DOI: 10.5965/223811712332024537

Revista de Ciências Agroveterinárias 23 (3): 2024
Universidade do Estado de Santa Catarina

Prevalence and risk factors for hemoparasites in cattle in León, Nicaragua

Detecção molecular e fator de risco de hemoparasitas em bovinos no município de León, Nicarágua

Byron Flores *[1(ORCID 0000-0002-1932-3227)], Jessica Sheleby-Elías [2(ORCID 0000-0001-7370-5763)], Brenda Mora-Sánchez [1(ORCID 0000-0002-5179-8660)], Xaviera Dávila [1(ORCID 0000-0003-0149-4498)], Ariel Díaz [1(ORCID 0009-0008-2432-4524)], William Jirón [1(ORCID 0000-0002-5778-5721)]

[1]Universidad Nacional Autónoma de Nicaragua-León (UNAN-León), León, Nicaragua. *Author for correspondence: byronfloressomarriba@gmail.com
[2]Universidad Nacional Agricultura-Catacamas, Olancho, Honduras.

Submission: 19/12/2023 | Acceptance: 13/04/2024

ABSTRACT

Hemoparasite infections are a substantial challenge to agriculture, worldwide. Infected cattle suffer malnutrition, stunting, decreased milk production, and reproductive loss, which can result in great economic loss. The aim of this study was to characterize the predominant hemoparasites affecting cattle in Nicaragua, whose economy is largely dependent on agriculture, and define associated epidemiological factors. Blood from 68 bovines in the municipality of León were analyzed by microscopy and molecular techniques. PCR revealed *Anaplasma marginale* in 33.82% (IC 95%: 21.84-45.80), 19.11% (IC 95%: 9.03-29.19) of animals were infected with *Babesia* spp, and 8.82% (95% CI: 1.34-16.30) were simultaneously co-infected with *Babesia* spp and *A. marginale*. However, *A. phagocytophilum*, *Leishmania* spp., or *Trypanosoma* spp were not detected (0%, 95% CI: 0.0-5.20). *A. marginale* was more frequently detected in males (p=0.041) and calves (p=0.041). This is the first study to report the prevalence of hemoparasites in cattle using molecular diagnosis in western Nicaragua.

KEYWORDS: hemoparasites, cattle; Nicaragua; *Anaplasma* spp.; *Babesia* spp.

RESUMO

Agentes infecciosos chamados hemoparasitas destroem as hemácias em bovinos. O objetivo deste estudo foi detectar os principais hemoparasitas em bovinos no município de León, Nicarágua, e os fatores epidemiológicos associados à sua presença. Foram coletadas 68 amostras de sangue bovino em EDTA e realizada PCR a partir de uma amostra conglomerada selecionada para representar os bovinos do município de León. A análise de PCR revelou que 33,82% (IC 95%: 21,84-45,80) dos animais foram positivos para *A. marginale*, 19,11% (IC 95%: 9.03-29,19) dos animais estavam infectados com *Babesia* spp, 8,82% (95% de IC: 1,34-16,30) estavam infectados com *Babesia* spp. e *A. marginale*, no entanto, nenhum animal foi considerado positivo para *A. phagocytophilum*, *Leishmania* spp. ou *Trypanosoma* spp (0%, IC 95%: 0,0-5,20). A. marginale foi detectada com maior frequência em machos (p=0,041) e bezerros (p=0,041). Este é o primeiro estudo que relata a prevalência de hemoparasitas em bovinos, aplicando diagnóstico molecular no oeste da Nicarágua.

PALAVRAS-CHAVE: hemoparasitas, bovinos; Nicarágua; *Anaplasma* spp.; *Babesia* spp.

Blood-borne parasites are a substantial cause for concern in the agricultural setting, from subsistence farming to commercial production. Hemoparasites in cattle have a major impact on the economy and public health. In addition to the challenges to animal health and welfare, many of these pathogens also pose a risk to human health through zoonotic spread. Agents such as *Anaplasma* spp, *Leihsmania* spp, *Trypanosoma* spp, *Babesia* spp, and *Ehrlichia* spp are among the most prevalent, disproportionately affecting communities in tropical and sub-tropical regions.

