Revista Veterinaria. 2025; 36(1): 1-7
https://doi.org/10.30972/vet.3618109
Artículo Original

Revista Veterinaria
Facultad de Ciencias Veterinarias-UNNE
ISSN: 1668-4834 / ISSN-e: 1669-6840

The seroprevalence and risk factors associated with bovine neosporosis (*Neosporum caninum*) in Belisario Quevedo, Cotopaxi-Ecuador

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Abstract
Bovine neosporosis is a parasitic disease caused by the protozoan *Neosporum caninum*, leading to abortions in cattle, reduced milk production, and reproductive disorders. In Ecuador's rural sectors, reports of this disease have been very limited. Therefore, this study aimed to determine the seroprevalence of *N. caninum* using indirect ELISA (ELISA-i) in Belisario Quevedo, a region with high livestock activity, and to identify the risk factors associated with seropositivity. Serum samples were analyzed using the IDEXX NEOSPORA Ab kit, detecting antibodies in 30% of the cases. Additionally, the chi-square test revealed a significant association ($p<0.025$) between *N. caninum* infection and risk factors, specifically age and reproductive method (natural mating vs. artificial insemination). The seroprevalence of *N. caninum*, along with these findings and associated risk factors, has a significant impact on the livestock industry due to its adverse effects, primarily on reproduction. Therefore, it is crucial to implement preventive measures and monitoring strategies to prevent the spread of bovine neosporosis.

Key words: Bovine neosporosis, seroprevalence, ELISA-i, risk factors.

Seroprevalencia y factores de riesgo asociados a la neosporosis bovina (*Neosporum caninum*) en Belisario Quevedo, Cotopaxi-Ecuador

Resumen. La neosporosis bovina es una enfermedad parasitaria causada por el protozoario *N. caninum*, que ocasiona abortos en los bovinos, disminución de la producción de leche y problemas reproductivos. A pesar de que, en Ecuador, su reporte ha sido muy limitado, el presente estudio tuvo como propósito determinar los anticuerpos contra *N. caninum* mediante la prueba de ELISA-i en el Belisario Quevedo, lugar de alta actividad ganadera, y a la vez establecer los factores de riesgo intrínsecos y extrínsecos con la seropositividad. Las muestras de suero sanguíneo recogidas se determinaron con el kit IDEXX NEOSPORA Ab. En los resultados se detectó un 30% de anticuerpos. Por otro lado, el chi-cuadrado mostró que entre los factores de riesgo y *N. caninum*, se observó una diferencia significativa ($p<0,025$) para la variable edad, y el método reproductivo: monta vs. inseminación artificial. La seroprevalencia de *N. caninum* asociada a estos hallazgos y factores de riesgo tienen un impacto considerable en la industria ganadera debido a sus efectos adversos esencialmente en la reproducción; por lo tanto, es crucial implementar métodos de prevención y medidas de monitoreo para evitar la diseminación de la enfermedad neosporosis bovina.

Palabras clave: Neosporosis bovina, seroprevalencia, ELISA-i, factores de riesgo.

INTRODUCTION

The Bovine neosporosis is a multisystemic disease caused by the protozoan *Neospora caninum*, it is an intracellular parasite that spreads and remains in some livestock areas up to 90%, causing infections in cattle, dogs, horses, goats, sheep and deer (Dubey et al. 1998, Dubey 2003). The dog is considered the main host of *N. caninum*, which has a life cycle that includes the following phases: tachyzoites and tissue cysts (occurring in the cells of intermediate hosts), oocysts (occurring in definitive hosts) (Dubey et al. 2002). The dogs excrete approximately

Recibido: 15 agosto 2024. **Aceptado:** 30 enero 2025

500,000 oocysts, constituting the main source of infection for cows (Godin et al. 2002). The transplacental transmission rate can range from approximately 30 to 60%; however, it is suspected that not all infected cows transmit the infection to their offspring (Modrý et al. 2013).

