–159.
25. Segura-Correa V.M., Solis-Calderon J.J., Segura-Correa J.C.: Seroprevalence of and risk factors for leptospiral antibodies among cattle in the state of Yucatan, Mexico. Trop Anim Health Prod 2003, 35, 293–299.
26. Statistical Yearbook of Kazakhstan, 2009: www.stat.kz..
27. Swai E.S., Schoonman L., Machang'u R.S.: Prevalence and factors associated with bovine leptospirosis in small scale dairy farms in Tanga region, Tanzania. Bull Anim Health Prod Afr 2005, 53, 51–59.
28. Tansuphasiri U., Chanthadee R., Phulsuksombati D., Sangjun N.: Development of a duplex–polymerase chain reaction for rapid detection of pathogenic *Leptospira.* South Asian J Tropic Med Pub Health 2006, 37, 297–308.
29. Wasiński B., Sroka J., Wójcik-Fatla A., Zając V., Cisak E., Knap J., Sawczyn A., Dutkiewicz J.: Occurrence of leptospirosis in domestic animals reared on exposed or non-exposed to flood areas of eastern Poland. Bull Vet Inst Pulawy 2012, 56, 489–493.
30. Zharmagambetova Z.: Agricultural Development Program 2013 –2020. USDA Foreign Agricultural Service, Global Information Network, 2013, pp. 1–80.