International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies

http://TuEngr.com

PAPER ID: 11A15P

DIAGNOSES OF LEPTOSPIROSIS IN ANIMALS

Gulnara Nurlygayanova [1*], Vasily Belousov [1], Alisa Varentsova [1], Nadezhda Ozheredova [2], Oleg Chernykh [3]

[1] *Department of Short Research Papers, Federal State Budgetary Institution «Central Scientific and Methodical Veterinary Laboratory», RUSSIA.*
[2] *Department of Epizootology and Microbiology, Stavropol State Agrarian University, RUSSIA.*
[3] *Department of Microbiology, Epizootology and Virology, Kuban State Agrarian University named after I. T. Trubilina, RUSSIA.*

ARTICLEINFO

Article history:
Received 01 June 2020
Received in revised form 04 September 2020
Accepted 14 September 2020
Available online 15 September 2020

Keywords:
Dark-Field Microscopy; Microagglutination Reaction; PCR; Bacteriological studies; Microagglutination Reaction; Infectious jaundice; Microscopic agglutination test (MAT).

ABSTRACT

There have been presenting a comparative analysis of the results of leptospirosis diagnosis by methods used in the Russian Federation's veterinary laboratories. Using the microagglutination reaction, specific antibodies to leptospira were detecting in 136862 animals. A total of 64686 urine samples was examined with dark-field microscopy. Applying PCR, the leptospira genome is detected in pathological material in 57 cases. The PCR method in the water of open reservoirs cannot detect the pathogenic leptospira genome. In the study of biological material, the leptospira genome was found in 248 cases.

To increase the efficiency of diagnosis and reduce the time of diagnosis for leptospirosis, it is advisable to conduct parallel bacteriological studies of the material in combination with PCR analysis. Micro-agglutination reaction and molecular genetic analysis (PCR) are the most inforeaction micro-agglutinative and technologically advanced laboratory diagnostic methods.

Disciplinary: Biology, Epizootology, and Microbiology, Bioscience, Veterinary Sciences.

1 INTRODUCTION

The work devoted to the laboratory diagnosis of leptospirosis (see Figure 1). This infectious disease common to animals and humans, causing damage to livestock and human health (Bolotsky et al., 2009; Soboleva et al., 2017). In the Russian Federation (RF), animal leptospirosis is widespread almost everywhere, due to the presence on its territory of natural, anthropurgic, and mixed foci of the causative agent of the disease. This disease is not only in rural areas but also in cities, due to natural-geographical and climatic features. Currently, more than 130 animal species can be sources of

*Corresponding author (G.Nurlygayanova). Tel: +7-926996523 Email: Nurlygayanova@gmail.com Volume 11 No.15 ISSN 2228-9860 eISSN 1906-9642 CODEN: ITJEA8 Paper ID:11A15P http://TUENGR.COM/V11B/11A15P.pdf DOI: 10.14456/ITJEMAST.2020.304

this infection (Ananyina 2010).

