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Immunological Response to the Use of Leptospira Bacterins and Its Correlation with Serovases Present in Dairy Cattle from FMVZ-BUAP

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Abstract

Currently, vaccination techniques have been used for the prevention of leptospirosis considered a zoonosis, and in this way to avoid a major problem, in this investigation we worked with dairy cattle from the zootechnical post of the FMVZ-BUAP, due to this a vulnerable and economically important population; 20 blood sera from dairy cattle were analyzed, 12 were from producing cows with a history of vaccination and 8 from unvaccinated calves, using the Micro Plate Agglutination (MAT) method, which allowed us to detect antibodies against 12 leptospira serovars: Icterohaemorrhagiae, Bratislava, *Pyogenes, Grippotyphosa, Canicola, Pomona, Wolffi, Hardjo, Tarassovi*, of which three were national isolates H-89, *Palo Alto, Portland Vere*; through the analysis of these samples, information was obtained on the status, knowledge and identification of the serovars that are present in the post, and it was found that the *Bratislava serovar* has a greater number of positive cases in unvaccinated animals (87.5 %), the *Portland vere serovar* had 100% of positive cases in vaccinated animals and 62.5% in non-vaccinated animals, likewise for the rest of the serovars. The prevalence for the serovars *Icterohaemorragiae* and *Palo Alto* was 35%, *Pyogenes* and *Canicola* was 15%, *Grippotyphosa* and *Pomona* 10%. While for the serovars, *Wolffi, Hardjo, Tarassovi* and H-89 in the two groups of animals there were 100% negative cases. A comparative analysis was carried out between serovars of vaccinated animals with respect to those not vaccinated with the applied vaccines and their relationship in terms of immunological protection.

Keywords: Leptospirosis, Immune response, Bacterins, Serovars

Introduction

Leptospirosis is a zoonotic disease with a worldwide distribution that causes significant morbidity and mortality in human and animal populations. Leptospira interrogans is one of the main causes of animal diseases [1]. This systemic disease of humans and domestic animals, mainly dogs, cattle, and swine, is characterized by fever, renal and hepatic failure, pulmonary manifestations, and reproductive failure. Leptospirosis is caused by a spirochete of the Leptospira genus and was first described in 1886 by A. Weil. It occurs throughout the world, but is more common in tropical and subtropical areas. (Undersecretary of Prevention and Health Promotion). The analysis of the data in the Mexican Republic indicates, through the endemic channel, that leptospirosis occurs regularly throughout the year. However, according to the endemic index, there is an increase in cases in the months of August, September and October [2]. After infection, Leptospira are found in the blood and invade virtually all organs and tissues.

The transmission of leptospirosis can be through urine, semen, milk, placental. Cattle infected with Leptospira constitute an active reservoir for the spread of the zoonotic disease, especially for humans who are in direct contact with these animals.

When females become infected, they present abortions, mummifications, stillbirths, calves born weak, mastitis and sometimes reproductive failure, resulting in economic losses over production. For this reason, leptospirosis control requires specialized attention [3]. Despite this being a public health disease, it is not given the importance it really should be. Data from 2013 reveal annual reports of more than 500,000 severe cases and mortality of more than 10% in humans; (Pan American Health Organization). If only in the state of Puebla there is a study on the prevalence of Leptospira serovars that affect cattle in some states of the Mexican Republic, Veracruz, Puebla and Tabasco [4]. This disease is controlled and prevented through complementary measures, antibiotic treatment, vaccination, hygienic-sanitary prophylaxis; in order to avoid economic losses in production. Molecular methods appear as important tools for the diagnosis of chronic silent leptospirosis in domestic animals, the reference serological method for the identification of serovars present is the microscopic agglutination test (MAT) used to diagnose the disease in both individuals and in herds [5]. Reinforcing its evident impact not only on animal reproduction but also in the context of Health [6].

