La granja:
Revista de
Ciencias de la Vida
pISSN:1390-3799; eISSN:1390-8596
http://doi.org/10.17163/lgr.n31.2020.08

Scientific paper/ Artículo científico

VETERINARY SCIENCES

Antibiotic resistance of Salmonella spp, Escherichia coli isolated from alpacas (Vicugna pacus) with and without diarrhea

Resistencia antibiótica de Salmonella spp, Escherichia coli aisladas de alpacas (Vicugna pacus) con y sin diarrea

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Article received on May 12th, 2019. Accepted, after review, on January 27th, 2019. Published on March 1st, 2020.

Resumen

A nivel mundial, el problema de resistencia a antibióticos es considerado de prioridad sanitaria pública y veterinaria, por ello el objetivo de esta investigación fue evaluar la presencia de resistencia antibiótica frente a Salmonella sp., y Escherichia coli provenientes de crías de alpacas con y sin diarrea. La investigación fue de tipo descriptivo transversal múltiple. Se recolectaron 300 muestras de heces por hisopado rectal de crías de alpacas entre 10 a 60 días nacidas con y sin cuadros diarreicos provenientes de Comunidades Campesinas de Huancavelica-Perú. La presencia de Escherichia coli y Salmonella spp. Se identificó mediante pruebas bioquímicas la susceptibilidad antibacteriana por método Kirby Bauer y se evaluaron 8 antibióticos usuales del mercado veterinaria. El 100% de muestras con diarreas fueron positivas a Escherichia coli, 40,0% Salmonella spp., 20% Escherichia coli-Salmonella spp. y muestras sin diarrea 57,0% positivas a Escherichia coli, 24,0% Salmonella spp., 19.0% E. coli-Salmonella spp. Las cepas de Escherichia coli y Salmonella spp. fueron resistentes a Ampicilina (10,4± 0,3), (9,3± 0,2); Novomicina (11,1± 0,2), (11,2± 0,1); Tetraciclina (8,2± 0,1), (9,2± 0,3); Penicilina (9,1± 0,4), (11,1± 0,3); Gentamicina (10,1± 0,4), (10,2± 0,3) provenientes de muestras con diarrea y en muestras sin diarrea resistentes a Gentamicina (10,3± 0,1), (8,2± 0,1); Tetraciclina (9,2± 0,4), (8,2± 0,4); Ampicilina (11,2± 0,1), (9,3± 0,2); Penicilina (10,2± 0,4), (10,1± 0,3). Las cepas de Salmonella spp., y Escherichia coli aisladas de crías de alpacas con y sin diarreas evidencian resistencia antibacteriana a múltiples antibióticos usados en la veterinaria.

Palabras clave: alpaca, Salmonella spp, Escherichia coli, resistencia antibiótica.

Abstract

The problem of antibiotic resistance is considered a public and veterinary sanitary priority worldwide, for that reason the aim of the study was to evaluate the presence of antibiotic resistance against Salmonella spp., Escherichia coli coming from alpaca calves with and without diarrhea. The research was cross-sectional descriptive. 300 stool samples per rectal swab were collected from alpaca calves aging from 10 and 60 days with and without diarrhea from Peasant Communities in Huancavelica – Peru. The presence of Escherichia coli and Salmonella spp. was identified by conventional biochemical test, antibacterial susceptibility by Kirby Bauer method and 8 usual antibiotics from the veterinary market were evaluated. 100% of samples with diarrhea were positive to Escherichia coli; 40.0% Salmonella spp.; 40% Escherichia coli -Salmonella spp., and samples without diarrhea 48.3% positive to Escherichia Coli; 14.0% Salmonella spp.; 9.3% Escherichia coli -Salmonella spp. The CMI in Escherichia coli and Salmonella spp. strains were resistant to Ampicillin (10.4 ± 0.3), (9.3 ± 0.2); Novomycin (11.1 ± 0.2), (11.2 ± 0.1); Tetracycline (8.2 ± 0.1), (9.2 ± 0.3); Penicillin (9.1 ± 0.4), (11.1 ± 0.3); Gentamicin (10.1 ± 0.4), (10.2 ± 0.3) from samples with diarrhea and in samples without diarrhea resistant to Gentamicin (10.3 ± 0.1), (8.2 ± 0.1); Tetracycline (9.2 ± 0.4), (8.2 ± 0.4); Ampicillin (11.2 ± 0.1), (9.3 ± 0.2); Penicillin (10.2 ± 0.4), (10.1 ± 0.3). Salmonella spp., Escherichia coli strains isolated from alpaca calves with and without diarrhea show antibacterial resistance to multiple antibiotics used in veterinary.

