

Serological evidence of exposure to some zoonotic microorganisms in cattle and humans with occupational exposure to livestock in Antioquia, Colombia

Evidencia serológica de exposición a algunos microorganismos zoonóticos en bovinos y humanos con exposición ocupacional a ganadería en Antioquia, Colombia

Evidências sorológicas de exposição a alguns microrganismos zoonóticos em bovinos e em humanos com exposição ocupacional ao gado na Antioquia, Colômbia

Marcela Patricia Eraso-Cadena ¹
Licet Paola Molina-Guzmán ^{1,2}
Ximena Cardona ³
Jaiberth Antonio Cardona-Arias ²
Leonardo Alberto Ríos-Osorio ²
Lina Andrea Gutierrez-Builes ¹

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Abstract

Bacteria belonging to Anaplasma, Ehrlichia, Rickettsia and Coxiella genera are considered emerging pathogens and livestock is one of the contexts where the transmission of these microorganisms can occur. The goal of this study was to determine serological evidence for the exposure to these bacteria in cattle and humans with occupational exposure to livestock in the subregions North and Magdalena Medio, Antioquia, Colombia, and to explore related factors. A cross-sectional study was conducted in 48 livestock farms distributed in six municipalities from both subregions: Belmira, Entreríos and San Pedro de los Milagros (North), and Puerto Berrío, Puerto Nare and Puerto Triunfo (Magdalena Medio). Blood samples from 332 people and 384 bovines were evaluated by serology (IgM and IgG) screening for bacteria from the Anaplasma, Ehrlichia, Rickettsia, and Coxiella genera. Seropositivity in humans from both regions was 42.4% (95%CI: 31.2-55.1) for Anaplasma, 74.2% (95%CI: 66.0-84.4) for Ehrlichia, 72.5% (95%CI: 62.1-82.0) for Rickettsia, and 60.7% (95%CI: 59.7-69.1) for Coxiella burnetii. In cattle, seropositivity was 31.6% (95%CI: 19.9-44.2), 66.8% (95%CI: 55.2-78.1), 64.6% (95%CI: 53.8-74.5), and 61.6% (95%CI: 51.9-69.2), respectively. History of biting by ticks, milking, vaccination, having dogs and hens in the residence, as well as the consumption of raw milk derivatives were some factors associated with the infection by the bacteria studied. The results suggest a previous and recent exposure to these zoonotic bacteria genera in people with occupational exposure to livestock, as well as in cattle in the two studied subregions.

Zoonoses; Serology; Cattle; Occupational Health

Correspondence

L. A. Gutierrez-Builes
Grupo Biología de Sistemas, Escuela de Ciencias de la Salud,
Facultad de Medicina, Universidad Pontificia Bolivariana,
Calle 78 B # 72 A 109, Bloque B (Lab de investigaciones),
Medellín / Antioquia – 050034, Colombia.
lina.gutierrezb@upb.edu.co

¹ Facultad de Medicina, Universidad Pontificia Bolivariana, Medellín, Colombia.

² Escuela de Microbiología, Universidad de Antioquia, Medellín, Colombia.

³ Departamento de Asistencia Técnica, COLANTA, Medellín, Colombia.



Introduction

Tick-transmitted infectious diseases in humans are considered zoonotic diseases ¹. The livestock industry is one of the settings where such microorganisms are often detected, and the World Health Organization (WHO) considers this agricultural activity as one of the seven professional groups that is especially exposed to various zoonotic agents, given their close relationship with animals or their by-products, and the ongoing bidirectional transmission of diverse microorganisms ².

In Colombia, livestock farming occurs over the national territory as a major economic activity. The department of Antioquia hosts the largest bovine population of the country, where the Urabá, North, and Magdalena Medio subregions stand out as the largest producers at department level ³. Tick-borne diseases cause large economic losses, affecting cattle health and reducing the quality of by-products from the production system, such as meat, milk, and skins ⁴. The World Organization for Animal Health (OIE) suggests that some of these infectious diseases should be compulsorily reported for timely diagnosis and control due to their impact on animal welfare ⁵. However, Colombian surveillance programs often present underreporting regarding the etiological and clinical suspicion of tick-borne diseases, further, adequate diagnostic and therapeutic alternatives are currently unavailable.

Tick-borne diseases in humans, such as anaplasmosis, ehrlichiosis, rickettsiosis, and coxiellosis, develop with nonspecific symptoms like fever, headache, myalgia, and arthralgia ⁶. This clinical picture overlaps with other epidemiologically relevant pathologies recognized in the Colombian environment, such as malaria and dengue ⁷. Consequently, this similarity in symptoms may lead to an underestimation of the diagnosis and inadequate treatment of cases, which are usually recorded as unspecified fevers.

Ticks are obligate hematophagous ectoparasites associated with the transmission of viruses, bacteria, and protozoa that affect both human and animal health ⁸. Wild and domestic animals are part of their life cycle. The distribution of ticks at heights over 2,600 meters above the sea level (m.a.s.l.) ⁹ and the microorganism-transmission capacity at the transovarial and transstadial stages inherent to its life cycle, created the conditions for the maintenance of a steady population size over time, therefore increasing the risk of pathogenic microorganism transmission to diverse hosts ¹⁰. In the Colombian livestock context, different tick species have been found to be naturally infected by *Babesia* ¹¹, *Rickettsia* ¹², *Anaplasma* and *Ehrlichia* ¹³ genera, making them potential biological vectors. In addition, there are reports of human clinical cases of babesiosis ¹⁴, anaplasmosis ¹⁵, ehrlichiosis ¹⁴ and coxiellosis ¹⁶ associated with livestock activities.

Considering the representativeness of the North and Magdalena Medio subregions in livestock production in the department of Antioquia, Colombia, this study sought to determine, through serology screening, the level of exposure of cattle and people occupationally engaged in livestock activities to some tick-transmitted zoonotic agents, and to explore the associated factors with their detection in the studied subregions.

