



Directora en Edición:
- Ignacia de los Ríos
MSc. Sheila Serrano Vicens

Frecuencia:
2 números / año
ISSN: 1390-3799
e-ISSN: 1390-8846
DOI
10.17763 / lgr



Granja

Q4

Agricultural and
Biological Sciences
(miscellaneous)
best quartile

SJR 2019
0.12

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UNIVERSIDAD NACIONAL DE HUANCAVELICA

FACULTAD DE CIENCIAS DE INGENIERIA ESCUELA PROFESIONAL DE ZOOTECNIA

TESIS: MODALIDAD ARTICULO CIENTIFICO-INDESADA

ANTIBIOTIC RESISTANCE OF SALMONELLA SPP, ESCHERICHIA
COLI ISOLATED FROM ALPACAS (*Vicugna pacus*) WITH AND
WITHOUT DIARRHEA

LÍNEA DE INVESTIGACIÓN
SALUD ANIMAL

PRESENTADO POR:

Bach. CARHUAPOMA DELACRUZ, Victor

PARA OPTAR EL TITULO PROFESIONAL DE:
INGENIERO ZOOTECNISTA

ASESOR

Dr. Nicasio Valencia Mamani

HUANCAVELICA- PERU
2020



Contacto

Prolg. Sebastián Barranca
s/n –Huancavelica

983968467

yachayruacc@hotmail.com





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ACTA DE SUSTENTACIÓN VIRTUAL DE ARTÍCULO CIENTÍFICO

En la ciudad de Huancavelica, a los veinticuatro días (24) del mes de noviembre del año 2020, a horas diez horas (10:00 a.m.), se reunieron los miembros del Jurado Calificador conformado por los docentes: **Mg. Melanio JURADO ESCOBAR (PRESIDENTE)**, **Dr. Nicasio VALENCIA MAMANI (SECRETARIO)**, **Ing. Paul Herber MAYHUA MENDOZA (VOCAL)**, designados con Resolución de Decano N° 047-2020-FCI-UNH, de fecha 26 de octubre del 2020, a fin de proceder con la sustentación y calificación virtual mediante el aplicativo MEET del artículo científico titulado: "ANTIBIOTIC RESISTANCE OF SALMONELLA SPP, ESCHERICHIA COLI ISOLATED FROM ALPACAS (Vicugna pacus) WITH AND WITHOUT DIARRHEA", presentado por el Bachiller CARHUAPOMA DE LA CRUZ, Víctor, para optar el **Título Profesional de Ingeniero Zootecnista**. Finalizada la sustentación virtual a horas 11:50 A.M., se comunicó al sustentante y al público que los Miembros del Jurado que abandonaran el aula virtual para deliberación por parte de los Jurados, se llegando al siguiente resultado:

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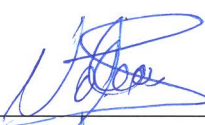
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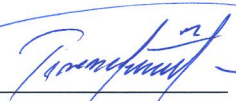
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ANTIBIOTIC RESISTANCE OF SALMONELLA SPP, ESCHERICHIA COLI ISOLATED FROM ALPACAS (*Vicugna pacus*) WITH AND WITHOUT DIARRHEA

RESISTENCIA ANTIBIÓTICA DE SALMONELLA SPP, ESCHERICHIA COLI AISLADAS DE ALPACAS (*Vicugna pacus*) CON Y SIN DIARREA

Victor Carhuapoma Dela Cruz^{*1} , Nicasio Valencia Mamani² , Teresa Huamán Gonzales² , Rufino Paucar Chanca² , Epifanio Hilario Lizana²  and Jorge L. Huere Peña³ 

¹ Scientific Multidisciplinary of Engineering Research Center, National University of Huancavelica. Huancavelica, Dos de Mayo, Acobamba 09380, Perú.

² EP Zootechnics, National University of Huancavelica. Huancavelica, Dos de Mayo, Acobamba 09380, Perú.

³ Environmental Engineering, National University of Huancavelica. Huancavelica, Dos de Mayo, Acobamba 09380, Perú.