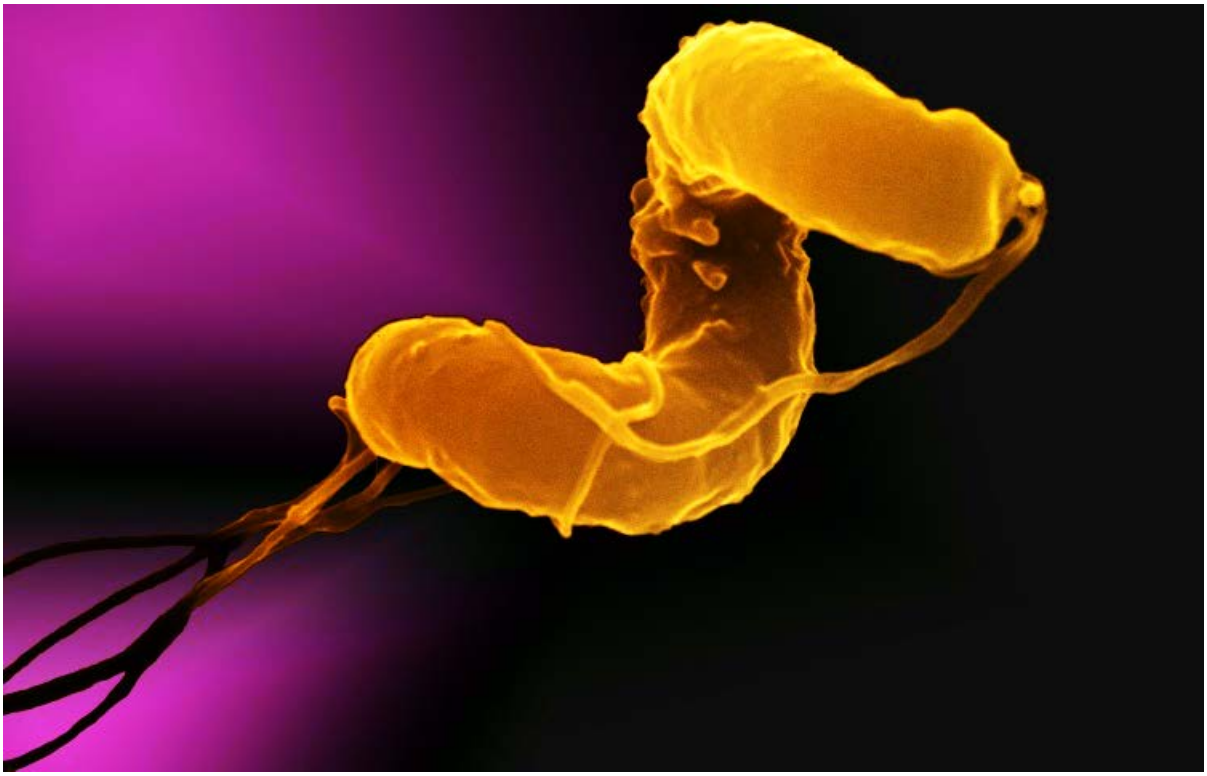

Figure 1: Leptospirosis or infectious jaundice: a natural focal disease.

For the period from 2016-2018, according to the Rospotrebnadzor and the Inforeaction of the microagglutination-analytical center of the Veterinary Control Department of the Federal Center for Animal Health, 469 people became ill with leptospirosis, and 470 dysfunctional cases were registered, in which 5,653 were detecting sick productive animals.

Domestic and foreign scientists note that the infection process in most animals proceeds in a latent form, which complicates timely diagnosis and contributes to latent leptospirosis. Leptospironiferous animals secrete the pathogen with urine into the environment and contaminate, infecting healthy people and animals. The excretion of leptospira with urine can last up to 1.5 years and for life in rodents (Los'-Yatsenko et al., 2011; Bal, et al., 1994). To effectively combat the spread of this infection, timely and complete identification of animals with a hidden form of the disease is necessary, which is a significant and urgent problem both in Russian Federation and in the world (Belousov et al., 2018; Budihal and Perwez 2014).

There is a need to improve laboratory diagnostic methods for animal leptospirosis used in testing laboratories of the Russian Federation. Our work aims to analyze the results of leptospirosis laboratory diagnosis in animals for the 2016-2018 period. Determine the effectiveness of various methods using in practice.

2 MATERIALS AND METHODS

Laboratory diagnosis of animal leptospirosis in the Russian Federation based on a complex of bacteriological, serological, and molecular genetic studies. This diagnosis was carried out throughout the Russian Federation by GOST 25386-91.

An analysis of the annual reporting data in the 4-vet form showed that the veterinary laboratories of the Russian Federation examined material selected from horses, cattle and small cattle, pigs, other animal species (dogs, cats, rabbits, deer, fur animals, rats, wild boars, buffaloes, seals, zoo animals, different species).

For intravital examination for leptospirosis, studies have been conducting of blood samples and blood serum, urine, and sperm. Samples of pathological material were taken from aborted fetuses, from animal corpses, and during diagnostic slaughter (parenchymal organs, transudate from the chest and abdominal cavities, pericardial fluid, bladder with contents, cerebrospinal fluid).

Bacteriological studies include post-mortem autopsy, injection of material on nutrient media, dark-field and luminescent microscopy, the staging of a biological sample.