Keywords: alpaca, Salmonella spp., Escherichia coli, antibiotic resistance.

Suggested citation: Carhuapoma, V., Valencia,N., Huamán, T., Paucar, R., Hilario, E. and Huere, J.L. (2020). Antibiotic resistance of Salmonella spp, Escherichia coli isolated from alpacas (*Vicugna pacus*) with and without diarrhea. La Granja: Revista de Ciencias de la Vida. Vol. 31(1):111-120. http://doi.org/10.17163/lgr.n31.2020.08.

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1 Introduction

The breeding of alpacas for the high Andean families of Peru is an activity with socioeconomic importance, since it provides high protein meat with low cholesterol content and its fiber has a high demand in the national and global market (Rosadio et al., 2012; Siuce et al., 2015). More than 85% of Alpaca breeding in Peru is distributed in the Peasant Communities and in small producers who live in extreme poverty and with lack of technical advice, thus facing significant economic losses from pathological problems and high mortality from parasitic, bacterial and viral etiologies (Lucas et al., 2016). High mortality rates in alpaca calves reach 12 to 50%, mainly due to enteropathogenic diarrheal problems of Escherichia coli and Salmonella spp. (Ramírez, 1990; Rosadio et al., 2012), and the resistance of antibacterials to Escherichia coli and Salmonella spp. causes global concern due to the serious impact on public health and animal production, conceived by conditions of inappropriate and indiscriminate use of antibacterials (Yagui, 2018).

From the perspective of animal production, the use of antibiotics has increased really fast, generating a risk of resistant bacteria (Centeno et al., 2018), because these bacteria may have undergone genetic modifications to their residency mechanism as an enzymatic inactivation, altered receptors and transportation of the altered antibiotic which make the permanence of bacterial genotypes resistant to Escherichia coli and Salmonella spp., and there are few animal production-oriented studies that could possibly be related to what was reported in human health (Schwarz et al., 2017).

Salmonella spp. and Escherichia coli are pathogens with clinical importance in the Animal Health in the Peruvian Andes, being the causal agent of pathologies that cause intestinal dysfunction, generating the neonatal diarrheal in alpacas, which makes difficult the therapeutic treatment (Silvera et al., 2012; Rosadio et al., 2012). Several studies report an increase in antimicrobial resistance and minimal sensitivity to different antibiotics to pathogenic microorganisms of Salmonella spp. and Escherichia coli in bird, pig, guinea pigs, bovine and silvestria animals such as monkeys, generating important losses of genetic and socioeconomic value in breeders (Oha, 2012; Medina et al., 2017; S., 2018). There are no related studies on the use of antibiotics in veterinary against Salmonella spp. and Escherichia coli from alpaca calves with and without diarrheal enteropathies, even though Peru constitutes a major producer worldwide of the textile and meat market, these microns being of emerging clinical importance in the production of alpacas.

It is believed that there may be cases of strains with resistance phenotypes similar to those in chicken, pig, bovine and domestic species (Ortiz, 2011), which would make it possible for the use of different antibacterial antibiotics in the therapeutic treatment in alpaca calves with diarrheal enteropathies (salmonellosis and Escherichia coli) that are not effective in high-zone Andeans communities of Peru. Because of the latter, there is a need to evaluate the antibiotic resistance of Salmonella spp. and Escherichia coli in alpaca calves with and without diarrheal enteropathies, which will allow future actions to be implemented to reduce the use of antimicrobials to prevent the generation, dissemination of antibiotic-resistant bacteria through the application of good health practices and the good use of antimicrobials.