Material and Methods

Study Area

The study area was the dairy cattle module of the Posta Zootécnica "El Salado FMVZ-BUAP"; It is located in the Salado that belongs to the municipality of Tecamachalco de Guerrero, Puebla, Its coordinates 18.8957418092, -97.6836947016, it is described as a warm-temperate climate.

Determination of Sample Size

A total population of 100 heads of bovines in production of the zootechnical post was considered, exclusively from dairy cows, for which the proportion estimation formula was applied to determine the sample size necessary to calculate the percentage of the population that could have or not the variable of interest using the following equation. n= z2pq/d2

Where n= sample size

Z= confidence level = 1.96 at 95%

P= Probability of the event occurring (estimated prevalence) 14%

Q= 1-p, probability that the event does not occur

D= estimated error (20%)

Substitution

n= (1.96)2(14) (100-1486)/202

n= (3.84) (14) (86)/400

n= 11.55

n= 12 cows

Type of Sampling

It was worked with 12 samples of dairy cows with a history of vaccination, which were chosen by means of a non-probabilistic sampling in which a non-random selection of the elements was carried out and the probability of being selected was unknown. Its importance lies in specific conditions in search of special characteristics, which make randomization impractical, as is the case of sick animals for cases and controls [7]. Through judgment and criteria sampling, samples were extracted from cows with a probability of finding the variable of interest, which in this case were samples of cows with a reproductive history, records of abortions, mummifications, and recurrent mastitis using the MAT test.

To complement the research work, it was taken into account to carry out a random sampling of an exposed population that is vulnerable, the calves, from the rearing area, without a history of vaccination, previously weaned in a range of 4 to 6 months of age, of which 8 samples were obtained.

Place and Time of Sampling

The samples were taken in the month of August, which includes summer seasons. Leptospirosis presents a seasonal frequency, increasing with increased rainfall and with occurrences of epidemics associated with changes in human behavior, water contamination with animals or wastewater (Ministry of Health 2012).

Study Population

Twelve blood samples were taken from the coccygeal vein of the cows in production of different ages, ranging from 1 to 6 years of age; As presented in Table 1, the selection of the study population was made based on their clinical history, record of reproductive history, abortions, calves born weak or mummifications. The following were 8 blood samples from the jugular vein of the weaned calves of the rearing area, and without vaccination record in a range of 4 to 6 months of age as shown in table 2.

Table 1: Population data registry

With a history of vaccination.

Vaccinated Cows	
Number´s Tag	**Age**
0.32	1 year -10 months
42	1 year -7 months
113	6 years -7 months
116	6 years -6 months
131	5 years -11 months
142	5 years -7 months
163	5 years -7 months
164	4 years -10 months
179	4 years -2 months
180	4 years -2 months
186	3 years -7 months
189	3 years -7 months

Table 2: Population data registry

Without a history of vaccination.

Non-Vaccinated Calves	
Number´s Tag	**Age**
0.93	6 months
0.94	6 months
0.95	6 months
0.96	5 months
0.97	5 months
0.98	5 months
0.99	4 months
100	4 months

Diagnostic Methodology

The samples were analyzed in a private laboratory, TAQ. Veterinary clinical analysis dedicated to ruminants. They were analyzed by the Microscopic Agglutination technique (MAT). Considered the standard technique for the diagnosis of this disease. The samples were analyzed by means of the MAT technique, since it is considered the reference test for the serological diagnosis of leptospirosis, it is considered a standard test; Mainly used to diagnose the disease in herds and individuals, it is very helpful in diagnosing acute infection. In this test live antigens are used, considering a positive sample when the titers are ≥ 1:100, the antibodies are produced a few days after infection and can last weeks, months and even years [5].