One of the primary methods for the intravital diagnosis of leptospirosis is the microagglutination reaction. For staging the microagglutination reaction as an antigen in the Russian Federation, live cultures of the leptospira standard set of 7-15 typical diagnostic strains used (the number of tensions in the collection depends on the status of the testing laboratory). The vital activity of the strains is maintained by regular reseeding every 12-15 days. Every quarter, laboratories monitor in the cross-reaction of microagglutination using group agglutinating sera. In Russian veterinary practice, the reaction was recorded according to a conditional system for assessing four crosses. A microagglutination reaction estimated to be two crosses or more is considered positive if there are specific antibodies in the blood serum titer of 1:50 and higher in unvaccinated and 1:100 and higher in vaccinated animals.

In laboratories equipped with modern and high-tech equipment, for the early diagnosis of leptospirosis, molecular genetic studies (PCR) are used. The tests carried out using commercial test systems by the requirements of the manufacturer of the test system for the determination of Leptospira interrogans DNA.

In the Russian Federation, enzyme-linked immunosorbent assay (ELISA) kits for the detection of antibodies to leptospira are presented on the diagnostic market. However, in routine laboratory practice, they did not find the full application.

3 RESULTS

Serological diagnosis: For the intravital diagnosis of leptospirosis in animals, the microagglutination reaction proposed by Martin and Pettit in 1916 remains a mass test. Using the microagglutination reaction, animals of various species vaccinated and unvaccinated against leptospirosis examined. The practical significance of the microagglutination reaction: the response is specific in any dilution of serum, a positive microagglutination reaction indicates the contact of a living organism with the pathogen in the past. The microagglutination reaction allows you to determine the level of antibodies to the pathogen at the level of the serogroup. With the help of the microagglutination reaction, the etiological structure of leptospira circulating in a specific territory studied. You can examine native samples of blood serum and dried on filter paper, which makes it possible to deliver to the laboratory samples taken in the field in remote farms.

However, there are certain disadvantages to the microagglutination reaction: the subjectivity of

*Corresponding author (G.Nurlygayanova). Tel: +7-926996523 Email: Nurlygayanova@gmail.com Volume 11 No.15 ISSN 2228-9860 eISSN 1906-9642 CODEN: ITJEA8 Paper ID:11A15P http://TUENGR.COM/V11B/11A15P.pdf DOI: 10.14456/ITJEMAST.2020.304

evaluating the results of the reaction; the possibility of cross-reactions; the work of laboratory specialists using a large number of live antigens; the need for regular reseeding of strains; the cost of working time for setting and accounting, especially when examining a large number of materials; the complexity and monotony of labor.

Table 1 presents the results of intravital studies of animals for leptospirosis in the microagglutination reaction and dark-field microscopy (See Figure 2) performed in veterinary laboratories of the Russian Federation.