Methods

Design and place of study

A cross-sectional study was performed in 48 farms in the North and Magdalena Medio subregions of Antioquia. For the North subregion, the study was conducted from September to October 2014, in the municipalities of Belmira, Entreríos and San Pedro de los Milagros, where the temperature range from 13 to 19°C, 79% relative humidity, and the altitude range is 1,850 to 2,540 m.a.s.l. For the Magdalena Medio subregion, from October to November 2015, in the municipalities of Puerto Berrío, Puerto Nare and Puerto Triunfo, where the temperature ranges from 32 to 43°C, 53% relative humidity, and the altitude ranges from 125 to 150 m.a.s.l. These environmental conditions were verified during field sampling using a portable meteorological station (Ambient WS-2080; Ambient Weather, Chandler, U.S.A.). The estimated sample size for cattle and humans was proportionally distributed among eight cattle herds from the six municipalities included in this study (48 total herds). Livestock herds were selected in each municipality using simple random sampling on databases provided by the

main livestock associations in each area. Figure 1 illustrates the geographical distribution of the studied areas. Information on geographical coordinates was collected on the field using the Trimble Juno 3B GPS equipment (Westminster, U.S.A.), and represented using the ArcGIS 10.1 software (<http://www.esri.com/software/arcgis/index.html>).

Information collection and processing of blood samples

This study included males and females over 18 years of age, who had contact with the bovine reference population, and who voluntarily agreed to participate in this research. All participants signed an informed consent form. A structured survey was conducted to collect and describe some socio-demographic, hygiene-sanitary and clinical aspects about each participant. The information about cattle included age, sex, breed and medical and veterinary management history. The blood samples from people and cattle were collected without any preservatives. A multiple test was conducted for the serological screening of cattle and humans, using a single cut-off point (dilution $\geq 1:16$) and a custom-made commercial kit prepared by Fuller Laboratories (Fullerton, U.S.A.). To detect IgM and IgG antibodies an indirect immunofluorescence assay (IFA – the analytical sensitivity and specificity were estimated by the manufacturer in 98-100%) was used. The plates contained fixed antigens of *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, *Rickettsia rickettsii*, and phase I and phase II *Coxiella burnetii*, obtained from axenic cultures of these microorganisms. Samples were processed, and results were analyzed according to the manufacturer's instructions. Results were read using an Eclipse 55i microscope (Nikon, Tokyo, Japan) with an Intensilight epifluorescence system (Nikon, Tokyo, Japan) using 40X objective lens. Each IFA was read by two independent observers, blind to the sample. In the case of ambiguous readings, a third reading was performed to reach a final consensus. When necessary, the sample was re-processed and read again by both analysts.

The socio-demographic, hygiene-sanitary and clinical characteristics reported by the participants and the registered characteristics of the studied bovine population, were described as means of absolute (n) and relative frequencies (%) for the qualitative variables, and summary measures for the quantitative variables. Associated factors with seropositivity were explored; crude odds ratio and adjusted odds ratio were estimated using binary logistic regression to control confounding factors. The bivariate (crude association measures) and multivariate analyses (regression-adjusted measures) considered a 0.05 statistical significance level and 95% confidence level. The standard entry method of simultaneous variables (the enter method) was used for the regression model; all independent variables were entered in the equation at the same time to determine confounding and highly correlated ones. Statistical analysis was performed using the software SPSS for Windows, version 22.0 (IBM SPSS, Armonk, U.S.A.). This study was endorsed by the Ethics Committee on Health Research of the Universidad Pontificia Bolivariana (Record n. 7, May 23, 2012).

Results

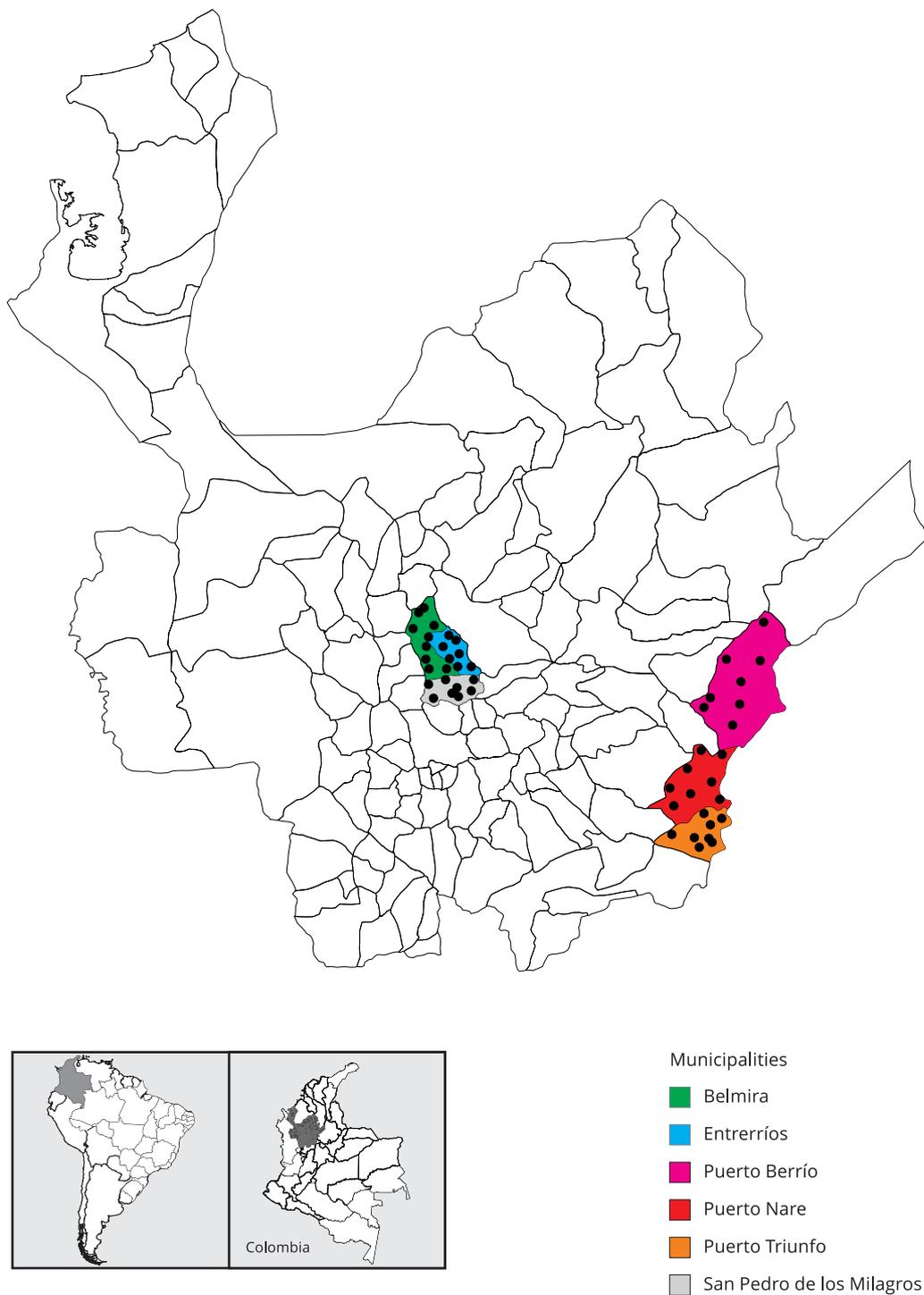
Socio-demographic, clinical, and health description of humans included in the study