The OIE code mentions that 1:100 is considered a positive titer in unvaccinated animals, but in vaccinated animals we will be considering 1:400 as positive titres as latent infection. The test interpretation criteria indicate that titles of 1:50 are suspicious and 1:100 or higher are positive. Titers from 1:100 to 1:200 are of importance mainly in non-vaccinated animals, higher titers with a single sample (=1:800) are usually indicative of infection. For this study we consider 1:100 for non-vaccinated animals as positive titres, and 1:400 for vaccinated animals.

Microscopic Agglutination Test

The MAT is a test that determines the agglutinating antibodies in a patient's serum by mixing several dilutions of it with live or dead (formolized) leptospires. Antileptospiral antibodies present in the serum cause leptospires to stick together in clumps. This clumping process is called agglutination and is observed using dark field microscopy [8]. It is a Leptospira agglutinating antibody test; cultured spirochetes are exposed to serial dilutions of the patient's serum, with a higher dilution reported to cause 50% agglutination of organisms [9]. It constitutes the reference test against which all other serological tests are evaluated. It measures seroconversion or the increase in antibody titers to Leptospira, so it is necessary to test suspicious samples both in the acute phase and in convalescence (serial samples).

The sensitivity of the test can be improved by using local isolates instead of reference strains, but the reference strains aid in the interpretation of results between laboratories. A titer of 1/100 is considered positive, but given the high specificity of MAT, lower titers may be taken as an indication of previous Leptospira exposure. After incubation, the serum/antigen mixtures are examined microscopically for agglutination and titers determined. 1:100,1:200,1:300,1:400 are considered normal vaccine titers. It uses a bacterium of live leptoviral strains, it is used to determine the antibody titer and for the tentative identification of the Leptospira serogroup involved, when epidemiological background information is available. Definitive identification of the infecting serovar or serogroup is not possible without isolation of the causative organism [10]. With dark-field microscopy, the scattering of light by very thin microorganisms, such as spirochetes, suspended in liquid allows them to be observed against a dark background.

Analyzed Variables

Two nominal categorical variables are qualitative variables: type of animal (vaccinated and non-vaccinated) and presence of leptospira serovars (negative and positive). The prevalence of different Leptospira serovars was analyzed in two groups of cattle: vaccinated and non-vaccinated animals. Resultados Through the analysis of the samples by means of the MAT test, it is expected to have positive results for Leptospira and to identify the serovars that are conditioning the presentation of the disease within the facilities of the zootechnical post, and thus analyze the use of biologicals in favor of the protection that are used in bovines to respond to the great problems that affect our animals. The results of these tests are presented below, tables 3, 4 and 5 for vaccinated cows and tables 6, 7 and 8 for unvaccinated heifers.

Table 3: MAT test results in vaccinated cows, Wolffi, Hardjo, Tarassovi, H-89 serovars.

L. Interrogans Serovars- Vaccinated Cows				
ID	**Wolffi**	**Hardjo**	**Tarassovi**	**H-89**
0.32	Negative	Negative	Negative	Negative
42	Negative	Negative	Negative	Negative
113	Negative	Negative	Negative	Negative
116	Negative	Negative	Negative	Negative
131	Negative	Negative	Negative	Negative
142	Negative	Negative	Negative	Negative
163	Negative	Negative	Negative	Negative
164	Negative	Negative	Negative	Negative
179	Negative	Negative	Negative	Negative
180	Negative	Negative	Negative	Negative
186	Negative	Negative	Negative	Negative
189	Negative	Negative	Negative	Negative

Table 4: MAT test result in vaccinated cows, Icterohaemorrhagiae, Bratislava, Palo Alto, Portland Vere serovars.