2 Materials and methods

According to the central limit theorem, a total of 300 samples of alpaca calves with diarrhea and 300 samples of calves without diarrhea of Huacaya breed from 10 to 60 days of birth, regardless their sex were used, since the birth population is unknown because it is progressive and temporary. Six Rural Communities of Huancavelica alpaca –Peru were taken into account, which were located above 4 200 m.a.s.l, with temperatures ranging from 5.6 to 8.5 °C, from January to March 2018. Likewise, the minutes of communal authorization and consent informed by the owners of the herds under study were also taken into account.

Samples were collected by triplicate per sampled animal by rectal swab in sterile cryovials suspended in means of Stuart transport (bufferted) at dawn (6.00 am to 7.30 am) without the presence of sun rays and with efficient biosecurity management, labeled and registered (Carhuapoma et al., 2018), transported in a refrigerant medium at a temperature 8-12 °C (technopor box with biological ice) to the Animal Health Laboratory (Microbiology

Area) of the National University of Huancavelica-Peru, for performing the bacteriological studies.

The inoculation (triplicate sample) was performed in threaded cap tubes enriched with brain heart infusion broth (BHI), making groups of 300 inocules without diarrhea and 300 inocules with diarrhea and incubated at 37 °C/24; enriched inoculs were sown independently by depletion in selective means of MacConkey Agar and Methylene Blue Eosin (EMB) for Escherichia coli and Salmonella-Shigella (SS), Xilosa lysine deoxycolate XHD for Salmonella spp. incubating it at 37 °C/ 48 h according to ISO 6579:2002.

For the optimal identification and differentiation of the strains of Salmonella spp. microscopic and macroscopic characterization of Escherichia coli (Gram coloration) were performed, such as: shape, consistency and elevation (Murcia, 2018). Suspicious colonies were inoculated in means of Triple Sugar Iron Agar (TSI), Lysine (LIA), Simmons Citrate (HS4), Sulfide-indole-Motility (SIM), Catalase and Voges-Proskauer and were incubated at 37 °C /24 hours. The results obtained were collated by the Manual of Systematic Bacteriology (Bergey's, 2008) and the Manual of Laboratory Procedures Zurita2013 for the final identification.

In relation to the isolation results, strains positive to Escherichia coli (300), Salmonella spp. (120) were selected from samples with diarrhea, and Escherichia coli (172), Salmonella spp. (72) from samples without diarrhea, from this pools of mother inoculum were prepared for Escherichia coli, Salmonella spp. and replicated to 320 independent inoculums for each study micron enriched in Brain Heart Infusion broth (BIH) and incubated at 37 °C /12 hours (Carhuapoma et al., 2018).

The antibiotic sensitivity was made using the Kirby Bauer Method, for this purpose strains of Escherichia coli, Salmonella spp. were cultured with a sterile swab homogeneously on Petri dishes with Agar Mueller Hilton, for a total of 320 strains per microorganism and distributed to 40 cultures by antibiotic as replications (observations of antibiotic sensitivity under study), in order to standardize the study, excluding following groups: with diarrheal enteropathies, Escherichia coli (n= 320 [40 replications/antibiotic]); Salmonella spp. (n= 320 [40 replications/antibiotic]); without diarrheal enteropathies, Escherichia coli (n= 320 [40 replications/antibiotic]); Salmonella spp. (n= 320 [40 replications/antibiotics]). The antimicrobial discs were placed independently infusing at 37 °C / 24 hours, most commonly used antibiotics were tested in the pharmaceutical-veterinary market (A-Gentamicin (30 μg), B-Novomycine (5 μg), C-Tetracycline (30 μg), D-Enrofloxacin (10 μg), E-Ampicillin (10 μg), F-Amikacin (30 μg), G- Ceftriaxone (30 μg) and H-Penicillin(10 μg)), subsequently, the growth inhibition halos were read and the results were interpreted in reference to the cut-off points proposed by the European Committee on Antimicrobial Susceptibility Testing manual (EUCAST, 2018).