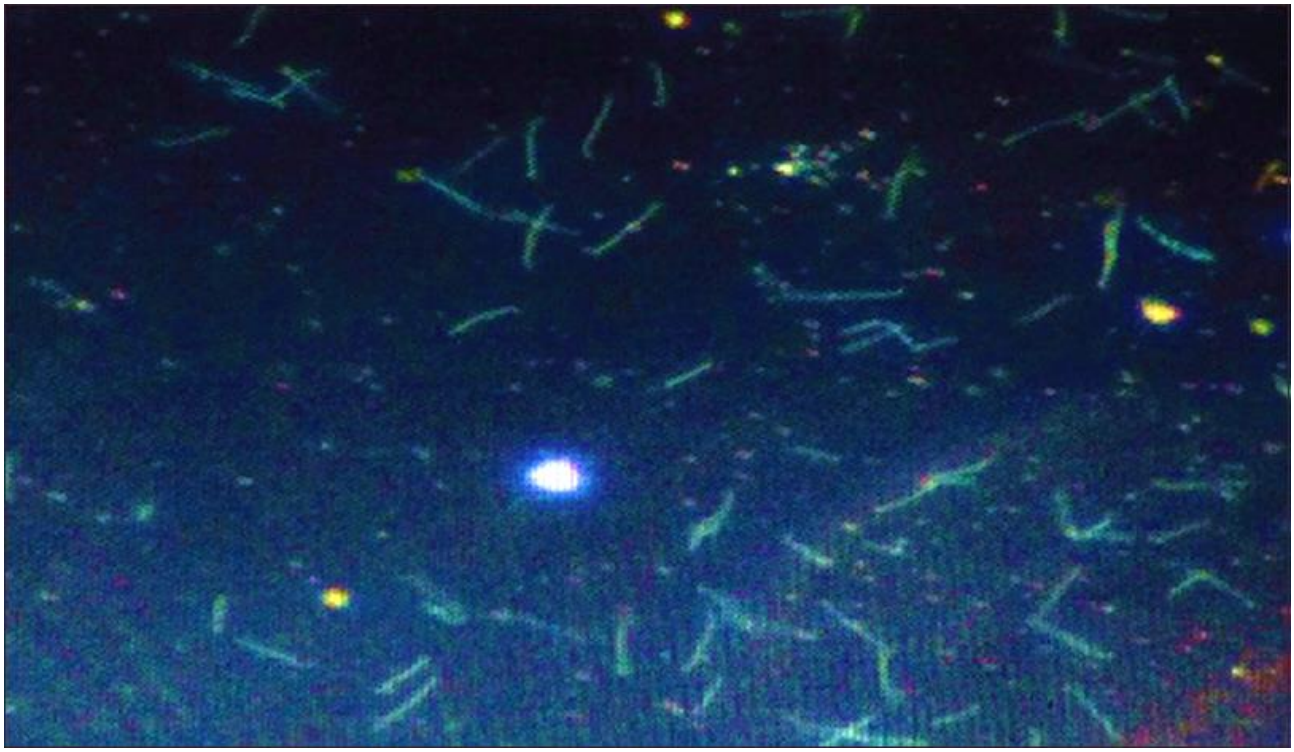

Figure 2: Leptospira under dark field microscope (x400).

Table 1: The results of animal studies on leptospirosis by the method of microagglutination reaction and dark-field microscopy

Animal species	Diagnostic Method / Material Name					
	Microagglutination reaction (blood serum)			Darkfield microscopy (urine)		
	total samples	total positive	part of positive (%)	total samples	total positive	part of positive (%)
Horses	191725	15420	8.0	2051	5	0.2
Cattle	1627894	92465	5.7	37256	93	0.2
Small cattle	425262	6748	1.6	623	2	0.3
Pigs	1216911	19424	1.6	23168	-	-
Other types	49621	2805	5.6	1588	128	8.1
Total	3511413	136862	3.9	64686	228	0.3

The data in Table 1 indicate that in total 3511,413 blood serum samples were received in the veterinary laboratories of the Russian Federation for testing leptospirosis in the microagglutination reaction, 136 862 (3.9%) identified as responding positively. The most significant number of seropositive animals found among horses 8.0%, cattle 5.7%, and other species 5.6%.

To identify leptospiratory animals, a total of 64 686 urine samples were studied by dark field microscopy in the Russian Federation, and 228 (0.3%) were obtained positive. The percentage of

identified leptospiratory animals was: horses and cattle, 0.2% each; small cattle 0.3%; other species 8.1% (dogs, cats, fur-bearing animals, zoo animals, different species). At the same time, not a single case of leptospiogenesis among the swineherd was revealed, which indicates the correct regimes and patterns of use of antileptospirosis vaccines in growing swine herds.

Note that out of the total number of leptospiratory animals (102 cases) detected by all Russian Federation veterinary laboratories during 2018, 73.5% of positive cases were diagnosed in the testing laboratories of the Central Scientific and Methodological Veterinary Laboratory (FSBN TsNMVL). In 2018, 1,231 urine samples were received at the Federal State Budgetary Institution of the Central Scientific Medical Laboratory for Microscopy; leptospiratory animals were detected in cattle and dogs.

Bacteriological diagnostics: The importance of the bacteriological method in the operational diagnosis is not great, because the method is time-consuming and time-consuming. However, the isolation of cultures of leptospira is evidence of the leptospirosis etiology of the disease during diagnosis, an intravital examination of animals to clarify the diagnosis, control the quality of treatment, puts forward a bacterioscopic study.

At the same time, there are certain disadvantages of the method: long cultivation of leptospira; the need to identify and differentiate selected cultures, special requirements for special nutrient media, and the quality of the dishes used. Against the background of the use of antibiotics, the sensitivity of the method decreases. An essential condition for successful diagnosis by this method is the temperature and time of delivery of the material to the diagnostic laboratory.