It is well known that *N. caninum* is one of the main agents causing abortions diagnosed worldwide. In Latin America, Brazil, Argentina and Uruguay registered diagnoses of *N. caninum* (Piaggio et al. 2007). These abortions can be endemic when herds present a high abortion rate, around 5-10% throughout the year, and it can persist for years (Saenz 2008, Dubey and Schares 2011). Or it can be an "abortion storm", that is less common and observed when 10 to 12.5% of pregnant cows on a farm abort within 6 to 8 weeks (Cruz 2011). The abortion is recurrent and occurs from 3 months of gestation regardless of age. In pregnant cows, *N. caninum* causes reduction of the amount of milk by approximately 1 kg per cow, death, resorption, and fetal mummification. In the case of calves, they show weakness, neurological signs, low weight, inability to stand up, difficulty to suckle, flexed or hyperextended limbs. And surviving calves are considered clinically healthy but chronically infected, the latter being responsible for the maintenance and dissemination of the disease (Dubey et al. 2009, Pérez and Rojas 2021, Llivisaca 2023).

In epidemiological studies is important and necessary to identify the risk factors that contribute to the persistence. In the case of *N. caninum* in livestock the risk factors are age, breed, sex, origin, presence of definitive hosts and abortions, they are crucial elements for the development of control strategies and/or prevention of the disease (Girata 2016). Although neosporosis causes serious economic, reproductive and productive losses in livestock areas around the world, there are currently no effective treatments or vaccines available to prevent this infection in cattle (García et al. 2014).

Thus, in the world there are researchers to employ for epidemiological monitoring of *N. caninum*, using serological and molecular techniques for this purpose. For example, Kenya in West Africa reported seroprevalences of 24.1% for the year 2023 (Olum et al. 2020); likewise, in the region of Khomas in Namibia in Southern Africa was detected at cattle level a seroprevalence of 25% in the year 2020. In the same way, in the United Kingdom at southeast of Scotland reported in 2019, the presence of *N. caninum* DNA in bovine fetuses in at least one sample, and an association between *N. caninum* and abortion of 18% (Bartley et al. 2019). And in Latin American countries such as Uruguay reported in 2020, a seroprevalence of N. caninum at the animal level of 22.3% and 96% at the herd level. It also showed that the presence of dogs is associated with infection with an Odds ratio of 1.43 (Macchi et al. 2020), and another study in the capital of Argentina, economic losses have been estimated at US$ 33 million annually for dairy cattle and US$ 12 million annually for beef cattle. Around 9% of bovine abortions in the Buenos Aires province are attributed to *N. caninum.* (Campero et al. 2023).

On the other hand, in Ecuador there are several studies related to the subject, however there is no information on the prevalence of neosporosis, nor data on the risk factors associated with bovine neosporosis in Belisario Quevedo. Thus, high prevalences have been reported in different provinces and places of Ecuador, such as the study carried out in 2004 in the provinces of the Northern Andean Region, which reported a seropositivity of 42% (Lozada 2004). Another example is the province of Chimborazo, where a prevalence of 70.88% of cows developed seroprevalence against *N. caninum* (Baquero Tapia et al. 2022).

Likewise, in five provinces of Ecuador (Manabí, Santo Domingo de los Tsáchilas, Pichincha, Napo and Orellana), they showed a prevalence of 27.3% (Guzmán Ordoñez 2017). Another study conducted in Guamote indicated a prevalence of 15.22% (Guamán 2022). In this context, where different studies have shown relatively high prevalences in our country, and since it is well known that Belisario Quevedo is considered a rural area where the population is dedicated to dairy farming for their economic livelihood, we felt the need to propose the following objectives: (i) to find the presence of *N. caninum* seroprevalence and (ii) to correlate the risk factors associated with *N. caninum* in the Belisario Quevedo, Cotopaxi-Ecuador by using the enzyme-linked immunoadsorption assay technique.

MATERIALS AND METHODS

Location. The research was carried out in milk producing cows of the Belisario Quevedo, located in Latacunga canton of the province of Cotopaxi, from November 2023 to February 2024. In the epidemiological study we applied the cross-sectional and correlational types, for which a survey was elaborated considering intrinsic factors such as: age, breed, origin of animals and extrinsic factors such as: reproductive method and presence of dogs.