Three hundred and thirty-two people were analyzed in total; 189 subjects from the North subregion and 143 from the Magdalena Medio subregion. In the Northern area, the study group consisted of 166 (87.8%) men and 23 (12.2%) women with a median age of 40 years. Most of the analyzed population resided in rural areas (82.5%) and milking was the most frequent activity on the farm (74.1%). The presence of ticks at the farm was reported by 18% of the population and 5.8% reported a history of tick bites. Preparation of foods derived from raw milk was reported by 51.3% of the individuals and usual consumption of foods derived from raw milk by 83.1%. Regarding the presence of domestic animals in the household, dogs were the most reported (71.4%), followed by cattle (66.7%) and horses (41.8%).

Most individuals from the Magdalena Medio subregion were men (82.5%) with a median age of 42 years. Regarding the place of residence, 83.2% of the participants lived in rural areas. The main work activities performed at the farm included cattle corralling (44.8%), milking (44.1%), and direct routine contact with bovine biological fluids while performing farm-related tasks (66.2%). The participants

Figure 1

Geographic location of the municipalities included in this study.



Note: geographical localization of the cattle farms included in this study is represented by dots within each of the six municipalities in the Department of Antioquia, Colombia. Overall, 48 farms (8 farms per municipality), 332 individuals (189 in the North subregion and 143 in the Magdalena Medio subregion) and 384 cattle (192 in the North subregion and 192 in the Magdalena Medio subregion) were sampled.

reported high values for the presence of ticks in the farms (85.3%), as well as a history of tick biting (62.9%) and involvement of immature ticks in biting (53.1%). Regarding the possession of domestic animals in the household, dogs (70.6%) and cattle (70.6%) were the most frequent. Preparation of foods derived from raw milk was reported by 50.3% of the individuals, and 59.4% reported their consumption on a regular basis (Table 1).

Table 1

Socio-demographic, clinical and sanitary characteristics of the participants.

Variables	North		Magdalena Medio	
	Median	IR	Median	IR
Age (years)	40	31-48	42	32-50
Time working with cattle (years)	19	10-30	20	10-28
Time working with cattle (hours/day)	8	4-9	8	1-8
	n	%	n	%
Sex				
Male	166	87.8	118	82.5
Female	23	12.2	25	17.5
Site of residence				
Urban	33	17.5	24	16.8
Rural	156	82.5	119	83.2
Education level (years)				
≤ 5	117	61.9	95	66.4
6-11	59	31.2	40	28.0
≥ 12	13	6.9	8	5.6
Occupational history *				
Contact with cattle	175	92.6	112	78.3
Contact with bovine biological fluids	156	82.5	94	66.2
Presence of ticks in the farm	34	18.0	122	85.3
History of tick bites	11	5.8	90	62.9
History of immature ticks found in their bodies	9	4.8	76	53.1
Farm occupations				
Milking	140	74.1	63	44.1
Cattle corralling	111	58.7	64	44.8
Cattle bath	97	51.3	35	24.5
Vaccination	93	49.2	61	42.7
Fumigation	61	32.3	41	28.7
Cattle slaughtering	33	17.5	17	11.9
Farm barbed-wiring	31	16.4	36	25.2
Animals in the residence place				
Dog	135	71.4	101	70.6
Cattle	126	66.7	101	70.6
Horse	79	41.8	84	58.7
Hen	76	40.2	93	65.0
Cat	73	38.6	93	65.0
Pig	57	30.2	47	32.9
Sheep	16	8.5	7	4.9
Goat	9	4.8	3	2.1

(continues)

Table 1 (continued)

Variables	North		Magdalena Medio	
	n	%	n	%
Hygiene-sanitary characteristics				
Hand washing before eating and cooking	185	97.9	134	93.0
Preparation of food derived from raw milk	97	51.3	72	50.3
Consumption of food derived from raw milk	157	83.1	85	59.4
Consumption of raw milk	80	42.3	30	21.0
Self-report of medical history *				
Headache	62	32.8	55	38.5
Fever	52	27.5	49	34.3
Chills	36	19.0	47	32.9
Fatigue	34	18.0	21	14.8
Stomachache	32	16.9	25	17.5
Myalgia	25	13.2	17	11.9
Nausea	24	12.7	21	14.7

IR: interquartile range.

* Corresponds to a self-report made by each participant of the study about the occupational history and after asking if he/she thinks that some of the mentioned signs and/or symptoms occur frequently.