L. Interrogans Serovars- Vaccinated Cows				
ID	**Icterohaemorrhagiae**	**Bratislava**	**Palo Alto**	**Portland Vere**
0.32	1:50	1:100	1:100	1:1600
42	1:200	1:200	1:100	1:400
113	1:400	1:800	1:800	1:1600
116	1:50	1:200	1:100	1:800

131	1:200	1:100	1:50	1:800
142	1:1600	1:1600	1:1600	1:1600
163	1:200	1:200	1:50	1:400
164	1:200	1:50	Negative	1:400
179	1:400	1:400	1:800	1:1600
180	1:1600	1:400	1:1600	1:400
186	1:800	1:400	1:1600	1:800
189	1:200	1:200	1:600	1:800

Table 5: MAT test results in vaccinated cows, Pyogenes, Gryppotyphosa, Canicola, Pomona serovars.

L. Interrogans Serovars- Vaccinated Cows				
ID	**Pyogenes**	**Gryppotyphosa**	**Canicola**	**Pomona**
0.32	1:100	1:200	1:200	1:200
42	1:100	1:100	1:200	1:200
113	1:100	1:200	1:200	1:200
116	1:400	1:200	1:400	1:200
131	1:50	1:100	1:200	1:100
142	1:800	1:400	1:1600	1:1600
163	1:100	1:50	1:100	1:50
164	1:100	Negative	1:100	1:50
179	1:200	1:200	1:200	1:200
180	1:50	1:50	1:50	1:200
186	1:200	1:100	1:400	1:100
189	1:400	1:400	1:200	1:100

Table 6: MAT test results in unvaccinated calves, Wolffi, Hardjo, Tarassovi, H-89 serovars.

L. Interrogans Serovars- unvaccinated Cows				
ID	**Wolffi**	**Hardjo**	**Tarassovi**	**H-89**
0.93	Negative	Negative	Negative	Negative
0.94	Negative	Negative	Negative	Negative
0.95	Negative	Negative	Negative	Negative
0.96	Negative	Negative	Negative	Negative
0.97	Negative	Negative	Negative	Negative
0.98	Negative	Negative	Negative	Negative
0.99	Negative	Negative	Negative	Negative
100	Negative	Negative	Negative	Negative

Table 7: esultados de prueba MAT en becerras no vacunadas, serovariedades Icterohaemorrhagiae, Bratislava, Palo Alto, Portland Vere.

L. Interrogans Serovars- unvaccinated Cows				
ID	**Icterohaemorrhag-iae**	**Bratislava**	**Palo Alto**	**Portland Vere**
0.93	Negative	1:50	Negative	1:100
0.94	Negative	1:100	Negative	Negative
0.95	Negative	1:100	1:50	1:100
0.96	Negative	1:100	Negative	Negative
0.97	1:50	1:400	1:50	1:100
0.98	1:100	1:100	1:100	1:200
0.99	Negative	1:100	Negative	1:50
100	1:100	1:200	1:50	1:100

Table 8: Results of MAT test in unvaccinated calves, Pyogenes, Gryppotyphosa, Canicola, Pomona serovars.

L. Interrogans Serovars- unvaccinated Cows				
ID	**Pyogenes**	**Gryppotyphosa**	**Canicola**	**Pomona**
0.93	Negative	Negative	Negative	Negative
0.94	1:50	Negative	Negative	Negative
0.95	Negative	Negative	1:50	Negative
0.96	Negative	Negative	Negative	Negative
0.97	Negative	Negative	Negative	Negative
0.98	Negative	Negative	1:50	Negative
0.99	Negative	Negative	Negative	1:50
100	Negative	Negative	Negative	1:100

Discussion

The serovars with the highest prevalence in dairy cattle from the FMVZ-BUAP zootechnical post were the *Portland vere* strain, Bratislava, followed by Icterohaemorrhagiae and the *Palo Alto* strain *(icterohaemorragiae*), with some similarity with what was mentioned in a study, where *Palo Alto* strain (*Icterohaemorrhagiae*), Sinaloa ACR strain *(Portland Vere)* and Bratislava [11] are considered the most relevant in a temperate region. For the *Portland vere serovar*, 100% of positive cases were found in vaccinated animals and 62.5% in non-vaccinated animals. In this case, with the applied bacterin it seems that it favored the presence of this serovar, we must consider the factors that condition the presence of a specific serovarity or serovars such as the environment, host, and climatic factors.