Sample size. The sample size was calculated with the simple random sampling formula (Ortega 2018), with a margin error of 2,5%, a confidence level of 97,5%, and an expected prevalence of 12%. The sample size was calculated based on the information obtained in a census conducted for the sector resulting in 164 dairy cows, due to the fact that the total population were around 161 874 dairy cows (Iza 2020), and we considering only animals from 2 to 9 years of age. The selection of cattle, individuals and the application of the epidemiological survey was carried out randomly and systematically.

Sample collection and processing. Each sample was punctured in the coccygeal vein to obtain approximately 10 ml of venous blood through the vacuum system in vacutainer tubes without anticoagulant. The punctured area was cleaning by ethyl alcohol 70° antiseptic. Each tube was transported in coolers at 4 °C to the parasitology laboratory of the Veterinary Clinic of the Technical University of Cotopaxi (UTC), where they were centrifuged at 5.5 thousand revolutions for 15 minutes. Finally, the blood serums were placed in duplicate It is important to point out that samples were taken in accordance with the regulations stipulated by the Agency for Phytosanitary and Zoosanitary Regulation and Control of Ecuador (AGROCALIDAD 2021), since at the moment there is no Bioethics Committee

in our institution (*Reglamento para la conformación, aprobación y el seguimiento de Comités de Ética para la investigación con animales en el Ecuador*, 2021).

The blood serums were read at the Parasitology Laboratory of the Faculty of Agricultural Sciences and Natural Resources of the UTC. The presence or absence of seroprevalence against *N. caninum* was determined using the ELISA-i technique, with the BioTek 800 TS Absorbance Reader, and the commercial kit with the IDEXX Neospora Ab Kit, following the manufacturer's instructions. To determine the presence or absence of antibodies and to establish the serostatus of the animals (positive, suspicious, negative), the results were based on the reading of the results with a spectrophotometer at a wavelength of 450 nm. Finally, BioTek Gen5 software was used for quantitative analysis, and according to the manufacturer's instructions the following was considered: values ≤ 30 are negative, values of 40 are suspicious and values ≥ 40 were considered as positive. (IDEXX Laboratories 2024).

Statistical Analysis. Data were analyzed with chi-square using the software R (R Core Team 2022). We used the package abind (Plate and Heiberger 2016), with a 95% confidence interval. Contingency tables were used to record and correlate the association between risk factors and Neosporosis seropositivity, using the R-study statistical analysis program, where significance was established through the interpretation of the p-value and the risk was calculated as oddsratio (OR) (Jiménez and Chávez 2014, González Díaz 2016). The OR's standard error and 95% confidence interval were calculated with the statistical package 'epitools' (Aragon 2020).

RESULTS

In the Table 1 shows the results of seroprevalence in the Belisario Quevedo. It is showing a 30% of seroprevalence, corresponding to 49 seropositive dairy cows; while the suspected cases represented 4%, with 4 suspected cases representing 2%, and 68% of seronegative. Confidence interval values detected no statistical differences between seropositive, seronegative and suspect animals.

Table 1. Prevalence of seroprevalence against *N. caninum*, detected by the ELISA method. Source (Author).

ELISA method	Frequency	% of frequency	95 % IC
Seropositive	49	30	-122.19 - 196.19
Seronegative	111	68	-53.94 - 86.60
Suspected	4	2	-53.94 - 86.60
Total	164	100	

In Table 2 and Figure 1b, they show that the association between age and seropositivity of *N. caninum*, the animals aged 6 to 8 years, have an odds ratio of 31.06, indicating that the probability of acquiring bovine neosporosis is 31.06 times higher than the other age groups, in second place, they are in cows aged 4 to 6 years with a odds ratio 3.56, and the lowest seroprevalence is in the age of 2 to 4 years with a 3% prevalence.