Description of some characteristics and history of clinical and veterinary management of cattle

Three hundred and eighty-four bovines were evaluated in total. In the North subregion, the studied bovine population were 192 females, most of them born at the farm, with a median age of 60 months. Regarding the breeds tested in this region, 79.2% were *Bos taurus* and 20.8% were crossbreeds (Table 2). The proportions of male and female bovines studied in Magdalena Medio (192 cattle) were similar. The median age was 20 months. Studied breeds under mainly included *Bos indicus* animals (43.2%), different crossbreeds (34.4%), *B. taurus* (18.2%) and Colombian creole breeds (4.2%) (Table 2).

Results of the serological screening in humans and cattle from both subregions

Seropositivity was obtained for *Anaplasma*, *Ehrlichia*, *Rickettsia*, and *Coxiella* genera in individuals and cattle from the six municipalities analyzed. When analyzing the frequency of positive samples for both types of antibodies at the same time, 11.7% (45/384) was found for *Anaplasma*, 46.4% (178/384) for *Ehrlichia*, 41.1% (158/384) for *Rickettsia*, and 27.1% (104/384) for *Coxiella*; on the other hand, the human population showed a 29.2% (97/332) frequency for *Anaplasma*; 59.9% (199/332) for *Ehrlichia*, 52.1% (173/332) for *Rickettsia*, and 31.9% (106/332) for *Coxiella* (Table 3).

The highest frequency of anti-*Anaplasma* spp. IgM antibodies in people was detected in San Pedro de los Milagros, where 84.1% (53/63) of the samples were positive. Anti-*Ehrlichia* spp. IgM antibodies were mostly found in Entrerriós in 98.4% (62/63), and anti-*Rickettsia* spp. IgM antibodies were found in equal proportions in Belmira and San Pedro de los Milagros with 100% (63/63) positivity. For anti-*C. burnetii* IgM antibodies, the highest frequency was found in Belmira, with 98.4% (62/63). Regarding the detection of IgG-type antibodies in every studied bacterial genus, the highest frequency of anti-*Anaplasma* spp. IgG was found in the municipality of Belmira, with 60.3% (38/63). Anti-*Ehrlichia* spp. IgG was equally frequent in Entrerriós and San Pedro de los Milagros (84.1%, 53/63). Analyzed samples from San Pedro de los Milagros showed the highest seropositivity for anti-*Rickettsia* spp. and anti-*C. burnetii* IgG, with 81% (51/63) and 60.3% (38/63) frequencies, respectively.

In cattle, the highest frequency of anti-*Anaplasma* spp. IgM antibodies was found in Puerto Berrío, with 64.1% (41/64), whereas detection percentages of anti-*Ehrlichia* spp. IgM were observed in three municipalities alike: Entrerriós, Belmira and Puerto Berrío, with 87.5% (56/64). The highest frequency

Table 2

Characterization of the studied cattle population.

Variables	North		Magdalena Medio	
	Median	IR	Median	IR
Age (months)	60	42-81	20	9-60
Time cattle has been in the farm (months)	60	41-72	14	8-48
	n	%	n	%
Sex				
Male	-	-	96	50.0
Female	192	100.0	96	50.0
Breed *				
<i>Bos taurus</i> **	152	79.2	35	18.2
<i>Bos indicus</i> **	-	-	83	43.2
Different crossbreeds	40	20.8	66	34.4
Colombian creole breeds **	-	-	8	4.2
Type of livestock production				
Meat	-	-	98	51.0
Milk	192	100.0	59	30.7
Breeding	-	-	23	12.0
Dual purpose	-	-	12	6.3
Place of origin of the bovine				
Born at the farm	168	87.5	148	77.1
Born in another farm	24	12.5	44	22.9
History of clinical and veterinary management				
None/Non-diseased	187	97.4	192	100.0
Low milk production	2	1.0	-	-
Fever	1	0.5	-	-
Weight loss	1	0.5	-	-
Milk fever and hypocalcemia	1	0.5	-	-

IR: interquartile range.

* Breeds were categorized to better note the frequencies;

** *Bos indicus*: within the group of bovines of Indian origin also known as zebu cattle, breeds like Brahman, Zebu, Gyr, and Simbrah were included; *Bos taurus*: bovines of European origin, within this group breeds like Holstein, Jersey, Ayrshire, Simmental, Brown Swiss, Normande, Senepol, and Swedish Red were included; Colombian creoles: Bon and Romosinuano.

of anti-*Rickettsia* spp. IgM antibodies was detected at equal values in Belmira and San Pedro de los Milagros (96.9%, 62/64). For anti-*C. burnetii* IgM, the highest frequency was found in Belmira, where 100% (64/64) of samples tested positive. Regarding the detection of IgG antibodies in cattle, the highest frequency of anti-*Anaplasma* spp. IgG was 45.3% (29/64), and 82.8% (53/64) for anti-*Ehrlichia* spp. IgG, both proportions were detected in Puerto Berrío. The highest frequencies of anti-*Rickettsia* spp. IgG antibodies were 54.7% (35/64), equally detected in San Pedro de los Milagros and Puerto Berrío. For anti-*C. burnetii* IgG, the highest frequency (42.2%, 27/64) was found in Entrerriós.

Exploratory analysis of variables related to serological evidence of exposure in the studied human and animal populations

No IgM antibody associated factors were observed in the four microorganisms analyzed in both sub-regions after a multivariate adjustment. However, after analyzing data on the detection of IgG antibodies in individuals from the North subregion, a significant increase in exposure to *Anaplasma* spp.

Table 3

Frequencies of IgM/IgG antibodies detected simultaneously for the genera *Anaplasma*, *Ehrlichia*, *Rickettsia* and *Coxiella* in people and cattle from the North and Magdalena Medio subregions of the department of Antioquia, Colombia.