*Corresponding author: yachayruacc@hotmail.com



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¹ Scientific Multidisciplinary of Engineering Research Center, National University of Huancavelica. Huancavelica, Dos de Mayo, Acobamba 09380, Perú.

² EP Zootecnica, National University of Huancavelica. Huancavelica, Dos de Mayo, Acobamba 09380, Perú.

³ Environmental Engineering, National University of Huancavelica. Huancavelica, Dos de Mayo, Acobamba 09380, Perú.

*Corresponding author: yachayruacc@hotmail.com

Article received on May 12th, 2019. Accepted, after revision, on January 27th, 2020. Published on March 3rd, 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 5 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 copas de *Escherichia coli* y *Salmonella* spp. fueron resistentes a Ampicilina (10,4 ± 0,3), (9,3 ± 0,2); Novomicina (11,2 ± 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 copas 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 ± 0.2), (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 pacos*) with and without diarrhea. La Granja: Revista de Ciencias de la Vida. Vol. 31(1):111-120. <http://doi.org/10.17183/lgr.v31.2020.08>.

Orcid IDs:

Victor Carhuapoma Dela Cruz: <https://orcid.org/0000-0002-4330-6899>

Nicolas Valencia Mamani: <https://orcid.org/0000-0002-9408-7770>

Teresa Huamán González: <https://orcid.org/0000-0003-2945-3874>

Rafael Paucar Chancas: <https://orcid.org/0000-0001-6820-6185>

Epifanio Lizana Hilario: <https://orcid.org/0000-0002-9909-2996>

Jorge L. Huere Poma: <https://orcid.org/0000-0002-3114-8134>

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; Sisco 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 (Silveira 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 Andean 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 (buffered) 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 inocules were sown independently by depletion in selective means of MacConkey Agar and Methylene Blue Eosin (EMB) for *Escherichia coli* and *Salmonella-Shigella* (SS), Xylose lysine desoxycolate 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 (LLA), 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 (BHI) 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 hales 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 microorganisms 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.

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 demonstra-

ted 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± 0.3 ^b	11.2± 0.1 ^b
C- TETRACYCLINE	40	0.0	16.2± 0.2 ^a	8.2± 0.1 ^{a,b}	40	0.0	15.1± 0.2 ^a	9.2± 0.3 ^{a,b}
D- ENROFLOXACIN	40	20.2± 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± 0.1 ^{a,c}	10.4± 0.3 ^b	40	0.0	16.2± 0.2 ^{a,c}	9.3± 0.2 ^a
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± 0.2 ^{a,c}	0.0	40	23.3± 0.3 ^a	0.0	0.0
H- PENICILLIN	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 ca- chetic 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*-*Salmonella* sp in alpaca calves, thus the existence of *Salmonella* spp. and *E. coli*-*Salmonella* spp. would be due to causal agents of pathologies that cause intestinal dysfunction, generating the neonatal acute diarrheal in alpacas (Mose 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 Egaiguren 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		<i>Escherichia Coli</i>		N		<i>Salmonella</i> 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 ± 0.3^b	0.0	40	0.0	14.4 ± 0.2^b	10.3 ± 0.3^b
C- TETRACYCLINE	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	$20.2 \pm 0.3^{a,b}$	13.2 ± 0.2^b	0.0	40	$20.1 \pm 0.2^{a,b}$	14.3 ± 0.2^b	0.0
E- AMPICILLIN	40	0.0	$13.2 \pm 0.3^{a,c}$	11.2 ± 0.1^a	40	0.0	$12.1 \pm 0.4^{a,c}$	9.3 ± 0.3^a
F- AMIKACIN	40	22.1 ± 0.4^b	$14.2 \pm 0.3^{a,c}$	0.0	40	21.1 ± 0.4^b	0.0	0.0
G- CEFTRIAZONE	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- PENICILLIN	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.

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; Succi 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 Typhosis. Cordero Ruiz 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 multi-resistance 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 topoisomerase IV (Romero 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 Trimethoxypirim (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, Novomycin 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.