The prevalence for the serovars Icterohaemorrhagiae and *Palo Alto* was 35%, that of *Pyogenes* and *Canicola* was 15%, that of *Grippotyphosa* and *Pomona* 10%. It is relevant considering these pathogenic serovars, Icterohaemorrhagiae, *Canicola, Pomona, Grippotyphosa* and Australis are mentioned as part of the most pathogenic [12], considering that the commercial bacterin protects against five serovars, among which are *Icterohaemorrhagiae, Gryppotyphosa, Canicola* and *Pomona*, and when positive titles are found against these serovars, it really follows that the bacterin is not being effective and therefore is not providing protection to dairy cattle. To this we must mention that various non-specific serovars were isolated from bovines. The main reservoirs of the *grippothyposa* and *Icterohaemorrhagiae* serovars are rodents and wild animals. The *pomona* serovar is frequently found in pigs [13].

The prevalence of one or another serovar may vary according to the geographical area and the species of infected animal. In cattle, the *Hardjo, Wolffi* and *Tarassovi* serovars are the most frequent in Mexico [14] we differ since in dairy cattle from the zootechnical post no titers were found against these *Wolffi, Hardjo, Hardjo (prajitno*) strains. Strain H-89, for which there is no similarity, with what the author reports, there was no agglutination for these serovars. Serovars *hardjo*, *icterohaemorrhagie*, *pomona*, and *canicola* are considered to be maintained in cattle, rodents, porcine, and canine, respectively. However, they may have incidental hosts, and thus *Leptospires* belonging to a particular serovar are nonetheless not host-specific [15].

The results between vaccinated animals against those not vaccinated. For the Bratislava serovar, there is a greater number of positive cases in unvaccinated animals (87.5%) than in vaccinated animals (41.7%), but in both cases the result is positive [16-21].

Conclusion

It was confirmed that the FMVZ-BUAP dairy cattle have antibodies against leptospira, and they are antibodies present to more than one leptospira serovar. In addition, antibodies were found against two national strains, *Palo Alto, Portland Vere*, the second being the most relevant. High percentages of seropositives were found for the Bratislava, *Portland Vere* serovars, followed by the Icterohaemorrhagiae strain (*Palo Alto*). Negative results were found against the serovars that normally affect the bovine species, *Wolffi, Hardjo, Tarassovi. Hardjo* Prajitno strain H-89. Vaccines made in other countries do not provide adequate protection, and with this it can be deduced that vaccinating them with strains that are not present has no effect. The use of a preventive medicine protocol should be implemented in dairy cattle at the FMVZ-BUAP post. The use of biosafety protocols must be implemented for the personnel who work in the facilities.