Subregion/ Municipality	Analyzed samples	Anaplasma		Ehrlichia		Rickettsia		Coxiella	
		#	% (95%CI)	#	% (95%CI)	#	% (95%CI)	#	% (95%CI)
People									
North									
Entrerríos	63	27	42.9 (30.5-56.0)	52	82.5 (70.9-91.0)	47	74.6 (62.1-84.7)	27	42.9 (30.5-56.0)
Belmira	63	28	44.4 (31.9-57.5)	47	74.6 (62.1-84.7)	36	57.1 (44.1-69.5)	30	47.6 (34.9-60.6)
San Pedro de los Milagros	63	34	54.0 (40.9-66.6)	49	77.8 (65.5-87.9)	51	81.0 (69.1-89.8)	32	50.8 (37.9-63.6)
Total	189	89	47.1 (39.8-54.5)	148	78.3 (71.7-84.0)	134	70.9 (63.9-77.3)	89	47.1 (39.8-54.5)
Magdalena Medio									
Puerto Berrío	50	2	4.0 (0.5-13.7)	27	54.0 (39.3-68.2)	17	34.0 (21.2-48.8)	9	18.0 (8.6-31.4)
Puerto Triunfo	37	6	16.2 (6.2-32.0)	21	56.8 (39.5-72.9)	13	35.1 (20.2-52.5)	6	16.2 (6.2-32.0)
Puerto Nare	56	- *	- *	3	5.4 (1.1-14.9)	9	16.1 (7.6-28.3)	2	3.6 (0.4-12.3)
Total	143	8	5.6 (2.4-10.7)	51	35.7 (27.8-44.1)	39	27.3 (20.2-35.4)	17	11.9 (7.1-18.4)
Total (people)	332	97	29.2 (24.4-34.4)	199	59.9 (54.5-65.3)	173	52.1(46.6-57.6)	106	31.9 (26.9-37.2)
Cattle									
North									
Entrerríos	64	4	6.3 (1.7-15.2)	29	45.3 (32.8-58.3)	26	40.6 (28.5-53.6)	23	35.9 (24.3-48.9)
Belmira	64	2	3.1 (0.4-10.8)	33	51.6 (38.7-64.3)	33	51.6 (38.7-64.3)	24	37.5 (25.7-50.5)
San Pedro de los Milagros	64	11	17.2 (8.9-28.7)	37	57.8 (44.8-70.1)	34	53.1 (40.2-65.7)	25	39.1 (27.1-52.1)
Total	192	17	8.9 (5.24-13.8)	99	51.6 (44.3-58.8)	93	48.4 (41.2-55.7)	72	37.5 (30.6-44.8)
Magdalena Medio									
Puerto Berrío	64	20	31.3 (20.2-44.1)	47	73.4 (60.9-83.7)	33	51.6 (38.7-63.3)	11	22.0 (8.9-28.7)
Puerto Triunfo	64	7	10.9 (4.5-21.3)	25	39.1 (27.1-52.1)	22	34.4 (23.0-47.3)	14	21.9 (12.5-34.0)
Puerto Nare	64	1	1.6 (0.04-8.4)	7	10.9 (4.5-21.3)	10	15.6 (7.8-26.9)	7	10.9 (4.5-21.3)
Total	192	28	14.6 (9.9-20.4)	79	41.1 (34.1-48.5)	65	33.9 (27.2-41.0)	32	16.7 (11.7-22.7)
Total (cattle)	384	45	11.7 (8.7-15.4)	178	46.4 (41.3-51.5)	158	41.1 (36.2-46.3)	104	27.1 (22.7-31.8)

#: number of positive samples; 95%CI: 95% confidence interval.

* Without positive data for IgM/IgG antibodies detected at same time.

infection was observed, which is directly related to the length of time (in years) of work with cattle and to the direct contact with cattle in work activities performed in farms. However, in the Magdalena Medio subregion such relation involved the participation in livestock corralling. The exposure to *Rickettsia* spp. in the North subregion showed greater likelihood in individuals who performed milking and vaccination, as well as in participants who reported having dogs in their residence. In the Magdalena Medio subregion, no associated factors with *Rickettsia* spp. infection were observed. Regarding exposure to *Ehrlichia* spp., no associated factors in the North subregion were found; however, in Magdalena Medio, individuals who had direct occupational contact with cattle presented a higher probability of exposure to infection by this microorganism. Participants from the North subregion who reported tick bite history, participation in livestock slaughter and cohabitation with hens in their residence showed a higher probability of presenting serological evidence of exposure to *C. burnetii*. Data analysis from Magdalena Medio showed a greater probability of exposure to infection by this zoonotic agent in people consuming raw milk products.

In the Magdalena Medio subregion, it was observed that serological evidence (IgG antibodies) for exposure to *Anaplasma* spp. and anti-*Rickettsia* spp. in cattle significantly increased with age. Exposure

to *C. burnetii* increased significantly and was related to the period cattle had been in the farms (regardless of their origin), thus resulting in the detection of IgG antibodies in bovines from the North sub-region (Table 4).

Table 4

Exploratory analysis of variables related with seropositive (IgG antibodies) to *Anaplasma*, *Ehrlichia*, *Rickettsia* and *Coxiella* in the analyzed people and cattle from the North and Magdalena Medio subregions of the department of Antioquia, Colombia.