Table 2: The results of studies of pathological material and aborted fetuses for leptospirosis

Material	Number of materials, samples	Diagnostic Method / Number of Studies					Total positive
		histological	fluorescence microscopy	bacteriological	biological	PCR	
Horses							
histological material	249	-	-	-	-	249	1
abortion fetus	5	-	-	-	-	5	-
Cattle							
histological material	1198	-	21	12	10	1177	7
abortion fetus	1283	-	770	662	642	451	1
Small cattle							
histological material	116	2	18	18	18	98	-
abortion fetus	23	-	-	-	-	23	-
Pigs							
histological material	1706	1	4	4	-	1609	14
abortion fetus	1878	-	-	-	-	1876	21
Other species							
histological material	495	2	11	3	-	484	35
abortion fetus	6953	5	824	699	670	5972	79

Currently, in veterinary laboratories of the Russian Federation, bacteriological studies of pathological material and aborted fetuses for leptospirosis are carried out in insignificant amounts due

*Corresponding author (G.Nurlygayanova). Tel: +7-926996523 Email: Nurlygayanova@gmail.com Volume 11 No.15 ISSN 2228-9860 eISSN 1906-9642 CODEN: ITJEA8 Paper ID:11A15P http://TUENGR.COM/V11B/11A15P.pdf DOI: 10.14456/ITJEMAST.2020.304

to changes in management forms, violation by owners of a personal subsidiary, and peasant farms of the requirements of veterinary rules. It is impossible to exclude the problematic climatic conditions of Russia, the remoteness of animal welfare from veterinary laboratories. Table 2 presents the results of studies on leptospirosis of pathological material (pathological material) and aborted fetuses (abortion) by bacteriological methods and the molecular genetic method (PCR).

The data presented in Table 2 indicate that 6,953 samples of material received in the veterinary laboratories of the Russian Federation for research on leptospirosis, according to which 8,170 studies were carried out. In contrast, bacteriological studies, in any case, did not establish leptospirosis. Seventy-nine positive results obtained using PCR, including in the study of pathological material, the leptospira genome was detected in 57 cases (1.5%), and in the study of aborted fetuses, 22 cases (0.7%). The leptospira DNA detection rate for animal species was: horses 0.4%, cattle 0.3%, pigs 1.0%, other species 7.1%.

Thus, the parallel use of traditional diagnostic methods with modern methods (PCR) reduces the time of diagnosis and significantly increases its effectiveness.

We conducted comparative experimental studies on the effectiveness of the detection of leptospira in the test material in animals infected with leptospirosis at the scientific and production base of the Central Scientific and Methodological Veterinary Laboratory. A total of 127 golden hamsters weighing up to 25 g, highly sensitive to this serogroup, were infected with leptospira serogroup Grippotyphosa. The leptospira culture is administered to animals intraperitoneally and subcutaneously. After six days, the animals were killed according to the requirements of bioethics. The liver and kidneys examined using various methods: microscopic, luminescent microscopic, bacteriological (plating on albumin nutrient medium), biological (reinfection of hamsters with suspension from hamsters and kidneys of hamsters, followed by isolation of leptospira on culture media), and molecular genetic method (PCR).

Using microscopy in a dark field of the drug “crushed drop” it was possible to detect leptospira in only 63% of cases, by sowing on albumin medium in 56.7% of cases, by placing a bioassay on golden hamsters 94.5%, luminescent microscopy 96.8%, by PCR method 98.0% of cases.

Thus, in working conditions for the detection of leptospira, one can successfully apply a biological test on golden hamsters, followed by isolation of leptospira in culture media, luminescence microscopy, and PCR. The highest efficiency observed when used in the diagnosis of biological samples. To increase the efficiency of leptospira isolation, we recommend that laboratories use albumin media.