References

- Barbeza, D. and Suarez, J. (2012). Sensibilidad a la fosfomicina ante los diferentes agentes patógenos aislados en animales domésticos en Venezuela. In *Memorias de la XVI Reunión Científico Técnica de la Asociación Argentina de Veterinarios de Laboratorios de Diagnóstico (AAVLD)*, Mar del Plata (Buenos Aires) Argentina. Buenos Aires (CF).
- Barrios-Arpi, M., Morales, S., and Villacaqui-Ayllon, E. (2016). Susceptibilidad antibiótica de cepas de *Escherichia coli* en crías de alpaca con y sin diarrea. *Revista de Investigaciones Veterinarias del Perú*, 27(2):381–387. Online: <https://bit.ly/3a8CiKa>.
- Bergey's (2008). *Manual of systematic bacteriology*, second edition volume three the firmicutes edition.
- Carhuapoma, D., Mayhua, M., Valencia, M., and Lizana, H. (2018). Antibacterial in vitro of effect urtica dioica and piper angustifolium in alpacas (*Vicugna pacos*) with diarrheal enteropathies. *MOJ Anat Physiol*, 5:160–162. Online: <https://bit.ly/2T4HLw1>.
- Carhuapoma, V., Valencia, N., Paucar, R., Mayhua, P. H., Lizana-Hilario, E., et al. (2019). Efecto de *Escherichia coli* y *Salmonella* spp en el crecimiento y mortalidad de crías de alpacas (*Vicugna pacos*). *Revista de Investigaciones Veterinarias del Perú*, 30(2):946–953. Online: <https://bit.ly/2l2EeZ2>.
- Castillo, G., Koga, Y., Alvarado, A., Tinoco, R., and Fernández, D. (2014). Aislamiento e identificación bioquímica de cepas de *Pasteurella multocida* y *Gallibacterium anatis* en aves de producción con signos respiratorios. *Revista de Investigaciones Veterinarias del Perú*, 25(4):516–522. Online: <https://bit.ly/2T4F4e0>.
- Cebra, C. K., Mattson, D. E., Baker, R. J., Sorn, R. J., and Dearing, P. L. (2003). Potential pathogens in feces from unwashed llamas and alpacas with diarrhea. *Journal of the American Veterinary Medical Association*, 223(12):1806–1808. Online: <https://bit.ly/2T65rKS>.
- Centeno, S., Salvatierra, G., and Calle, S. (2018). Detección de fenotipos de resistencia accut, blos y ampc en cepas de *Salmonella enterica* aisladas de infecciones en animales. *Revista de Investigaciones Veterinarias del Perú*, 29(2):580–587. Online: <https://bit.ly/2HZbW00>.
- Cordero Ruiz, D. M., García Pérez, A. L., Barral González, R. T., Jiménez Armada, J., and Rojas Hernández, N. (2002). Comportamiento de la infección nosocomial en las unidades de terapia en un período de 5 años. *Revista cubana de Higiene y Epidemiología*, 40(2):79–88. Online: <https://bit.ly/32w3B87>.
- De la Fuente, M., Castañeda, C., Cortes, V., and Espino, A. (2015). Análisis comparativo de la susceptibilidad de cepas de *Salmonella* sp aisladas de vegetales (*Lactuca sativa*) contra antibióticos, bacteriocinas de *Bacillus thuringiensis* y risina. In *XII Congreso Nacional de Ciencia y Tecnología de Alimentos*, pages 1–4, Salamanca España. Universidad Guanajuato- Facultad de Ciencias Biológicas.
- EUCAST (2018). Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST version 8.0. Technical report, European Committee on Antimicrobial Susceptibility Testing.
- Gatica Eguiguren, M. d. I. A. and Rojas, H. (2018). Gestión sanitaria y resistencia a los antimicrobianos en animales de producción. *Revista Peruana de Medicina Experimental y Salud Pública*, 35:118–125. Online: <https://bit.ly/2Vu4evw>.
- Lee, Y. J., Kim, K. S., Kwon, Y. K., and Tak, R. B. (2003). Biochemical characteristics and antimicrobials susceptibility of *Salmonella gallinarum*