The prevalence of microrganisms of Escherichia coli, Salmonella spp. present in alpaca calves with and without diarrhea was performed by comparing Means and Distribution Frequency (descriptive statistic) through a multiple cross-sectional descriptive level research. In order to determine antibiotic and micron-organism antibiotic sensitivity, the analysis of variance and the Tukey test was performed ($P<0.01$), using 8*2 multi-factorial design and the SPSS v. 20 program.

Table 1. Distribution percentage of Escherichia coli, Salmonella spp. and Escherichia coli-Salmonella spp. associates, isolated from samples with diarrhea (n= 300) and without diarrhea (n= 300) of alpacas calves.

Types of samples	Escherichia Coli				Salmonella spp.				E. coli-Salmonella spp.			
	P (+)	N (-)	% (+)	% (-)	P (+)	N (-)	% (+)	% (-)	P (+)	N (-)	% (+)	% (-)
With Diarrhea	300	0	100	0	120	180	40	60	60	0	20	0
Without diarrhoea	172	128	57	43	72	228	24	76	56	224	19	81

Legend: P (+) Positive, N (-) Negative.

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3 Results

Out of the 300 samples tested from alpaca calves with diarrhea, 100% (300/300) were positive to Escherichia coli, 40.0% (120/300) to Salmonella spp. and 20% (120/300) to Escherichia coli-Salmonella spp., with the highest percentage of the presence of Escherichia coli and Salmonella spp. Likewise, out of the 300 samples from alpaca calves without diarrhea, 57.0% (172/300) were positive to Escherichia coli, 24.0% (24/300) to Salmonella spp. and 19.0% (56/300) associated with E. coli-Salmonella spp. (Table 1), with lower prevalence of Escherichia coli and Salmonella spp. in samples of alpaca calves with diarrhea.

Pathogenic microorganisms of Escherichia coli and Salmonella spp. found in rectal samples of alpaca calves with diarrheal enteropathies demonstrated statistical differences (P<0.01) in halos diameters, inhibiting resistance against antibiotics Gentamicin, Novomycin, Tetracycline, Ampicillin and Penicillin; demonstrating antibiotic multibiotic resistance for both microorganisms, while antibiotic sensitivity in both microorganisms were seen in the antibiotics Enrofloxacin, Amikacin and Ceftriaxone, demonstrating diameters of halos inhibition within the sensitivity standards range (Table 2), in a total of 8 antibiotics evaluated.

Escherichia coli and Salmonella spp. isolated from samples of alpaca calf stools without diarrheal enteropathies had significant differences (p<0.01) of antibiotic resistance against Gentamicin, Tetracycline, Ampicillin, Penicillin and Novomycin, and were sensitive to Novomycin, Enrofloxacin, Amikacin and Cephaline (Table 3).

Table 2. Means and standard deviation of antibiotic resistance of Escherichia coli (n= 320) and Salmonella spp. (n= 320) from alpaca calves with diarrhea.