A greater and more granular epidemiologic clarity could translate to improved control efforts. An important aspect of hematoparasites is that they may be transmitted by various vectors, including ticks, hematophagous flies or mosquitoes. Vector population dynamics depend largely on specific demographic

and environmental characteristics of an area, with different factors favoring population growth and dispersion to facilitate pathogen spread (JAIMES-DUEÑEZ et al. 2018b, ROBI et al. 2021, JAIMES-DUEÑEZ et al. 2018a), and transmission among herds and between different species of animals, including between bovines and humans (SALAMANCA-CARREÑO et al. 2018). It is well established that the tropics bear a disproportionate burden of parasitic diseases and stand to gain the most from efficient and effective control measures. Vector populations are also more concentrated in tropical regions than elsewhere. In South America alone, the economic impact of babesiosis and anaplasmosis control is an estimated $875 million (HOVE et al. 2018). Importantly, characteristics of tropical regions are not homogenous across the globe, making it important to establish the local epidemiology as a foundation for the most strategic efforts for disease control.

During the infectious cycle of blood-borne parasites, red blood cells are destroyed, creating a cascade of ill health and stunting. Infected cattle suffer wasting, losing up to 30% body weight. Mild production drops by an average of 1.16 L/day for each infected cow (RASHID et al. 2019), Malnutrition is a further concern, as has already been described with trypanosomiasis, attributed to hepatocellular damage and resulting albumin deficiency (HOTA et al. 2019). Bovine hemoparasitosis can have serious reproductive implications, causing abortions or can even be transmitted through semen (COUTO et al. 2022).

Hemoparasites affecting cattle can also be transmitted to other mammals, including humans. Zoonotic spread of tick-borne rickettsial parasites, such as *Anaplasma* spp, and protozoal parasites transmitted by hematophagous flies, such as *Trypanosoma* spp and *Leishmania* spp, are long-standing and serious problems for both human and veterinary public health.

Nicaragua is an agricultural country with more than 6 million heads of cattle, and cattle ranching has represented one of the main economic endeavors for many years. More than 90% of cattle are in the hands of small producers (VERGARA-CAMUS & KAY 2017), who may suffer the most significant costs when their animals suffer hemoparasite infections. An estimated 60% of cattle mortality in Nicaragua is associated with hemoparasitosis, mainly in dry areas, where the low availability of pasture decreases grazing area and increases vulnerability (INTA NICARAGUA 2021).

Despite this, no studies have been carried out to characterize the burden of specific hemoparasites affecting cattle in Nicaragua or to determine related epidemiological factors. Such information is crucial for informing evidence-based prevention and control measures, which would ultimately improve the health and welfare of human and animal populations. Therefore, this study aims to use molecular techniques to identify and describe the most important bovine hemoparasites in the municipality of León, Nicaragua, and identify risk factors for infection. The overarching goal is to provide an evidence base for partnerships that bridge the human and animal health domains to reduce the burden of vector-borne parasites in Nicaragua.

A descriptive pilot study was conducted during the raining season, August to October 2022, among cattle in rural communities of Leon, Nicaragua. A sample of 68 cattle selected from the total estimated bovine population of 27295 (ANUARIO ESTADÍSTICO 2019 2021), was determined sufficient for a pilot on prevalence, with a confidence level of 90%, and precision of 10%. An agnostic prevalence (50%) was assumed since the study aimed to detect various hemoparasites, and the calculation was performed in EPIDAT 4.1 (PÉREZ & BRAYANA 2020). A cluster sampling (rural community) technique was employed, first randomly selecting 4 of the 14 known rural communities in the study area: Abangasca (n= 15), Chacaraseca (n=34), El Convento (n=4) and Las Chacaras (n=15). Within each community, animals were selected by simple random sampling.

Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes. Blood was drawn from the ear vein for preparation of blood smears and from the jugular vein for complete blood count (CBC) and DNA extraction. Data was collected on each animal (breed, age, sex, and tick control) at the same time as specimen collection, using a standardized instrument developed for this study.

For all subjects, CBCs were performed as described by Verbrugge et al (VERBRUGGE & HUISMAN 2015). Blood smears were prepared with the rapid Panoptic staining method and examined by microscopy with an OLYMPUS CX31 optical microscope (100x) to identify *Anaplasma spp, Babesia spp, Ehrlichia spp, Leishmania spp, and Trypanosoma spp.*

The QIAamp DNA Kits (Qiagen, Hilden, Germany) were used to extract and purify DNA from 200 µl of whole blood with a final elution volume of 50 µl, and a negative control (nuclease-free water). Conventional PCR was performed to detect *Leishmania* spp, *T. vivax*, *T. cruzi*, *T. brucei evansi*, *Anaplasma* spp., *A. phagocytophilum*, and *A. marginale* using the primers described in Table 1.

Table 1. PCR primers for the molecular diagnosis of hemoparasites in bovines.

Hemoparasites	Sequences 5´- 3´	Gen	Products (pb)	Reference
Leishmania spp	CCTATTTTACACCAACCCCCAGT GGGTAGGGGCGTTCTGCGAAA	kinetoplast	120	(SHANG et al. 2011)
Trypanosoma vivax	CGCAAGTGGACCGTTCGCCT ACGCGGGGCGAACAGAAGTG	Proline racemase	239	(FIKRU et al. 2014)
Trypanosoma brucei evansi	ACAGTCCGAGAGATAGAG CTGTACTCTACATCTACCTC	minicircle	436	(GETAHUN et al. 2022)
Trypanosoma cruzi	CGAGCTCTTGCCCACACGGGTGCT CCTCCAAGCAGCGGATAGTTCAGG	kinetoplast	188	(MATEUS et al. 2019)
Anaplasma phagocytophilum	AACGGATTATTCTTTATAGCTTGCT GGCAGTATTAAAAGCAGCTCCAGG	16S rRNA	546	(PANTHAWONG et al. 2020)
Anaplasma spp	CACATGCAAGTCGAACGGATTATTC TTCCGTTAAGAAGGATCTAATCTCC	16S rRNA	932	(PANTHAWONG et al. 2020)
Anaplasma marginale	TAAGAATTAAGCATGTGACCGCTGAC GTGTTCGTTGGGGTGTGATAGATGAG	msp5	652	(CORONA et al. 2001)

For a reaction volume of 50 µl, we combined 25 µl of Master Mix 2X (Promega, USA), 12 µl of nuclease-free water, 4 µl of each primer ($1x10^3$ nM) and 5 µl of sample DNA. We added a negative control (nuclease free water) and a positive control (DNA from banked positive specimens for each agent, provided by the Veterinary Center for Diagnosis and Research, Nicaragua). PCR was performed with the Applied Biosystem 2720 thermocycler, raising the temperature to 94 °C for 10 minutes, followed by 40 cycles (95 °C for 50 seconds, 55 °C for one minute, 72 °C for one minute), and a final extension for seven min at 72 °C. To visualize the PCR products, electrophoresis was performed on a 1.3% agarose gel stained with ethidium bromide, applying 10 µl of the amplification product in each well and visualized by ultraviolet light transilluminator.

No animal experimentation was performed. All cattle studied were managed according to the "*Law for the protection and welfare of domestic animals and domesticated wild animals (747)*" (NORMAS JURÍDICAS DE NICARAGUA 2011). Blood sampling is a routine practice used for laboratory diagnosis and was performed by experienced veterinarians. Informed consent was obtained from each owner.