- isolated in korea. *Journal of veterinary science*, 4(2):161–166. Online: <https://bit.ly/2FvHlay>.
- Lucas, J. R., Morales, S., Barrios, M., Rodríguez, J., Vázquez, M., Lira, B., Torres, B., Casas, E., and Espinoza, J. (2016). Patógenos involucrados en casos fatales de diarrea en crías de alpaca de la sierra central del Perú. *Revista de Investigaciones Veterinarias del Perú*, 27(1):169–175. Online: <https://bit.ly/2l00Gll>.
- Mancera Martínez, A., Corpus, O., de Lourdes, M., Tenorio Gutiérrez, V. R., and Vázquez Navarrete, J. (2004). Determinación de la existencia de plásmidos en aislamientos de salmonella enteritidis (fagotipos 4 y 8) y su análisis en la resistencia antimicrobiana. *Técnica Pecuaria en México*, 42(3):325–332. Online: <https://bit.ly/3caGXNH>.
- Medina, C., Morales, S., and Navarrete, M. (2017). Resistencia antibiótica de enterobacterias aisladas de monos (*Ateles*, *Callicebus* y *Leontideus*) en semi-captiverio en un centro de rescate, Perú. *Revista de Investigaciones Veterinarias del Perú*, 28(2):418–425. Online: <https://bit.ly/3a7L2jW>.
- Morales, S., Siu, E., Ramírez, P., and Navarro, A. (2017). Determinación de serotipos de escherichia coli aisladas de crías de alpacas (*Vicugna pacos*) con y sin diarrea en huancavelica. *REDVET. Revista Electrónica de Veterinaria*, 18(9):1–14. Online: <https://bit.ly/2T4MNbT>.
- Moro, J., Manchego, A., Sandoval, N., Ramírez, M., Pezo, D., Chio, K. L., and Rivera, H. (2011). Detección genómica y expresión de péptidos antimicrobianos (α -y β -defensinas) en mucosa intestinal de crías de alpaca (*Vicugna pacos*). *Revista de Investigaciones Veterinarias del Perú*, 22(4):324–335. Online: <https://bit.ly/385VOpB>.
- Murcia, M. (2018). Identificación de enterobacterias. Link.
- Oña, R. (2012). *Anatomía patológica de las diarreas infecciosas en crías de alpaca (Lama pacos) en la SAIS Aricoma Ltda*. 57. PhD thesis, Tesis de Médico Veterinario y Zootecnista: Facultad de Medicina Veterinaria, Univ. Nacional del Altiplano, Puno. Perú.
- Ortiz, S. (2011). *Evaluación de algunos métodos de control de la mortalidad en crías de alpaca (Lama pacos) en explotaciones familiares*. PhD thesis, Tesis de Médico Veterinario. Lima: Universidad Nacional Mayor de San Marcos.
- Pinto Jiménez, C. E., Martín Espada, C., and Cid Vázquez, M. D. (2010). Camélidos sudamericanos: estado sanitario de sus crías. *Revista Colombiana de Ciencias Veterinarias*, 4(1):37–50. Online: <https://bit.ly/2SQANdJ>.
- Quesada, A., Reginatto, G. A., Ruiz Español, A., Colantonio, L. D., and Burrone, M. S. (2016). Resistencia antimicrobiana de salmonella spp aislada de alimentos de origen animal para consumo humano. *Revista Peruana de Medicina Experimental y Salud Pública*, 33:32–44. Online: <https://bit.ly/2Tprnko>.
- Ramírez, A. (1990). Colibacilosis entérica en crías de alpacas. *Avances sobre investigación en salud animal camélidos sudamericanos*. LINMSM. Bol Div N°, 23:64.
- Ríos, W. (2012). *Determinación del perfil de resistencia antibiótica de Salmonella entérica aislada de cerdos fumados en un matadero de Lima Metropolitana*. PhD thesis, Tesis de Médico Veterinario. Lima: Universidad Nacional Mayor de San Marcos.
- Rivera Calderón, L. G., Delgado, P. A. M., Urbano, M. F. C., and Coy, F. A. C. (2012). Resistencia de la salmonela a los antimicrobianos convencionales para su tratamiento. *Revista CES Medicina Veterinaria y Zootecnia*, 7(1):116–129. Online: <https://bit.ly/2w0bw5V>.
- Romeu Álvarez, B., Salazar Jiménez, P., Lugo Moya, D., Rojas Hernández, N. M., and Eslava Campos, C. A. (2012). Susceptibilidad antimicrobiana de aislamientos de escherichia coli procedentes de ecosistemas dulceacuícolas. *Revista Cubana de Medicina Tropical*, 64(2):132–141. Online: <https://bit.ly/32iPKC>.
- Rosadio, A., Yaya, L., Véliz, A., Quispe, T., et al. (2012). Efecto protector de una vacuna polivalente anticlostridial sobre la mortalidad neonatal en alpacas. *Revista de Investigaciones Veterinarias del Perú*, 23(3):299–306. Online: <https://bit.ly/2Tt02of>.
- Ruiz, D., Suárez, M. C., and Uribe, C. (2014). Susceptibilidad antimicrobiana in vitro de cepas de