Similarly, Table 2 and Figure 1a show the association between the reproductive method and seropositivity of *N. caninum*, where the reproductive method by mating has a odds ratio of 4.79 and artificial insemination has a odds ratio of 1, indicating that natural mating has a 4.79 of probability of being infected by chronically infected cows than in cows that have been artificially inseminated. However, although the OR suggests an association between age, natural mating and seropositivity, given that the p-value indicates that this association is not statistically significant. This suggests that the results could be due to chance, and it cannot be deduced that there is a real relationship between these risk factors and the presence of *N. caninum*.

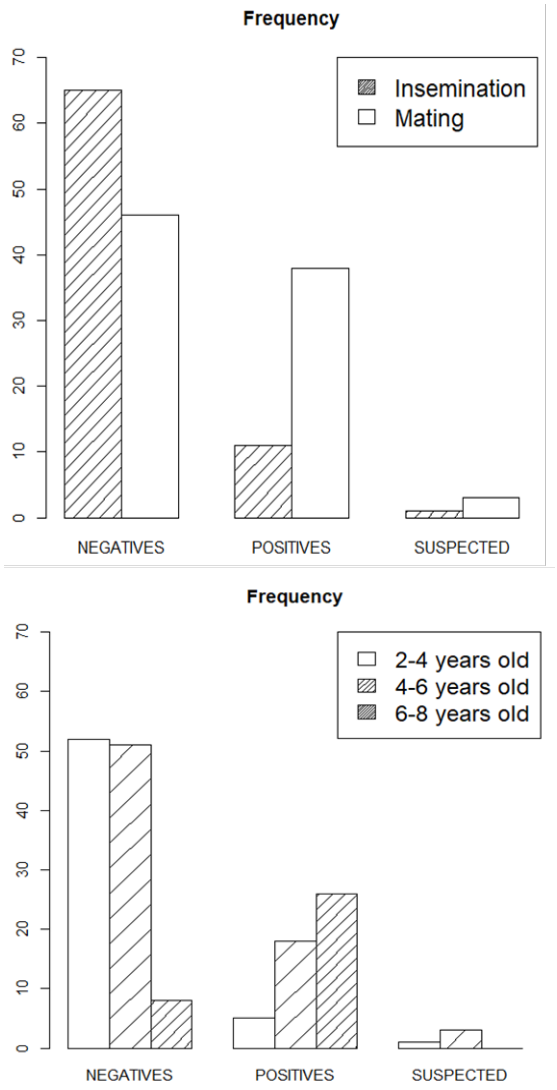

Figure 1. Frequency barplot: a) Frequency by type of reproduction; b) Frequency by age (n=164). The p-value is from the chi-square test. Source (Author).

On the other hand, Table 2 analyzes the association between breed, origin (source), altitude, reproduction method and presence of dogs vs *N. caninum* seropositivity, where the trend with the highest concentration of seropositive is in the Holstein breed with 14%. The statistical analysis showed a p>0.05, which determines that there is no significant difference for the breed factor. Likewise, it is observed that seropositivity is higher

in animals that born and bred on the same farm with 17% seroprevalence compared to those obtained at the livestock fair with 13% seropositive. This could be due to the predominance of congenital transmission in the cows studied, but statistically, a p>0.05 was obtained, which indicates that there is no significant difference between the variables analyzed, therefore, no association is established.

In the same way, Table 2 analyzes the association between altitude and *N. caninum* seropositivity where the p>0.05 therefore it is determined that there is no association. And finally, the association between the number of dogs and *N. caninum* seropositivity was evaluated, reaching 23%; it is worth mentioning that there were both owned and unowned dogs on the farms. Statistically, it was determined that there was no significant difference (p>0.05), so it is inferred that the transmission must be vertical.

Table 2. Odds ratio analysis for risk factors associated with Neosporosis bovina status of dairy cows in Belisario Quevedo, Cotopaxi.