For statistical analysis, numerical variables were analyzed by the Shapiro Wilk normality test and presented as means with standard deviations. Categorical variables are described as proportions with 95% confidence intervals (95% CI). Inferential statistics were applied to determine bivariate associations between independent and dependent variables by applying Fisher's exact test. In the case of numerical variables, a student t test was applied.

As a result of the direct smear, 73.52% (95% CI: 62.30-84.75) animals had structures compatible with *Anaplasma* spp. and 19.11% (95% CI: 9.03-29.19) were infected by *Babesia* spp. Coinfection with *Anaplasma* spp and *Babesia* spp simultaneously were observed in 10.76 % (95% CI: 3.37-20.15). *Trypanosoma* spp, *Ehrlichia* spp, or *Leishmania* spp were not observed in any cattle (0%, 95% CI: 0.0-5.2).

By PCR, 33.82 % (95% CI: 21.84-45.80) of cattle were positive for *A. marginale* (Figure 1), and 19.11 % (95% CI: 9.03-29.19) were infected with *Babesia* spp. Coinfections with *Babesia* spp and *A. maginale* were observed in 8.82% (95% CI: 1.34-16.30). None were found positive for *A. phagocytophilum*, *Leishmania* spp., or *Trypanosoma* spp (0%, 95% CI: 0.0-5.20).

The analysis to identify factors associated with hemoparasite infection, we focused our analysis on *A. marginale* and *Babesia* spp (Table 2). *A. marginale* was statistically more common among male cattle (p=0.041) and among calves (p=0.041). No epidemiologic risk factors for *Babesia* spp were revealed by this analysis. On clinical analysis, a higher basophil fraction was noted in cattle infected with *Babesia* spp (p=0.038), while no features were associated with *A. marginale* infection (Table 3).