- salmonella spp. en granjas de ponedoras comerciales del departamento de antioquia. *Revista Colombiana de Ciencias Pecuarias*, 19(3):297–305. Online: <https://bit.ly/2T7yz91>.
- S., M. (2018). Identificación, serotipificación y resistencia de cepas de salmonella entérica aisladas de cuyes (cavia porcellus) clínicamente enfermos. *REDVET - Revista electrónica de Veterinaria*, 19(1).
- Salvatierra, G., Pinto, C., Inga, E., Siuce, J., and Calle, S. (2015). Detección de salmonella sp en carcasas porcinas en camales de lima, Perú. *Revista de Investigaciones Veterinarias del Perú*, 26(4):682–688. Online: <https://bit.ly/3ab3Q1L>.
- Schwarz, S., Loeffler, A., and Kadlec, K. (2017). Bacterial resistance to antimicrobial agents and its impact on veterinary and human medicine. *Advances in Veterinary Dermatology*, 8:95–110. Online: <https://bit.ly/3865Elc>.
- Silveira, E., Perales, R., Rodríguez, J., López, T., Cavidia, C., Agapito, J., and Palacios, C. (2012). Presencia de escherichia coli o157 en crías de alpacas (vicugna pacos). *Revista de Investigaciones Veterinarias del Perú*, 23(1):98–104. Online: <https://bit.ly/2TykQZl>.
- Siuce, J., Manchego, A., Sandoval, N., More, J., Chick, K.-L., Pezo, D., and Rivera, H. (2015). Expresión de defensinas en yeyuno de crías de alpacas (vicugna pacos) con enteropatías. *Revista de Investigaciones Veterinarias del Perú*, 26(2):317–327. Online: <https://bit.ly/2TlZaWn>.
- Tacchini A, M. D. M., Caraffini F, A., Montamat C, M. S., Spitale A, N., Bosio D, Y., and Minguez G, A. (2010). Empiema causado por salmonella typhimurium. *Revista chilena de enfermedades respiratorias*, 26(2):91–94. Online: <https://bit.ly/3ccG6xz>.
- Talavera Rojas, M., Reyes Rodríguez, N. E., Lagunas Berrubé, S., Fernández Rosas, P., Morales Erasto, V., and Soriano Vargas, E. (2011). Variabilidad genética de aislamientos de salmonella typhimurium (grupo b) obtenidos de hígados de pollo destinados para consumo humano. *Revista mexicana de ciencias pecuarias*, 2(4):Online: <https://bit.ly/3c6Y1Ei>.
- Whitehead, C. E. and Anderson, D. E. (2006). Neonatal diarrhea in llamas and alpacas. *Small Ruminant Research*, 61(2-3):207–215. Online: <https://bit.ly/3abW6g4>.
- Yagui, M. (2018). Resistencia antimicrobiana: nuevo enfoque y oportunidad. *Revista Peruana de Medicina Experimental y Salud Pública*, 35(1):7–8. Online: <https://bit.ly/3814xtb>.