Variable	OR *	95%CI
People		
North subregion		
<i>Anaplasma</i> spp.		
Time working with cattle (in years)	1.04	1.00-1.08 **
Direct occupational contact with cattle	3.43	1.00-11.71 **
<i>Rickettsia</i> spp.		
Participation in milking	2.64	1.08-6.43 **
Participation in vaccination	4.07	1.53-10.84 **
Having dogs in the place of residence	6.14	2.26-16.66 ***
<i>Coxiella burnetii</i>		
Participation in livestock slaughtering	3.76	1.35-10.45 **
History of tick bites	6.18	1.12-34.07 **
Having hens in the place of residence	3.61	1.32-9.79 **
Magdalena Medio subregion		
<i>Anaplasma</i> spp.		
Participation in livestock corralling	3.43	1.22-9.63 **
Time working with cattle (in years)	1.90	1.21-3.26 **
<i>Ehrlichia</i> spp.		
Direct occupational contact with cattle	2.59	1.02-6.57 **
Participation in fumigation	0.34	0.15-0.78 **
<i>Coxiella burnetii</i>		
Consuming foods derived from raw milk	4.04	1.58-10.34 **
Time working with cattle (in years)	6.16	2.28-16.4 ***
Cattle		
North subregion		
<i>Coxiella burnetii</i>		
Time cattle has been in the farm	2.45	1.02-6.08 **
Ticks control method	1.69	1.13-3.27 ***
Magdalena Medio subregion		
<i>Anaplasma</i> spp.		
Age	1.79	1.12-3.63 **
Ticks control method	1.21	1.31-8.26 **
<i>Rickettsia</i> spp.		
Age	1.99	1.15-6.57 **
Type of livestock production	0.75	0.73-8.58 **

95%CI: 95% confidence interval; OR: odds ratio.

Note: for *Coxiella burnetii* were considered seropositive results against both phase I and II IgG antibodies.

* Adjusted by logistic regression;

** p < 0.05;

*** p < 0.01.

Discussion

In this study, serological signs of exposure to *Anaplasma*, *Ehrlichia*, *Rickettsia* and *Coxiella* genera were detected in bovines and in people working in cattle raising in the North and Magdalena Medio subregions. This finding denotes the circulation of these zoonotic agents in the livestock context in the studied subregions, suggesting that their role as potential etiological agents of associated infectious diseases must be considered to establish some type of differential diagnosis for both cattle and humans.

IFA is the most used technique for screening the studied type of infections; however, this test is hindered by the interference of cross-reactions between different microorganisms. For example, species from the genus *Anaplasma* exhibit seroreactivity between them, as well as with other *Rickettsia* and *Ehrlichia*, given the existing phylogenetical relation that was demonstrated through the analysis of the 16S rRNA gene¹⁷. Likewise, there are reports of the occurrence of coinfections of *C. burnetii* and species from the *Rickettsia* genus¹⁸. Therefore, for cases that require more specific species differentiation, future studies should explore serological screening methods with molecular techniques like polymerase chain reactions and in vitro culture¹⁹. In addition, it is important to consider that the cut-off point used in this study was employed for screening and research in apparently healthy people working at farms; higher cut-off points values, measured in paired serum (seroconversion), should be employed in clinical diagnosis and treatment, according to the dynamics of antibodies production of each zoonosis²⁰. Accordingly, if higher cut-off points were used, the frequencies reported in this study are expected to be lower since they would indicate active diseases, not exposure in the livestock context.

A. phagocytophilum is considered the etiologic agent of human granulocytic anaplasmosis. In Colombia, there are reports of cases in people working at livestock farms in the departments of Bolívar¹⁵, Córdoba and Sucre, where exposure to this zoonotic agent has been suggested²¹. Regarding cattle, reports claim the possibility of these animals being asymptotically infected by *Anaplasma* spp.²², in some instances, this species has been considered the cause of conditions such as fever, anemia, and of decrease in milk production depending on the animal's age or immune status²³. In species like *Anaplasma marginale*, a recognized etiological agent of bovine anaplasmosis, an extensive genetic strain diversity has been described²⁴, which may be related to various courses of clinical evolution in this type of infection. Considering IgG seropositivity associated factors for *Anaplasma* in bovines from the Magdalena Medio subregion, the findings of this study suggest that exposure increased significantly with age, similarly to Wesonga et al.²⁵.

Anaplasma has been detected in *Rhipicephalus microplus* cattle ticks, in *Dermacentor nitens* collected from horses¹³, and in *R. microplus*, *Amblyomma cajennense* and *Amblyomma maculatum* ticks collected in buffaloes²⁶, demonstrating a wide circulation in diverse hosts and potential vector species. Clinical reports show that *Anaplasma* transmission does not occur exclusively by tick bites, documents in the literature link this microorganism to transmission through blood transfusions²⁷, such transmission route for this microorganism is still poorly understood. Transplacental transmission has also been reported as the infection of calves has been observed since birth, thus becoming a potential infection source for other hosts²⁸. In addition, mechanical transmission has been reported through fomites contaminated with infected blood²⁹ or through diptera of the *Tabanus* and *Stomoxys* genera³⁰. Several animals, such as cattle, horses, goats, buffaloes, and canids are involved in the life cycle of *Anaplasma*. Infections have been reported in tropical countries like Colombia, Costa Rica and Brazil.

On the other hand, there is currently little evidence recording the circulation of the *Ehrlichia* species related to cattle or to the livestock context in Colombia, which contrasts with the exposure to *Ehrlichia* genus found in this study. However, literature reports exposure to *Ehrlichia ruminantium* in bovines from cattle systems in Tanzania³¹ and molecular detection of this microbial agent in cattle systems in Turkey³². In Colombia, *Ehrlichia* species has been detected in hosts such as dogs, and *Ehrlichia canis* infection has been identified as the cause canine monocytic ehrlichiosis³³; also, its relation to infections in human cases has been suggested. In countries like Costa Rica, the circulation of this species has been detected in samples from blood donors³⁴, while in Brazil, studies made in rural areas have shown exposure to *E. canis* in dogs and *E. chaffensis* in humans and horses³⁵. Some studies have shown the circulation of *Ehrlichia* sp. in bovine ticks (*R. microplus*), and in dog ticks (*Rhipicephalus san-*

guineus). *E. canis*, *Anaplasma platys* and *A. phagocytophilum* have been detected in dog ticks in tropical countries like Costa Rica ³⁶, where cases in humans have also been diagnosed. This microorganism has a high diversity of host species. *E. chaffeensis* has been described as the species responsible for human monocytic ehrlichiosis; further, it has also been identified as an etiological agent of infections in dogs and cattle, among other animals. Susceptibility to this zoonotic agent has been experimentally tested in bovine cattle, causing fever, lethargy and in some severe cases, the death of the animal ³⁷.