Table 3: The results of studies of biological material using PCR for leptospirosis

Material	Number of studies, samples				Total positive	The share of positive in%
	Years			Total, 2016-2018		
	2016	2017	2018			
Blood serum	2868	1886	1277	6031	5	0.08
Blood	4802	1155	1387	7344	232	3.2
Urine	339	1940	64	2343	11	0.5
Microagglutination Surgery	64	77	-	141	-	-
Total	8 073	5058	2728	15859	248	1.6

Molecular genetic studies using PCR are used to identify the genome of the causative agent of leptospirosis. The method is characterized by high specificity and sensitivity (from 10 to 1000 cells in the sample) and high diagnostic efficiency in the first week of the disease (starting from the first day), even against the background of antibiotic therapy, which allows you to quickly confirm or deny the clinical diagnosis by the laboratory method (Stoyanova et al, 2010).

The main disadvantages of the method are the receipt of false-positive results in the case of contamination of samples; also, DNA polymerase inhibitors may appear in the reaction mixture, which can lead to a false-negative effect,

Table 3 presents the results of studies of samples of biological material from animals by the PCR method to detect leptospira DNA, performed in veterinary laboratories of the Russian Federation.

The results of the analysis of Table 3 show that a total of 15859 samples of biological material were studied, of which 1965 samples were from horses, cattle 7739, small cattle 487, pigs 5066, other animal species 602. Results give a total of 248 positive (1.6%). The leptospira genome was detected: in horses in 82 cases (4.2%), cattle 47 cases (0.6%), small cattle 2 cases (0.4%), pigs 10 cases (0.2 %), other animal species 107 cases (17.8%).

Leptospira DNA found in blood serum 0.08% of cases, in the blood 3.2%, in urine 0.5%, in semen samples the result is negative.

Murgia et al. (1997) proposed to use PCR to identify pathogenic and saprophytic leptospira species in water. Table 4 presents the results of studies of water samples of open reservoirs for the presence of pathogenic leptospira, performed in veterinary laboratories of Russia.

Table 4: The results of the study of water samples by PCR

Material	Number of studies, samples				Total positive
	Years			Total (2016-2018)	
	2016	2017	2018		
Water	7	-	6	13	-

Table 4, in the veterinary laboratories of the Russian Federation, open water research not carried out regularly. In small quantities, the DNA of pathogenic leptospira for this period is not detected. It has been known that the negative results of the study samples are not grounds for excluding water as a transmission factor of the causative agent of infection since the concentration of leptospira in water samples is low. The biological method can also be used to study water (Malakhov et al., 2000).

4 DISCUSSION

Studies have shown that in the Russian Federation, leptospirosis of animals according to the results of serological reactions recorded in 3.9% of cases, including among horses 8.0%, cattle 5.7%, small cattle, and pigs 6% each, among other animal species 5.6%.

Carrier leptospiration in animals was detected by darkfield microscopy in 0.3% of cases, including 0.2% among horses and cattle, 0.3% in small cattle, and 8.1% in other animal species (dogs, cats), fur-bearing animals, zoo animals, and other species). Dogs can be a source of human infection.

In the analyzed period, leptospirosis was not established by the bacteriological method

*Corresponding author (G.Nurlygayanova). Tel: +7-926996523 Email: Nurlygayanova@gmail.com Volume 11 No.15 ISSN 2228-9860 eISSN 1906-9642 CODEN: ITJEA8 Paper ID:11A15P http://TUENGR.COM/V11B/11A15P.pdf DOI: 10.14456/ITJEMAST.2020.304

(microscopy, culture on culture media, biological sample, pathological autopsy).

In the PCR study of pathological material and aborted fetuses, leptospirosis detected in 1.5% and 0.7% of cases, respectively; in the study of biological material (microagglutination operation, blood, blood serum, urine) in 1.6% of cases.

Pathogenic leptospira in the water of open reservoirs by the PCR method is not detected.

5 CONCLUSION

Analysis of the research results showed that laboratory diagnosis of leptospirosis in animals in the Russian Federation carried out comprehensively, using traditional methods with molecular genetic. However, the success of the laboratory diagnosis of leptospirosis largely depends on the quality of sampling of material and the time of delivery to the laboratory for research.

To increase the effectiveness of diagnostic, preventive, and health-improving measures in case of animal leptospirosis, it is necessary:

1. For all animals that respond positively to the microagglutination reaction, additionally conduct urinalysis by dark field microscopy and molecular genetic method (PCR).

2. For the timely detection of patients with leptospirosis, animals use luminescent microscopy and polymerase chain reaction (PCR).