Variable	Category	Odds ratio	95 % IC	P-value
Age	2 to 4	1,00	Reference	
	4 to 6	**3.56**	1.29-11.70	3.12
	6 to 8	**31.06**	9.93	4.47
Breed (dairy cows)	Holstein	1.00	Reference	
	Mestiza	1.18	0.48-3.81	0.12
	Jersey	0.91	0.47-2.00	0.17
Origien (Source)	Cattle fair	1.00	Reference	
	Own cattle	0.94	0.47-1.88	0.93
Reproduction Method	Artificial insemination	1.00	Reference	
	Natural mating	**4.79**	2.27-10.82	9.19
Presence of dogs	Yes	1	Reference	
	No	1.26	0.58-2.91	0.45

Note: Odds ratio values are in bold.

DISCUSSION

In Belisario Quevedo there are no records of *N. caninum*, however, in Latacunga, according to Toaquiza (Toaquiza and Valencia 2022) in the year 2022, reports a 30% seroprevalence like the present project. However, according to Iza (2020) in Ignacio Flores other area of Latacunga, Iza reported a 12% seroprevalence in the year 2020, which compared to the present project has a lower prevalence, which is assumed due to the number of animals limited to 50 samples. Additionally, in Salcedo´s canton, Samaniego (2019) reported a 25% prevalence in 2019 with a sample size like this study.

In our research, it was found that there is an association between age and seropositivity (6 to 8 years) in contrast with the data obtained by Iza (2020), showing that the highest risk is found among animals sampled from 2 to 6 years of age.

Our results correlate age with the possibility of having greater contact with *N. caninum*. The same as the study reported by Guamán (2022), who stated that the highest risk was found in the 5 to 6 years age group. Corroborating that, the risk factor age according to Dyer et al. (2000) the older the more susceptible to infection by *N. caninum*. Waldner et al. (1998) suggest that horizontal transmission is of importance in dairy and beef cows.

Similarly, the reproductive method, natural mating, was reported to be a risk factor associated with the presence of bovine Neosporosis. However, according to Osoro et al. (2009), venereal transmission of *N. caninum* by natural mating seems very unlikely, at least in animals managed under adequate sanitary and nutritional conditions, suggesting a failure in the control of replacement of cows that may be infected or be daughters of seropositive dams.

In the case of artificial insemination, *N. caninum* DNA has been detected in semen, however, this route is not a source of transmission suggesting insemination in seropositive or chronically infected cows. This association presented in the results differs from that presented by Toaquiza and Valencia (2022) who determined the non-correlation between the reproductive method and *N. caninum.*

On the other hand, the breed risk factor was not significant in our research, but this result does not coincide with those of Toaquiza and Valencia (2022), where the Brown Swiss breed and the Holstein breed are more exposed to *N. caninum*. Similarly, Guamán (2022) and Iza (2020) reported that the Holstein breed is more susceptible to acquiring the infection. The last one was reported for the area of Ignacio Flores.

Therefore, it is assumed that there is no correlation between breed and the presence of *N. caninum*. Additionally, the Holstein breed predominates due to its adaptability to the characteristics of the area (Guamán 2022).

In the results of the origin of dairy cows, our results differ from those reported in the study by Escalona et al. (2010) who mention that animals born and raised on the same farm are more likely to be infected than those purchased at cattle fairs. However, the results did not reflect a significant difference in this research.

Finally, the presence of dogs was not associated with *N. caninum* seroprevalence, as did Iza (2020) who indicated that there is no probability of infection in the presence of dogs. Similar to Villar (2018), Pereira-Bueno et al. (2003) and Escalona et al. (2010) who related the number of dogs and seroprevalence in cattle, who found no significant values. Furthermore, it has been suggested that infection persists in the absence of a definitive host with predominant vertical transmission (Arauco et al. 2020).

Unlike McAllister et al. (2020) who reported that the presence of dogs on farms is an important element because these animals shed oocysts of the parasite in their feces, becoming a source of infection for the horizontal spread of neosporosis. But the findings indicate that in Belisario Quevedo cattle, the main form of transmission is through chronically infected cows, which according to Arauco et al. (2020) means that only reproductive females need to be infected, without the need for exposure to reinfection.