References

1. Hamond C, LeCount K, Putz EJ, Bayles DO, Camp P, et al. (2022) Bovine Leptospirosis Due to Persistent Renal Carriage of Leptospira borgpetersenii Serovar Tarassovi. Front Vet Sci 9: 848664.
2. Yescas JE, Rivero PN, Montiel HE, Valladares B, Peláez A, et al. (2020) Comportamiento epidemiológico de la leptospirosis en México durante el periodo 2013-2019. Revista de Salud Pública 22(4): 1-5.
3. Petrakovsky J, Bianchi A, Fisun H, Nájera AP, Pereira MM (2014) Animal Leptospirosis in Latin América and the Caribbean Countries: Reported Outbreaks and Literature Review (2002-2014). International Journal of Environmental Research and Public Health 11(10): 10770-10789.
4. Rosete FJV, Ríos UA, Zárate MJP, Socci EGA, Fragoso IA, et al. (2021) Prevalencia de diversos serovares de Leptospira interrogans en vacas no vacunadas en los estados de Puebla, Tabasco y Veracruz, México. Revista mexicana de ciencias pecuarias 12(4): 1305-1316.
5. (2021) Organización Mundial de Sanidad Animal (OIE) Leptospirosis. En Manual de las pruebas de diagnóstico y de las vacunas para los animales terrestres, Capítulo 3.1.12. OIE, París, Francia.
6. Azevedo MIN, Lilenbaum W (2021) An overview on the molecular diagnosis of animal leptospirosis. Letters in Applied Microbiology 72(5): 496-508.
7. Arango B (2010) Leptospira and Leptospirosis. Current Topics in Microbiology and Immunology pp:387.
8. (2008) Organización Mundial de la Salud; traducción del Centro Panamericano de Fiebre Aftosa. Leptospirosis humana: guía para el diagnóstico, vigilancia y control. - Rio de Janeiro: Centro Panamericano de Fiebre Aftosa -VP/OPS/OMS.
9. Vaden SL, Knoll JS, Smith FWK, Tilley LP (2011) Blackwell´S La Consulta Veterinaria En 5 Minutos Canina Y Felina: Prueba De Laboratorio Y Procedimientos De Diagnostico.1ª (Edn.), Editorial inter medica.
10. Hathaway SC, Little TW, Pritchard DG (1986) Problems associated with the serological diagnosis of Leptospira interrogans serovar hardjo infection in bovine populations. Veterinary Record 119(4): 84-86.
11. Luna AMA, Moles CLP, Banda RVM, Torres BJ (2012) Primer reporte del aislamiento de L. interrogans serovariedad L.Portland-Vere en México.

12. Torres CM, Hernández BS, Agudelo FP, Arroyave SE, Zavala CJ, et al. (2016) Artículos de revisión Revisión actual de la epidemiología de la leptospirosis Revisión actual de la epidemiología de la leptospirosis. Revista Médica del Instituto Mexicano Seguro Social 54.

13. Betancur HC, Orrego UA, Gonzales TM (2013) Seroepidemiología de la leptospirosis en bovinos con trastornos reproductivos en el municipio de Montería, Colombia. Revista médico veterinario p.26.

14. Méndez C, Benavides L, Esquivel A, Aldama A, Torres J, et al. (2013) Pesquisa serológica de Leptospira en roedores silvestres, bovinos, equinos y caninos en el noreste de México. Revista salud animal 35(1): 25-32.

15. Hernández P, Gómez AP (2011) Leptospirosis: una zoonosis que afecta a la salud pública y la producción pecuaria. Revista Ciencia Animal 1(4): 15-23.

16. Carmona GCA, León LL, Castillo S LO, Ramírez OJM, Ko A, et al. (2011) Detección de Leptospira santarosai y L. kirschneri en bovinos: nuevos aislados con potencial impacto en producción bovina y salud pública. Veterinaria México 42: 4.

17. Castro MJ, Zamora EL, Y Valladares BC (2001) Discusión de un caso diagnóstico. Nueva Época, Boletín Informativo de la Universidad Autónoma Del Estado de México. Facultad de Medicina Veterinaria y Zootecnia México.

18. Gómez LB, Saltarén CA, Díaz AMT, Robalino VMP, Lucero PSA (2018) Cepario autóctono de leptospiras en la prueba de micro- aglutinación. Correo Científico Médico 22(1): 50-65.

19. Nally JE, Hornsb RL, Alt DP, Bayles D, Wilson Welder, et al. (2018) Aislamiento y caracterización de leptospiras patógenas asociadas al ganado bovino. Microbiología Veterinaria 218: 25-30.

20. Proyecto de NOM-029-SSa-2014, Para la precención y control de la leptospirosis en el humano.

21. (2012) Secretaría de Salud. Subsecretaría de Prevención y Promoción de la Salud Dirección General de Epidemiología ISBN. Manual de procedimientos estandarizados para la vigilancia epidemiológica de la leptospirosis.