3. To increase the efficiency of isolating leptospira cultures, testing laboratories must use albumin nutrient media, as well as staging a biological sample on golden hamsters aged 20-30 days or rabbits aged 10-20 days.

6 AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding author.

7 REFERENCES

Ananyina, Yu.V. (2010). Leptospirosis of humans and animals: distribution trends and prevention problems. *Epidemiology and Vaccine Prevention, 2*(51), 13-16.

Bal, A.E., Gravekamp, C., Hartskeerl, T.R.A., De Meza-Brewster, J., Korver, H., Terpstra, W.J. (1994). Detection of Leptospires in Urine by PCR for Early Diagnosis of Leptospirosis. *Journal of Clinical Microbiology*, 1894-1898.

Belousov, V.I., Nurlygayanova, G.A., Varentsova, A.A., Bazarbayev, S.B. (2018). Laboratory diagnosis and improvement of the prevention of leptospirosis in animals in the Russian Federation. *Proceedings of the All-Russian Research Institute of Experimental Veterinary Medicine named after Ya.R. Kovalenko, 80*(2), 46-52.

Bolotsky I.A., Sementsov, V.I., Prutsakov, S.V., Vasiliev, A.K., Pakhomov, A.V. (2009). The epizootic role of wild animals in leptospirosis in the North Caucasus. *Journal of Veterinary Medicine of the Kuban, 3*, 15-16.

Budihal, S.V., Perwez, K. (2014). Leptospirosis Diagnosis: Competency of Various Laboratory Tests. *Journal of Clinical and Diagnostic Research, 8*(1), 199-202.

Los'-Yatsenko N.G., Karimov, I.Z., Pavlenko, A.L., Kozlovsky, O.A. (2011). Leptospirosis in the Crimea - a problem not only of infectious diseases. *Krimsky therapeutic journal*, 1, 83-88.

Malakhov, Yu.A., Panin, A.N., Soboleva, G.L. (2000). *Leptospirosis of animals*. Yaroslavl. DIA-press, 584.

Soboleva, G.L., Ananyina, Yu.V., Nepoklonova, I.V. (2017). Actual issues of leptospirosis in humans and animals. *Russian Veterinary Journal, 8*, 13-17.

Stoyanova, N.A., Tokarevich, N.K., Vaganova, A.N., Gracheva, L.I., Mayorova, S.O. (2010). *Leptospirosis: a manual for doctors*. Edited by Yu.V. Ananyina, SPb. NIIEM them. Pasteur, 116.

World Health Organization. (2003). *Human leptospirosis: guidance for diagnosis, surveillance, and control*. 109p.

Gulnara Nurlygayanova is a Veterinarian and Researcher of the Department of Leading Research Works. She is a Candidate of Veterinary Sciences. Her researches are Diagnostics of Infectious and Non-Infectious Diseases of Animals, Fish, and other Aquatic Organisms.

Professor Dr.Vasily Belousov is a Chief Researcher of the Central Scientific and Methodical Veterinary Laboratory. He holds a Doctor of Veterinary Sciences. His researches are Quality Control and Safety of Products of Animal and Plant Origin, Organization and Conduct of Laboratory Research.

Alisa Varentsova is Head of the Department for Coordination of Research Works, Candidate of Biological Sciences. She researches on an organization of laboratory works in Veterinary Medicine.

Professor Dr.Nadezhda Ozheredova is a Head of the Department of Epizootology and Microbiology. Her research includes Features of the Manifestation of Citrobacteriosis, candidiasis in Fish, and the Sanitary Assessment of Fish Products.

Professor Dr.Oleg Chernykh is Director of the Krasnodar Regional State Budgetary Institution "Kropotkin Regional Veterinary Laboratory". He holds a Doctor of Veterinary Sciences. He studies Infectious Diseases of Animals and Develops Therapeutic, Preventive Measures, and Veterinary and Sanitary Measures to Eliminate Infectious Animal Diseases.

*Corresponding author (G.Nurlygayanova). Tel: +7-926996523 Email: Nurlygayanova@gmail.com Volume 11 No.15 ISSN 2228-9860 eISSN 1906-9642 CODEN: ITJEA8 Paper ID:11A15P http://TUENGR.COM/V11B/11A15P.pdf DOI: 10.14456/ITJEMAST.2020.304