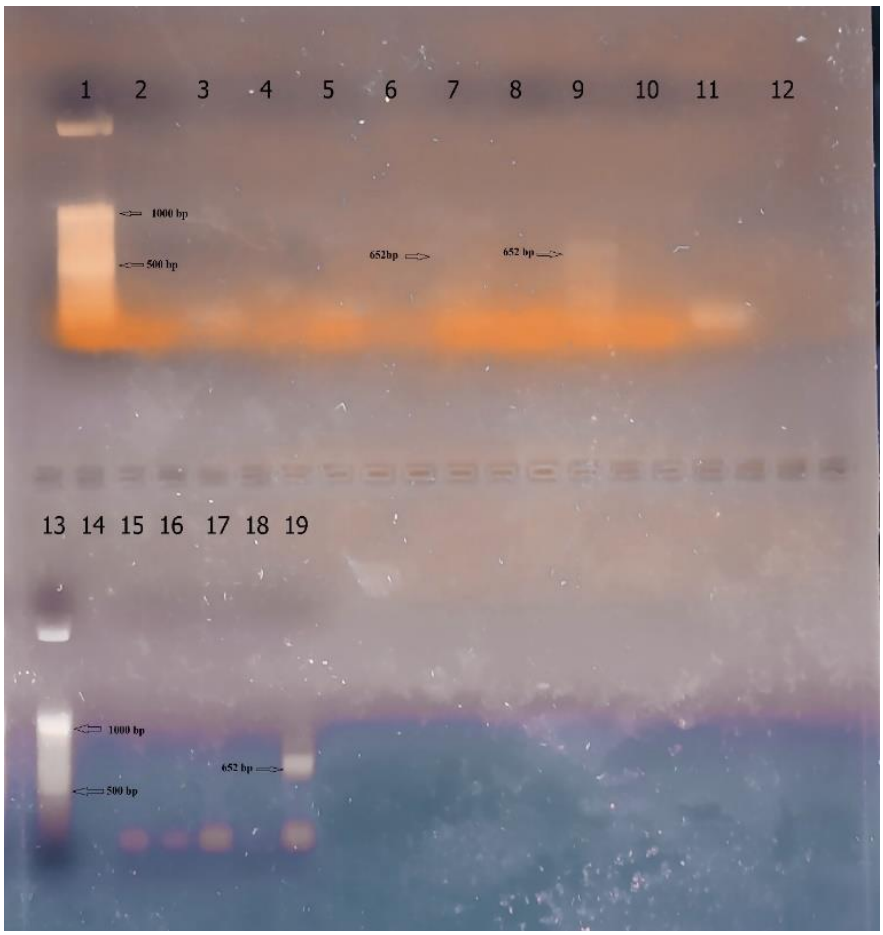

Figure 1. PCR results for the identification of *Anaplasma marginale*. Lines 1 and 13: molecular weight marker (100 bp), lines 7 and 9: positive samples to *Anaplasma marginale*, line 18: negative control, line 19: positive control.

Table 2. Epidemiological factors associated with *Anaplasma marginale* and *Babesia* spp infection in cattle in León.

Characteristic		*Anaplasma marginale* PCR		*Babesia* spp	
		Negative	Positive	Negative	Positive
Breed	Brahman	10	5	14	1
	Pardo	13	3	15	1
	Pardo/Brahman Mix	22	15	26	11
	Significance*	0.316		0.080	
Sex	Female	44	19	51	12
	Male	1	4	4	1
	Significance	0.041		0.665	
Age Category	Calf	2	5	5	2
	Cow	42	17	48	11
	Heifer	1	1	2	0
	Significance	0.041		0.750	
Ticks control	No	6	17	17	6
	Yes	12	33	38	7
	Significance	0.600		0.339	
Community	Abangasca	6	6	10	3
	Chácaraseca	25	10	27	8
	El Convento	4	1	4	1
	Las Chácaras	10	6	14	1
	Significance	0.554		0.549	

Table 3. Association of hematological parameters with *A. marginale* and *Babesia* spp infection in cattle in León.

Blood parameter	*A. marginale* PCR				*Babesia* spp		
	Results	Mean	SD	p-value	Mean	SD	p-value
Hematocrit %	Negative	30.42	4.26	0.442	29.84	5.18	0.393
	Positive	29.43	6.16		31.15	3.89	
Protein g/dL	Negative	7.56	0,52	0.338	7.52	0.67	0.759
	Positive	7.41	0,76		7.46	0.28	
White blood cells/ml (x10^3)	Negative	10.78	2.92	0.696	10.52	3.23	0.459
	Positive	10.44	4.11		11.29	3.86	
Red blood cells/ml (x10^6)	Negative	8.26	10,62	0.392	7.86	9.68	0.722
	Positive	6.53	2.14		6.89	1.72	
Lymphocytes %	Negative	30.84	10.85	0.996	32.11	12.63	0.092
	Positive	30.83	16.23		25.46	12.57	
N. Segmented %	Negative	41.64	17.56	0.918	40.71	18.09	0.461
	Positive	41.17	18.31		44.77	16.05	
Eosinophiles %	Negative	5.98	4.71	0.379	5.69	4.81	0.795
	Positive	4.91	4.58		5.31	4.13	
Monocytes %	Negative	20.44	15.70	0.824	20.49	15.58	0.784
	Positive	21.30	13.71		21.77	12.49	
Basophiles %	Negative	1.40	2.50	0.933	1.09	2.21	0.038
	Positive	1.35	2.23		2.62	2.81	

We found a high prevalence of *A. marginale*, *Babesia* spp, and their co-infection. This is similar to what was reported for Latin America, as a region, through a meta-analysis for *A. marginale* (48.9% [95% CI: 30.3–67.8%]), *Babesia* spp. (39.8% [95% CI: 24.6–57.2%]), and their coinfection (26.1% [95% CI: 9.1–55.8%]) (FERREIRA et al. 2022). In that study, a high prevalence of *A. marginale* was noted in Mexico (67.1%) and northern Brazil (71.9%). Overall Brazil had a prevalence was 36.6% for *A. marginale,* 62.6% for *Babesia* spp. and 8.2% for their coinfections, which is more similar to what we found in Nicaragua. *Babesia* spp., the prevalence was considerably divergent between regions, with the highest values also observed in the North region (97 .4%), and the lowest in the South region (9.5%),