CONCLUSIONS

The prevalence of seropositivity showed that the presence of *N. caninum* was relatively high, which demonstrated that the disease is in the production of dairy cattle in Belisario Quevedo, constituting a risk that affects reproductive, productive and economic efficiency.

On the one hand, one of the risk factors associated with the presence of *N. caninum* showed that the older the cattle, the greater the susceptibility to infection. In addition, natural mating also revealed to be a risk factor that conditions the persistence of bovine Neosporosis in the sector, factors that in the present study suggest a positive association with seropositivity, although the p-value indicated that this association is not statistically significant. Thus, suggesting that the results could be due to random chance. On the other hand, the presence of dogs, breed and altitude, showed no association with seropositivity. However, the presence of dogs should not be underestimated when implementing prevention and control planin order to avoid the persistence and spread of bovine neoporosis.

Acknowledgment. To the Universidad Técnica de Cotopaxi, to the small-scale dairy farmers of Belisario Quevedo for their cooperation and to PhD. Esthela Gonzalez Sarango for her your review and point of view of present research.

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REFERENCE

1. AGROCALIDAD. Reglamento para la conformación, aprobación y el seguimiento de Comités de Ética para la investigación con animales en el Ecuador, (testimony of AGROCALIDAD). 2021. https://www.agrocalidad.gob.ec/wp-content/uploads/2021/11/DAJ-2021454-0201.0227-1.pdf
2. Aragon TJ. Epitools: Epidemiology Tools. R package. version 0.5-10.1.2020. https://CRAN.R-project.org/package=epitools
3. Arauco Villar F, Mayorga Sánchez N, Cruz Flores D, Astohuamán Vilcahuamán J. Dinámica de seroconversión de diarrea viral bovina y neosporosis en hatos lecheros de la sierra central del Perú. *Rev Investig Vet Peru.* 2020; 31(3): e18727.
4. Baquero Tapia F, Díaz Monroy B, Vinueza Veloz P. Estudio de la Neosporosis en Bovinos de la provincia de Chimborazo, Ecuador. *Alfa*. 2022; 6(17): 224-238.
5. Bartley PM, Guido S, Mason C, Stevenson H, Chianini F, Carty H, Innes EA, Katzer F. Detection of *Neospora caninum* DNA in cases of bovine and ovine abortion in the South-West of Scotland. *Parasitology*. 2019; 146(7): 979-982.
6. Campero LM, Basso W, Moré G, Fiorani F, Hecker YP, Echaide I, Cantón GJ, Cirone KM, Campero CM, Venturini MC, Moore DP. Neosporosis in Argentina: Past, present and future perspectives. *Vet. Parasitol.* 2023; 41: 100882.
7. Cruz M. Identificación del parasito "*Neospora caninum*" en bovinos por medio del método de ELISA, en las haciendas ganaderas del cantón Tulcán en la Provincia del Carchi. Tesis de pregrado. 2011. Universidad de las Américas, Quito. Available in http://dspace.udla.edu.ec/handle/33000/2807
8. Dubey JP, Carpenter JL, Speer CA, Topper MJ, Uggla A. Newly recognized fatal protozoan disease of dogs. *J Am Vet Med Assoc*. 1988; 192(9): 1269-1285.
9. Dubey JP, Barr BC, Barta JR, Bjerkås I, Björkman C, Blagburn BL, Bowman DD, Buxton D, Ellis JT, Gottstein B, Hemphill A, Hill DE, Howe DK, Jenkins MC, Kobayashi Y, Koudela B, Marsh AE, Mattsson JG, McAllister MM, Modrý D, Lindsay DS. Redescription of *Neospora caninum* and its differentiation from related coccidia. Int *J Parasitol*. 2002; 32(8): 929-46.
10. Dubey JP. Review of *Neospora caninum* and neosporosis in animals. *Korean J Parasitol*. 2003; 41(1): 1-16.
11. Dubey JP, Jenkins MC, Kwok OCH, Zink RL, Michalski ML, Ulrich V, Gill J, Carstensen M, Thulliez P. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* seroprevalence in white-tailed deer (*Odocoileus virginianus*) from Iowa and Minnesota using four serologic tests. *Vet Parasitol.* 2009; 161(3-4): 330-334.
12. Dubey JP, Schares G. Neosporosis in animals—The last five years. *Vet Parasitol.* 2011; 180(1-2): 90-108.
13. Dyer RM, Jenkins MC, Kwok OCH, Douglas LW, Dubey JP. Encuesta serológica de la infección por *Neospora caninum* en un rebaño cerrado de ganado lechero en Maryland: riesgo de reactividad serológica