Antibiotic Types	N	Escherichia Coli Strains			N	Salmonella Sp strains.		
	320	S	I	R	320	S	I	R
A- GENTAMICIN	40	0.0	0.0	10.1 ± 0.4^{a}	40	0.0	16.2 ± 0.1^{a}	10.2 ± 0.3^{a}
B- NOVOMYCIN	40	0.0	0.0	11.1 ± 0.2^{b}	40	0.0	16.4 ± 0.5^{b}	11.2 ± 0.1^{b}
C- TETRACYCLINE	40	0.0	16.2 ± 0.2^{a}	$8.2\pm 0.1^{a,b}$	40	0.0	15.1 ± 0.2^{a}	$9.2\pm 0.3^{a,b}$
D- ENROFLOXACIN	40	$20.2\pm 0.3^{a,b}$	16.1 ± 0.6^{b}	0.0	40	19.3 ± 0.1^{b}	14.3 ± 0.1^{b}	0.0
E- AMPICILLIN	40	0.0	$14.1\pm 0.1^{a,c}$	10.4 ± 0.3^{c}	40	0.0	$16.2\pm 0.2^{a,c}$	9.3 ± 0.2^{c}
F- AMIKACIN	40	23.1 ± 0.4^{a}	0.0	0.0	40	19.1 ± 0.4^{a}	0.0	0.0
G- CEFTRIAXONE	40	21.3 ± 0.3^{a}	$15.2\pm 0.2^{a,c}$	0.0	40	23.3 ± 0.3^{a}	0.0	0.0
H- PENECILLIN	40	0.0	0.0	9.1 ± 0.4^{b}	40	0.0	0.0	11.1 ± 0.3^{a}

^{a}, ^{b}, ^{c} Different superscripts within columns indicate statistical difference to the Tukey test (p<0.01).
S Sensitive, I Intermediate, R Resistance.

4 Discussion

High prevalence rates of Escherichia coli (100%, 57), Salmonella spp. (40%, 19%) and Escherichia coli-Salmonella spp. (20%, 19%) in rectal samples of alpaca calves with and without diarrheal enteropathies may occur because alpaca producers may not be making the efficient and responsibly therapeutic use of the different veterinary antibacterials, and it is presumed that it could be related to the use of poor quality antibacterial products, making the pathological control difficult and increasing their incidence; this information agrees with the reported by Carhuapoma et al. (2018, 2019). In addition, the prevalence of these bacterial pathogenic microorganisms would be closely related to animals born with low immune levels and from mothers with cachetic conditions, which makes them very susceptible to Escherichia coli and Salmonella spp., being a pathology of clinical importance in the breeding of alpacas (Rosadio et al., 2012)

Lucas et al. (2016) identified Escherichia coli (8.0%), coronavirus (53.3%), rotavirus (36.6%), Salmonella spp. (18.3%) and associated bacteria and parasites 23.3%, viruses and bacteria 11.7% and triple associated 38.3% in alpaca calves with diarrhea. Morales et al. (2017), isolated Escherichia

coli 47.78% in alpacas with diarrhea and 58.33% without diarrhea, while Chuquizuta et al. (2017) detected Escherichia coli (40.84%), Salmonella spp. (39.27%) in dead guinea pigs and Carhuapoma et al. (2018) found the presence of Escherichia coli in 80% in alpaca calves with diarrhea. Carhuapoma et al. (2019) reported the presence of Escherichia coli and Salmonella spp. in 12% in males and females associated with Escherichia coli - Salmonella spp., 10% of a total of 104 calves with diarrhea; the results found in the study carried are of great importance in both microorganisms versus those reported.

Cebra et al. (2003), mentioned that salmonella in alpaca diarrhea is not a common cause, but Whitehead and Anderson (2006) detected various Salmonella species in animals with diarrhea; subsequently, Lucas et al. (2016) and Carhuapoma et al. (2019) show that the presence of salmonella in alpacas with diarrhea is very common in mixed herds (birds, pigs). This confirmation suggests that this bacterium is optional, making its cycle of epidemiological transmission very viable and its pathogenesis is common in domestic animals as well as in alpaca breeds.

Zambrano et al. (2013) identified Salmonella spp. in 23.5% and 32.4% samples of body surface and cloacal swab in broiler chicken. Salvatierra et al. (2015) identified Salmonella spp. 6.3 $\pm$ 2.4% in belly shells and 1.8% in sub-samples of head skin in swine, and Talavera Rojas et al. (2011) reported 1.34% of Salmonella group B (Typhimurium) in chicken liver samples for sale. The results found in the research are similar with some reported, but different with some others because there were more enteropathogens of Escherichia coli, Salmonella spp. and Escherichia coli-Salmonela sp in alpaca calves, thus the existence of Salmonella spp. and E. coli-Salmonela spp. would be due to causal agents of pathologies that cause intestinal dysfunction, generating the neonatal acute diarrheal in alpacas (More et al., 2011; Mancera Martínez et al., 2004; Ruiz et al., 2014), since Salmonella is an infection belonging to the enterobacteriaceae family (Tacchini A et al., 2010; Ríos, 2012) and there are very few studies of clinical cases of salmonellosis infestation in alpacas, even though this zoonotic pathology is of clinical importance in public health and it is presumed to be present in serovars Typhimurium and Enteritidis by biochemical, macromicroscopic, colonization and clinical manifestations observed in the study.