We found that male bovines may be at greater risk of *A. marginale* infection. This research is similar to a report from Pakistan on the higher prevalence of *Anaplasma* in males (26.25%) than females (16.62%) (FAROOQI et al. 2018). The higher prevalence in males can be attributed to the fact that most of these are intended for meat and farmers are negligent and neglect health care. However, this differs from the higher prevalence reported in female cattle in Pakistan (ASHRAF et al. 2021) and a study in Kenya reporting no sex difference (OKAL et al. 2020). One hypothesis is that females experience a higher prevalence of parasites with depressed immunity during pregnancy, due to stress and altered hormone levels (KAMANI et al. 2010). There is also a potential for increased exposure to infection through contaminated needles, through the practice of injecting hormones to let down milk (ATIF et al. 2012). Another risk factor for infection by *A. marginales* was young age, in agreement with what has been reported in Pakistan (KHAN et al. 2019). Young animals may have less robust or underdeveloped immune systems, compared to older animals (SHAKAR et al. 2018). One limitation of our analysis was the small number of males subjects available for our analysis, although this is not thought to be different than the sex distribution of the herds.

Hematological parameters did not provide additional insight into infected and uninfected animals, with respect to *A. marginalis*. However, infected animals presented with a hematocrit anemia and mildly depleted red blood count (RBC). This is consistent with the hemoparasite infection course and has also been reported elsewhere, in addition to lower packed cell volume (PCV) and Hb concentration (ESMAEILNEJAD et al. 2018). Moreover, these same researchers attributed the anemia they observed to oxidative stress that correlated with *Anaplasma* parasite load in the blood, reducing membrane symmetry, and increasing the osmotic fragility of erythrocytes that also make them susceptible to erythrophagocytosis. We also observed that *Babesia* infection was associated with a basophilic presentation, hypothesized by others to be a function of the immune response to tick infestation (UNIGWE et al. 2022).

We observed a higher prevalence of *Anaplasma* spp by microscopy than was detected by PCR. Since it is well established that molecular techniques are more sensitive and specific than direct observations of

blood smears, this was an unexpected finding. We expected to identify only a subset of true infections by microscopy, given that erythrocyte inclusion bodies are found only when the infection is acute with a high parasite load, making direct observation of this feature unlikely during early infection or with low parasite burden (PARODI et al. 2021). Microscopy is inherently subjective to the observer and requires trained personnel. False positives also sometimes occur with microscopy because the stain used can produce artifacts, and inclusion bodies that resemble *A. marginale* can appear in the smear. Still, we are not the first to report this phenomenon with *Anaplasma* in cattle, as a group in the Philippines also reported a higher detection frequency for blood smear (73.7%) compared to PCR (63.7%) (AQUINO et al. 2018). In our study, laboratory personnel are well practiced in laboratory identification of veterinary pathogens using a wide range of techniques, including microscopy and molecular methods, and we believe it unlikely to be explained by user error or sole subjectivity.

This is the first report on the prevalence of hemoparasites in cattle that applies molecular diagnosis in the country and the first attempt to define epidemiologic factors of bovine *A. marginale* infection in western Nicaragua. This foundational work will aid in designing prevention and control strategies of blood-borne parasites in Nicaragua. Other regions with similar demographic and ecological characteristics may also benefit from our experience. Finally, our study demonstrates feasibility for additional investigations into these and other parasitic and vector-borne diseases that ultimately can inform veterinary and vector programs that benefit public health and improve profitability of livestock farming for small producers.

FUNDING

This study was financed with Fondos Concursables de Investigación 2021, REF:STCNU/187/2021, from the Consejo Nacional de Universidades (CNU).