por grupos de producción. *Vet. Parasitol*. 2000; 90: 171-181.
14. Escalona J, García F, Mosquera O, Vargas F, Corro A. Factores de riesgo asociados a la prevalencia de Neosporosis Bovina en el municipio Bolívar del estado Yaracuy, Venezuela. *Zootec Trop*. 2010; 28(2): 201-12.
15. García J, Moreno G, Cruz A. Prevalencia de *Neosporosis caninum* y DVB en una finca con problemas resproductivos en Sopó (Cundinamarca). *Cien. Agri*. 2014; 11(1): 9-16.
16. Girata J. Estudio zootécnico de la Neosporosis bovina: análisis teórico de orientación para los ganaderos de Santander y Boyacá. Universidad Nacional Abierta y a Distancia UNAD. Tesis de pregrado. 2016. Available in https://repository.unad.edu.co/handle/10596/6861
17. González Díaz C. La significación estadística y los p-valores. *Rev Cubana Hig Epidemiol.* 2016; 54(3): 60-62.
18. Guamán M. Prevalencia de *Neospora caninum* en bovinos en el cantón Guamote. Tesis de Pregrado. Universidad Técnica de Cotopaxi. 2022. Available in http://repositorio.utc.edu.ec/handle/27000/9822
19. Guzmán Ordoñez LT. Seroprevalencia y factores de riesgo de la infección por agentes reproductivos del ganado bovino (*Brucella* spp., *Coxiella burnetii*, *Leptospira interrogans* serovar Hardjo y *Neospora caninum*) en explotaciones lecheras y de doble propósito de Ecuador. Tesis Doctoral, Universidad de Córdoba, Córdoba, Argentina. 2017; p. 174, 185.
20. IDEXX. *Neospora caninum* Antibody Test Kit. Laboratories. Cited 2024 Feb 6. Available in https://www.idexx.es/es/livestock/livestock-tests/ruminanttests/idexx-neospora-ab-test/
21. Iza P. Prevalencia de Neosporosis en Bovinos en el Cantón de Latacunga, Parroquia Ignacio Flores. Universidad Técnica de Cotopaxi. Tesis de Pregrado. 2020. Available in http://repositorio.utc.edu.ec/handle/27000/6765
22. Jiménez M, Chávez A. Tablas de contingencia. In: Metodología de la investigación, bioestadística y bioinformática en ciencias médicas y de la salud. 2014. 2nd ed. McGraw-Hill. Available in: https://accessmedicina.mhmedical.com/content.aspx?bookid=1721&sectionid=115931 563
23. Llivisaca D. Determinación de la prevalencia de neosporosis (*Neospora caninum*) en vacas mestizas en el Cantón Tiwintza. Tesis de pregrado. 2023. Escuela Superior Politécnica de Chimborazo. Available in: http://dspace.espoch.edu.ec/handle/123456789/19583
24. Lozada E. Determinación de la presencia de anticuerpos a *Neospora caninum* en hatos lecheros de la Sierra Norte del Ecuador. Universidad Central del Ecuador. Tesis de Pregrado. 2004. Available in https://www.dspace.uce.edu.ec/server/api/core/bitstreams/a100f085-a925-4490-ae8e-2a12e07d9aee/content
25. Macchi MV, Suanes A, Salaberry X, Fernandez F, Piaggio J, Gil AD. Epidemiological study of neosporosis in Uruguay an dairy herds. *Prev Vet Med*. 2020; 179: 105022.
26. McAllister MM, Björkman C, Anderson-Sprecher R, Rogers DG. Evidence of point-source exposure to *Neospora caninum* and protective immunity in a herd of beef cows. *J Am Vet Med Assoc*. 2020; 217(6): 881-887.