The antibiotic multi-resistance presented by Escherichia coli and Salmonella spp. against the antibacterials Gentamicin, Tetracycline, Ampicillin, Penicillin and Novomycin, show that the proven antibiotics possibly underwent modifications of its action mechanism such as: enzymatic inactivation of antibiotics, impermeability of the cell membrane or wall, expulsion by active mechanisms of the antibiotic and modification of the white site of the antibiotic in the bacteria, as reported in the literature, reducing the therapeutic options (Mancera Martínez et al., 2004; Schwarz et al., 2017; Ríos, 2012; Gatica Eguiguren and Rojas, 2018).

Table 3. Means and standard deviation of antibiotic resistance of Escherichia coli (n= 320) and Salmonella sp (n= 320) from alpaca calves without diarrhea.

Antibiotic Types	**N**	**Escherichia Coli**			**N**	**Salmonella spp.**		
	320	S	I	R	320	S	I	R
A- GENTAMICIN	40	0.0	16.2 ± 0.2^{b}	10.3 ± 0.1^{a}	40	0.0	17.2 ± 0.2^{a}	8.2 ± 0.1^{b}
B- NOVOMYCIN	40	22.1 ± 0.3^{b}	14.4 ± 0.5^{b}	0.0	40	0.0	14.4 ± 0.2^{b}	10.3 ± 0.3^{a}
C- TETRACICLINA	40	0.0	16.2 ± 0.2^{a}	$9.2 \pm 0.4^{a.b}$	40	0.0	16.2 ± 0.4^{a}	$8.2 \pm 0.4^{a.b}$
D- ENROFLOXACIN	40	$20.2 \pm 0.3^{a.b}$	15.2 ± 0.2^{b}	0.0	40	$20.1 \pm 0.2^{a.b}$	14.3 ± 0.2^{b}	0.0
E- AMPIICILLIN	40	0.0	$15.2 \pm 0.3^{a.c}$	11.2 ± 0.1^{c}	40	0.0	$17.1 \pm 0.4^{a.c}$	9.3 ± 0.3^{c}
F- AMIKACIN	40	22.1 ± 0.4^{a}	$14.2 \pm 0.3^{a.c}$	0.0	40	21.1 ± 0.4^{a}	0.0	0.0
G- CEFTRIAXONE	40	20.3 ± 0.3^{a}	$16.2 \pm 0.1^{a.c}$	0.0	40	22.3 ± 0.3^{a}	0.0	0.0
H- PENECILLIN	40	0.0	0.0	10.2 ± 0.4^{a}	40	0.0	0.0	10.1 ± 0.3^{a}

^{a}, ^{b}, ^{c} Different superscripts within columns indicate statistical difference to the Tukey test ($p<0.01$).
S Sensitive, I Intermediate, R Resistance.

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In addition, the high resistant of 8 antibiotics tested in the study would be linked to the poor application in the health management and the inappropriate use of antibiotics that have been practiced by producers in their herds, and by the irresponsibly massive distribution of drugs by national programs promoted by unskilled professionals, which makes the progressive trend of antibiotic resistance between humans and animals. Lucas et al. (2016), report that Salmonella spp. and Escherichia coli would possibly be resistant to first-line antibacterial such as Phosphomycin, Enrofloxacin, Ciprofloxacin, Gentamicin, Oxytetracycline, Penicillin, Ceftazidime and Trimethoprim-sulfamethoxazole because they are most commonly used indiscriminately by veterinarians from many years ago in cattle, chicken, sparrows and pigs; therefore, it would not be advisable to use antibiotics as first choice for the treatment of diarrhea in alpaca calves without carrying out susceptibility tests in laboratories (Pinto Jiménez et al., 2010; Siuce et al., 2015; Carhuapoma et al., 2019).