Regarding the results obtained in this study about the exposure of people and cattle from the studied regions to the *Rickettsia* genus, several research in Colombia have consistently suggested the circulation of spotted fever group rickettsiosis detected in equines (horses, mules and donkeys) and cattle ³⁸. *R. rickettsii* is considered the most virulent species in the group responsible for the Rocky Mountain spotted fever, whose vector-borne transmission can be transstadial and transovarial ⁶, with 10-60% of cases described as fatal if not treated rapidly. In countries like Costa Rica, the circulation of different *Rickettsia* species has been confirmed both in ticks associated to different hosts like horses, cattle, and dogs, and in humans ³⁹. In Panamá, spotted fever group rickettsiosis have been isolated in ticks from domestic animals in areas near the Colombian border ⁴⁰, suggesting their potential circulation in other tropical countries. In addition, the circulation of *A. cajennense* ticks extracted from skin and clothing of people has also been detected, suggesting the affinity of these arthropods for humans ¹². IFA is the most used technique to diagnose rickettsial diseases; however, the presence of cross-reactions between the different species of *Rickettsia* and with other bacterial genera must be considered. Although in vitro culture screening is the reference test for *Rickettsia*, its application is not common given the need for a Biosafety Level 3 Laboratory. Therefore, in clinical instances with symptomatic patients, other aspects must be considered for the diagnosis, such as seroconversion and higher cut-off points for antibody titers $\geq 1:64$ for IgG and $\geq 1:32$ for IgM ⁴¹.

Regarding the exploration of associated factors with the detection of anti-*Rickettsia* IgG antibodies, the results of this study suggest a higher probability of infection by this microorganism in people engaged with milking and vaccination activities in cattle farms, as well as in people who have dogs in their household. The presence of several animals at home, dogs and horses being the most frequent, was noted after the survey analysis. This information is important since these animals have been reported as hosts of several of the microorganisms tested in this study. This last observation coincides with a recent report performed in urban areas of Costa Rica, in which dogs were found to have a serological imprint of exposure to spotted fever group rickettsiosis, especially in places with known human infections. These findings hypothesize that dogs could be infection vectors in these scenarios ⁴², which highlights the importance of the associated factors identified in this study as variables to be considered by the human and animal health sectors in subsequent studies seeking to identify the transmission dynamics of this type of zoonotic agent in the Colombian environment.

The exploration of the associated factors with seropositivity for *C. burnetii* found in people evaluated in this study proved that there is a greater probability of exposure to this microorganism in people who reported tick biting history and the consumption of food derived from raw milk. These factors are biologically plausible because vector-borne transmission of *C. burnetii* is considered secondary, and the main transmission mechanisms such as inhalation, contact with biological fluids from infected animals, and ingestion of contaminated products have been described. *C. burnetii* has been detected in different biological fluids like urine, feces, milk, and placenta of infected animals; tropism of this microbial agent by cells of the reproductive system has been evidenced, thus affecting consumers of unpasteurized milk ⁴³. Corroborating these results, *C. burnetii* has been previously detected in bovine milk from Colombia ⁴⁴. In addition, serological evidence of exposure to this zoonotic agent in people engaged in livestock activities has also been reported in the departments of Córdoba and Sucre, in towns like La Ceja, Envigado and Caracoli, also being found in other Antioquia regions ⁴⁵. In some cases, *C. burnetii* has been linked to chronic cardiac conditions, such as endocarditis ⁴⁶, in people with occupational exposure to livestock. IFA is the most used method to diagnose *C. burnetii*; cross-reactions have also been detected, in this case, with *Bartonella* species ⁴⁷. All these aspects must be considered for its diagnosis, in addition to the patient's clinical history and seroconversion during clinical evolution.

In Latin America, *C. burnetii* infection has been reported in people living in rural areas dedicated to livestock activities in Minas Gerais, Brazil ⁴⁸. In Argentina, exposure to this zoonotic agent was

reported in people living under poor sanitary conditions with domestic animals, such as cats and dogs ⁴⁹. In countries like Spain, exposure to this microorganism has been detected in dairy cattle ⁵⁰ and in sheep. Regarding the detection of this agent in ticks, it has been described in the *Amblyomma* and *Rhipicephalus* genera of specimens collected in Brazil, Colombia, Kenya, and China ⁵¹. McDaniel et al. ⁵² suggests that coxiellosis, or Q fever, should be one of the highly suspected zoonotic diseases in people with occupational exposure to cattle, and that early diagnoses must be made to achieve successful treatments, given the serious medical conditions, like endocarditis, observed in 65% of cases. Based on these data, we suggest that *C. burnetii* infections should also be considered in the Colombian environment for a differential and etiological diagnosis in people occupationally exposed to livestock, especially if they are used to consuming raw milk and raw milk-derived foods.

Anthropogenic activities related to the livestock production system are known to favor the contact with animals and hence, the development of zoonotic diseases. Although the North and Magdalena Medio subregions of Antioquia present contrasting ecological and productive conditions, the exposure of cattle and humans to the studied microorganisms was detected in both subregions. Milk production is considered the main economic and agricultural activity in Northern Antioquia, while breeding and dual-purpose activities are more frequent in livestock systems in the Magdalena Medio subregion ⁵³.

Climate change and global warming have been described as enhancers of the geographical expansion of vectors related to infectious diseases in the livestock context ⁵⁴, benefiting the development of diseases that affect both animals and humans. Moreover, ungulates have been cited as the main vertebrate hosts of zoonotic pathogens when compared to animals like rodents, primates, and bats ⁵⁵. The distribution of zoonotic agents related to cattle has also been claimed to be similar throughout the Americas, as well as in Oceania, Africa, Asia, and Europe ⁵². Although the results obtained in this study are relevant, some of them remain as research questions to be answered with greater certainty in future longitudinal studies. For example, it could be important to determine how different cattle breeds, dairy farming vs breeding and dual-purpose activities, cattle management characteristics, weather, and tick infestation rates could be influencing the exposure dynamics to these zoonotic agents in the studied regions.