ACKNOWLEDGMENTS

We thank the small livestock farmers of the Municipality of León who accepted voluntary participation in the study.

REFERENCES

AQUINO AJB et al. 2018. Detection of Anaplasma marginale infection in a dairy cattle farm by stained blood smear examination and nested polymerase chain reaction. Philippine Journal of Veterinary and Animal Sciences 44: 68-75.

ANUARIO ESTADÍSTICO 2019-2021. Instituto Nacional de Información de Desarrollo (INIDE) [en línea]. https://www.inide.gob.ni/Home/Anuarios [accedido 6 abril 2022].

ASHRAF S et al. 2021. First report regarding molecular epidemiology and novel variant identification of Anaplasma centrale in cattle from Pakistan. Saudi Journal of Biological Sciences 28: 6488-6494.

ATIF FA et al. 2012. Prevalence of Tick-Borne Diseases in Punjab (pakistan) and Hematological Profile of Anaplasma Marginale Infection in Indigenous and Crossbred Cattle. Pakistan Journal of Science 64: 11-15.

CORONA B et al. 2001. Biotecnologia en la ganaderia. Latin American Archives of Animal Production [en línea]. Vol. 9, n.o Suplemento. Recuperado a partir de: https://ojs.alpa.uy/index.php/ojs_files/article/view/2981 [accedido 10 octubre 2022].

COUTO LFM et al. 2022. Presence of Trypanosoma vivax DNA in cattle semen and reproductive tissues and related changes in sperm parameters. Veterinary Parasitology 309: 109761.

ESMAEILNEJAD B et al. 2018. Evaluation of oxidative stress and antioxidant status, serum trace mineral levels and cholinesterases activity in cattle infected with Anaplasma marginale. Microbial Pathogenesis 123: 402-409.

FAROOQI SH et al. 2018. Molecular epidemiology of bovine anaplasmosis in Khyber Pakhtunkhwa, Pakistan. Tropical Animal Health and Production 50: 1591-1598.

FERREIRA GCM et al. 2022. Prevalence of bovine Babesia spp., Anaplasma marginale, and their co-infections in Latin America: Systematic review-meta-analysis. Ticks and Tick-borne Diseases 13:101967.

FIKRU R et al. 2014. A proline racemase based pcr for identification of Trypanosoma vivax in Cattle Blood. PLoS ONE 9: e84819.

GETAHUN MN et al. 2022. Molecular characterization of pathogenic African trypanosomes in biting flies and camels in surra-endemic areas outside the tsetse fly belt in Kenya. International Journal of Tropical Insect Science 42: 3729–3745.

HOTA A et al. 2019. Therapeutic efficacy of quinapyramine compounds in T. evansi affected cattle. The Pharma Innovation Journal 8: 467-471.

HOVE P et al. 2018. Detection and Characterisation of Anaplasma marginale and A. centrale in South Africa. Veterinary Sciences 5: 26.

INTA NICARAGUA. 2021. Manejo de enfermedades bovinas en época húmeda [en línea]. 12 agosto 2021. Recuperado a partir de: https://www.youtube.com/watch?v=UXBvzEv9noc [accedido 1 agosto 2022].

JAIMES-DUEÑEZ J et al. 2018a. Molecular surveillance and phylogenetic traits of Babesia bigemina and Babesia bovis in cattle (Bos taurus) and water buffaloes (Bubalus bubalis) from Colombia. Parasites & Vectors 11:510.

JAIMES-DUEÑEZ J et al. 2018b. Genetic, host and environmental factors associated with a high prevalence of Anaplasma marginale. Ticks and Tick-borne Diseases 9: 1286-1295.

KAMANI J et al. 2010. Prevalence and Significance of Haemoparasitic Infections of Cattle in North-Central, Nigeria. [en línea]. Recuperado a partir de: http://irepos.unijos.edu.ng/jspui/handle/123456789/697 [accedido 10 octubre 2022]. Accepted: 2015-03-04T11:20:56Z

KHAN NU et al. 2019. Prevalence and risk factors analysis associated with anaplasmosis in symptomatic cattle under field conditions in southern Khyber Pakhtoonkhwa, Pakistan. Pure and Applied Biology 8: 2119-2127.