Barboza and Suarez (2012), found antibiotic resistance in 30% against Gentamicin, Norfloxacin and Tetracycline in Salmonella spp. isolated from cases of avian Tiphosis. Cordero Ruíz et al. (2002), reported multi-resistance in 84.4% to Tetracycline, Gentamicin, Ampicillin and Amikacin in gram-positive and negative bacteria of 11 proven antibiotics, and De la Fuente et al. (2015) found 91% resistance in strains of Salmonella spp. to Ampicillin, Nitrofurantoin and 55% to Cephalotin and Chloramphenicol; while Quesada et al. (2016) report multiresistance of Salmonella spp. to Nalidixic acid antibiotics, Streptomycin, Tetracycline, Chloramphenicol, Ampicillin, Trimethoprim/sulfamethoxazole, Gentamicin, Ciprofloxacin and Cephalosporin, these reports are similar to the results found in this study as they match almost all the antibiotics studied and these same resistance behaviors can be generated in hot-blooded animals (Castillo et al., 2014), for this reason, the antibiotic resistance of Salmonella spp. and Escherichia coli could be an epidemiological alarm for veterinarians (Rivera Calderón et al., 2012), caused by a lack of practice of conducting sensitivity tests in laboratories and by poor pharmacological knowledge capacity of veterinarians and operational technicians (Barboza and Suarez, 2012; Carhuapoma et al., 2018).

The antibiotic sensitivity demonstrated by the strains of Escherichia coli and Salmonella spp. isolated from rectal swab from alpaca calves with and without diarrhea versus the antibacterials of Enrofloxacin, Amikacin, Novomycin and Ceftriaxone, seem to be microbial that were not long-term used or were indiscriminate in herds (Carhuapoma et al., 2018), and may not have generated the modification of their mechanisms of antibiotic action, as well as the genes of DNA gyrase and topoisomesase IV (Romeu Álvarez et al., 2012; Lee et al., 2003; Ruiz et al., 2014; Schwarz et al., 2017). Additionally, Barrios-Arpi et al. (2016) found Escherichia coli sensitive to Trimethoxyprim (98%), Gentamicin (95%) and Phosphomycin (88%), Ciprofloxacin (85%), Ceftadizime (79%), and resistant to Nitrofurantoin (85%) from healthy and sick alpaca calves, results that differ from the ones obtained in this study with 4 antibacterial (Enrofloxacin, Amikacin, Novomiacin and Ceftriaxone) that proved to be sensitive and that would be specific for the therapeutic use of alpacas with and without diarrheal enteropathies.

5 Conclusions

The isolations of Salmonella spp. and Escherichia coli from alpaca calves with and without diarrhea were shown to be multi-resistance to the most common antibiotics in the pharmaceutical-veterinary market and only 4 antibiotics were sensitive, thus, comparative studies are required in the high Andean areas of Peru for the prevention and dissemination of antibacterial resistance.

Ethical commitments: The research team declares that strict management of animal welfare has been carried out for this research under the protocols established before and during the collection of samples for the study.

Confidentiality of data: The research team stated that a strict methodological management has been done to obtain adequate bases, as a statistical model and SPSS v. 20 program for data processing.

Reported consent: For the execution of the investigation no informed consent was required as it was a laboratory study based on protocols without the handling of animals, but they were taken into

account for greater reliability of the study.

Funding source: No internal or external institutional funding has been granted for the implementation of the study.

Conflicts of interest: The authors declare no conflict of interest.

Acknowledgments

The authors thank the executive administrator of the Animal Health Laboratory of the National University of Huancavelica and the alpaca breeders –Peru; for providing the collaboration and facilities for the execution of this study.

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