27. Modrý D, Hofmannová L, Mihalca A, Juránková J, Neumayerová H, Gianluca D›Amico G. Field and laboratory diagnostics of parasitic diseases of domestic animals: from sampling to diagnosis. Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic; 2017. p. 7,17,18.
28. Olum MO, Mungube EO, Njanja J, Kidali J, Njenga E, Maichomo M, Tsuma V, Mugambi J. Seroprevalence of canine neosporosis and bovine viral diarrhea in dairy cattle in selected regions of Kenya. *Transbound Emerg Dis*. 2020; 67(S2): 154-158.
29. Ortega C. ¿Sabes cómo realizar un muestreo aleatorio simple de manera sencilla? *Question Pro*. 2018. Available in https://www.questionpro.com/blog/es/como-realizar-un-muestreo-aleatorio-simple/
30. Osoro C, Tamargo C, Hidaldo C, Martinez A, Pérez I. La monta natural como vía de transmisión de la neosporosis bovina. Experimentación en campo. Servicio Regional de Investigación y Desarrollo Agroalimentario. 2009. Available in http://www.serida.org/resultadopreliminar.php?id=00000206
31. Pereira-Bueno J, Quintanilla-Gozalo A, Pérez-Pérez V, Espi-Felgueroso A, Álvarez-García G, Collantes-Fernández E, Ortega-Mora LM. Evaluation by different diagnostic techniques of bovine abortion associated with *Neospora caninum* in Spain. *Vet Parasitol.* 2003; 111(2-3): 143-152.
32. Pérez D, Rojas O. Neosporosis en caninos y bovinos. *Rev. vet.* 2021; 32(2): 238-241.
33. Piaggio J, Delucchi L, Bañales P, Easton C. Actualización en Neosporosis. Educación Permanente. 2007. Available in: https://www.colibri.udelar.edu.uy/jspui/bitstream/20.500.12008/20256/1/FVET_PiaggioJ_2007_Act.Neosporosis.PDF
34. Plate T, Heiberger R. _abind: Combine Multidimensional Arrays_. R package version 1.4-5. 2016. Available in https://CRAN.R-project.org/package=abind
35. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2022. Available in: https://www.R-project.org/
36. Saenz SC. Evaluación del efecto del uso de la vacuna (*Bovilis Neogurad*) en la reducción de la tasa de abortos en cuatro haciendas de la sierra ecuatoriana. Tesis de pregrado. Universidad San Francisco de Quito. 2008. pp. 54. Available in: https://repositorio.usfq.edu.ec/bitstream/23000/691/1/88820.pdf
37. Samaniego K. *Neospora caninum* en bovinos de leche en cuatro sectores del cantón Salcedo- Cotopaxi mediante la prueba de ELISA. Tesis de Pregrado. Universidad Técnica de Cotopaxi. 2019. Available in http://repositorio.utc.edu.ec/handle/27000/6324

38. Toaquiza S, Valencia C. Estudio seroepidemiológico de *Neospora caninum* en bovinos de traspatio en los cantones de Latacunga, La Maná y Salcedo de la provincia de Cotopaxi. Tesis de Pregrado. Universidad Técnica de Cotopaxi. Universidad Técnica de Cotopaxi. 2022. Available in http://repositorio.utc.edu.ec/handle/27000/9630
39. Villar FA. Seroprevalencia y factores de riesgo de neosporosis bovina en el valle del Mantaro-Región Junín, Perú. *RIVEP*. 2018; 29(4): 1430-1439.
40. Waldner CL, Janzen ED, Ribble CS. Determination of the association between *Neospora caninum* infection and reproductive performance in beef herds. *J. Am. Vet. Med. Assoc*. 1998; 213: 685-690.