According to this study, time of work and direct contact with cattle (milking, vaccination, livestock slaughtering and corralling) were associated with the exposure to the studied zoonotic agents. Therefore, occupational health measures must be addressed in the livestock cattle context, taking biosecurity actions in the work field, such as the proper use of face masks, gloves, boots, preventive actions against tick bites, among others. Consumption of raw milk and foods derived from it must be considered when studying the transmission of this kind of zoonotic agents (as it has been demonstrated for *C. burnetii*) so control procedures are adequately followed. In addition, considering that people working in livestock activities in Colombia are exposed to the studied infections, the infectious agents involved should be included in differential and etiological diagnoses for clinical cases of acute febrile syndrome in people with this type of occupation. In general, the findings from this study indicate the exposure to these microorganisms in the livestock context. Consequently, promotion and prevention programs specifically aimed at this type of occupational exposure are required, thus addressing this problem in a comprehensive, cooperative and articulated manner in public and animal health sectors.

Contributors

M. P. Eraso-Cadena performed research, analyzed data and wrote the paper. L. P. Molina-Guzmán designed research, performed research and analyzed data. X. Cardona designed research and performed research. J. A. Cardona-Arias and L. A. Ríos-Osorio designed research and analyzed data. L. A. Gutierrez-Builes designed research, analyzed data and wrote the paper.

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Resumen

Las bacterias pertenecientes a los géneros *Anaplasma*, *Ehrlichia*, *Rickettsia* y *Coxiella* son consideradas patógenos emergentes y la ganadería es uno de los contextos donde se puede producir la transmisión de este tipo de microorganismos. El objetivo de este estudio fue determinar la evidencia serológica, debida a la exposición a estas bacterias en bovinos y humanos con exposición ocupacional a ganadería en las subregiones Norte y Magdalena Medio, Antioquia, Colombia, además de estudiar los factores relacionados. Se realizó un estudio transversal en 48 fincas ganaderas, distribuidas en seis municipios de ambas subregiones: Belmira, Entrerriós y San Pedro de los Milagros (Norte), y Puerto Berrío, Puerto Nare y Puerto Triunfo (Magdalena Medio). Las muestras de sangre de 332 personas y 384 bovinos fueron evaluadas mediante tamización serológica (IgM e IgG) para la detección de bacterias de los géneros *Anaplasma*, *Ehrlichia*, *Rickettsia*, y *Coxiella*. La seropositividad en humanos de ambas regiones fue 42,4% (IC95%: 31,2-55,1) en el caso de *Anaplasma*, un 74,2% (IC95%: 66,0-84,4) en *Ehrlichia*, un 72,5% (IC95%: 62,1-82,0) en *Rickettsia*, y un 60,7% (IC95%: 59,7-69,1) en *Coxiella burnetii*. En los bovinos, la seropositividad fue 31,6% (IC95%: 19,9-44,2), 66,8% (IC95%: 55,2-78,1), 64,6% (IC95%: 53,8-74,5), y 61,6% (IC95%: 51,9-69,2), respectivamente. El antecedente de haber sido mordido por garrapatas, ordeñar, vacunación, tener perros y gallinas en la residencia, así como el consumo de derivados de leche cruda fueron algunos de los factores asociados con la infección por las bacterias estudiadas. Los resultados sugieren la exposición previa y reciente a estas bacterias en personas con una exposición ocupacional a la ganadería, así como a los bovinos en las dos subregiones estudiadas.

Zoonosis; Serología; Bovinos; Salud Laboral

Resumo

As bactérias dos gêneros *Anaplasma*, *Ehrlichia*, *Rickettsia* e *Coxiella* são considerados patógenos emergentes, e a transmissão desses microrganismos pode ocorrer no contexto da pecuária. O estudo teve como objetivos determinar as evidências sorológicas de exposição a essas bactérias em bovinos e em humanos com exposição ocupacional ao gado nas sub-regiões Norte e Magdalena Médio, Antioquia, Colômbia, e explorar fatores associados. Foi realizado um estudo transversal em 48 fazendas de gado bovino distribuídas em seis municípios nas duas sub-regiões: Belmira, Entrerriós e San Pedro de los Milagros (Norte) e Puerto Berrío, Puerto Nare e Puerto Triunfo (Magdalena Médio). Amostras de sangue de 332 humanos e 384 bovinos foram analisadas com sorologia (IgM e IgG) para bactérias dos gêneros *Anaplasma*, *Ehrlichia*, *Rickettsia* e *Coxiella*. Os níveis de sorologia positiva em humanos das duas regiões foram de 42,4% (IC95%: 31,2-55,1) para *Anaplasma*, 74,2% (IC95%: 66,0-84,4) para *Ehrlichia*, 72,5% (IC95%: 62,1-82,0) para *Rickettsia* e 60,7% (IC95%: 59,7-69,1) para *Coxiella burnetii*. Nos bovinos, os níveis foram 31,6% (IC95%: 19,9-44,2), 66,8% (IC95%: 55,2-78,1), 64,6% (IC95%: 53,8-74,5) e 61,6% (IC95%: 51,9-69,2), respectivamente. Os fatores associados às bactérias estudadas foram: história de picada de carrapato, ordenha, vacinação, presença de cães e galinhas no domicílio e consumo de laticínios feitos com leite cru, entre outros. Os resultados sugerem exposição prévia e recente a esses gêneros bacterianos zoonóticos em pessoas com contato ocupacional com gado, assim como nos próprios animais, nas duas sub-regiões estudadas.

Zoonoses; Sorologia; Bovinos; Saúde do Trabalhador

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