MATEUS J et al. 2019. An animal model of acute and chronic chagas disease with the reticulotropic y strain of Trypanosoma cruzi That Depicts the Multifunctionality and Dysfunctionality of T Cells. Frontiers in Immunology [en línea]. Vol. 10. Recuperado a partir de: https://www.frontiersin.org/articles/10.3389/fimmu.2019.00918 [accedido 13 septiembre 2022].

NORMAS JURÍDICAS DE NICARAGUA. 2011. Ley para la protección y el bienestar de los animales domésticos y animales silvestres domesticados. Normas Jurídicas de Nicaragua [en línea]. 2011. Recuperado a partir de: http://legislacion.asamblea.gob.ni/normaweb.nsf/b92aaea87dac762406257265005d21f7/cf820e2a63b1b690062578b00074ec1b [accedido 9 agosto 2022].

OKAL MN et al. 2020. Anaplasma and theileria pathogens in cattle of Lambwe Valley, Kenya: A case for pro-active surveillance in the wildlife–livestock interface. Microorganisms 8: 1830.

PARODI P et al. 2021. Validation of a multiplex PCR assay to detect Babesia spp. and Anaplasma marginale in cattle in Uruguay in the absence of a gold standard test. Journal of Veterinary Diagnostic Investigation 33: 73-79.

PANTHAWONG A et al. 2020. Detection of Anaplasma spp. and Bartonella spp. from wild-caught rodents and their ectoparasites in Nakhon Ratchasima Province, Thailand. Journal of Vector Ecology 45: 241-253.

PÉREZ I & BRAYANA S. 2020. Criterios diagnósticos y terapéuticos de la ehrlichiosis canina. [en línea]. Recuperado a partir de: http://repositorioslatinoamericanos.uchile.cl/handle/2250/3176595 [accedido 23 enero 2022]. Accepted: 2020-09-21T21:42:38Z

RASHID M et al. 2019. A systematic review on modelling approaches for economic losses studies caused by parasites and their associated diseases in cattle. Parasitology 146: 129-141.

ROBI DT et al. 2021. Trypanotolerance Sheko cattle: an option for sustainable control of bovine trypanosomiasis. Open Veterinary Science 2: 81-90.

SALAMANCA-CARREÑO A et al. 2018. Interaction between environmental and racial factors on the prevalence of hemotropics in dual purpose bovine females in Araucanas flooded savannas, Colombia. Revista Cientifica, Facultad de Ciencias Veterinarias, Universidad del Zulia 28: 52-62.

SHAKAR A et al. 2018. Prevalence of Anaplasmosis among risk groups of cattle and buffaloes in Mirpur, Azad Jammu and Kashmir. Pakistan Journal of Science [en línea] 70. Recuperado a partir de: https://www.proquest.com/docview/2348765051/abstract/46D2079D2AE94EC2PQ/1 [accedido 11 octubre 2022].

SHANG L et al. 2011. The prevalence of canine Leishmania infantum infection in Sichuan Province, southwestern China detected by real time PCR. Parasites & Vectors 4: 173.

UNIGWE CR et al. 2022. Haematological Implications of Haemoparasitism among Slaughtered White Fulani Cattle at Bodija Abattoir, Ibadan, Oyo State, Nigeria. Nigeria Agricultural Journal 53: 47-54.

VERBRUGGE SE & HUISMAN A. 2015. Verification and standardization of blood cell counters for routine clinical laboratory tests. Clinics in Laboratory Medicine 35: 183-196.

VERGARA-CAMUS L & KAY C. 2017. The agrarian political economy of left-wing governments in Latin America: Agribusiness, peasants, and the limits of neo-developmentalism. Journal of Agrarian Change 17: 415-437.