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Characterization of pneumonia in alpacas (*Vicugna pacos*) in high Andean communities of Huancavelica, Peru

Caracterización de Neumonías en alpacas (*Vicugna pacos*) en comunidades altoandinas de Huancavelica, Perú

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Abstract

Pneumonic pathologies are clinically important in alpaca breeding; however, there is little information about pneumonic types and bacterial causes. The objective is to characterize pneumonias and bacteria causing pneumonias in neonates of dead alpacas in high Andean communities of Huancavelica, Peru. We sampled 365 dead alpaca pups under 45 days of age, from 10 communities, 216 of them were taken tracheal swabs for microbiological studies with peptone buffer because they were cases of pneumonia. Macroscopic identification of pneumonias was done by photodocumentation test. *Pasteurella multocida, Mannheimia haemolytica* were grown on Colombia agar enriched with alpaca blood; *Streptococcus pneumoniae* on Brain Heart Infusion agar supplemented with alpaca blood and identified by morphological and microscopic characterization, biochemical reactions and optokine. It was found 59.8% of mortality due to pneumonia and 40.2% by other causes, characterizing pneumonia by inflammation 55.1%, 44.9% by lesion and subtypes: Exudative 9.5%, Suppurative 3.8%, Proliferative 42.3%, Lobar 17.8%, Interstitial 26.6%, isolating strains *Streptococcus pneumoniae* 43.7%, *Mannheimia haemolytica* 14.9%, *Pasteurella multocida* 20.9%, predominant associates *Streptococcus pneumoniae-Pasteurella multocida* 10.7%. High frequency of types and subtypes of pneumonia associated to Gram (+) bacteria were observed as causal of pneumonia in alpaca pups, with predominance in three high Andean

communities of Huancavelica, Peru.

Keywords: Alpaca, camelids, cleavage, fertilization, oocytes, reproduction.

Resumen

Las patologías neumónicas son de importancia clínica en crianza de alpacas; sin embargo, el conocimiento de tipos neumónicos y las causas bacterianas es muy limitado. El objetivo es caracterizar las neumonías y las bacterias causantes de neumonías en neonatos de alpacas muertas en comunidades altoandinas de Huancavelica, Perú. La muestra consistió en 365 crías muertas de alpacas menores de 45 días de edad, procedentes de 10 comunidades; se tomaron hisopados traqueales en 216 de ellas para estudios microbiológicos con buffer peptonada por resultar casos de neumonías. La identificación macroscópica de neumonías se hizo mediante el test foto documentador. Se cultivaron la *Pasteurella multocida* y *Mannheimia haemolytica* en agar Colombia enriquecidas con sangre de alpaca; *Streptococcus pneumoniae* en agar Infusión Cerebro Corazón suplementadas con sangre de alpaca e identificadas mediante caracterización morfológica, microscópica, reacciones bioquímicas y optoquina. Se encontraron 59,8% de mortalidad por neumonía y 40,2% por otras causas, caracterizándose neumonía por inflamación 55,1%; 44,9% por lesión y subtipos: Exudativa 9,5%, Supurativa 3,8%, Proliferativa 42,3%, Lobar 17,8%, Intersticial 26,6%, aislándose cepas de *Streptococcus pneumoniae* 43,7%, *Mannheimia haemolytica* 14,9%, *Pasteurella multocida* 20,9%, asociadas con *Streptococcus pneumoniae-Pasteurella multocida* 10,7%. Se evidenció una alta frecuencia de tipos, subtipos de neumonías asociados a bacterias Gram $(+)$ como causales de neumonías en crías de alpaca con predomino en tres comunidades altoandinas de Huancavelica, Perú.

Palabras clave: Alpaca, camélidos, clivaje, fertilización, ovocitos, reproducción.

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1 Introduction

Alpaca breeding is an important source of economic income for alpaca producers in Andean communities (Carpio, 1991), and alpaca fiber is an essential resource for the breeder, due to its high demand in the textile industry, as it is considered a special fiber (Morales et al., 2017). Likewise, the garments produced are considered luxury items in the international market (Rosadio et al., 2012), and its meat is known for its high protein content with low cholesterol (Cirilo et al., 2012; Carhuapoma De la Cruz et al., 2020); therefore, this activity is paramount for the economy of families in the high Andean zone of Peru (Carhuapoma De la Cruz et al., 2020).

Despite the goodness presented in this species, there are multiple factors that predispose it to various infectious respiratory diseases, such as sudden changes in extreme microclimates (Rosadio et al., 2011), transport, long grazing routes, overcrowding, inadequate management in productive activities (sanitary, reproductive, shearing), stress and immune deficiencies (Svensson et al., 2003; Carbonero et al., 2011). Among these pathologies that affect alpaca pups more frequently in the winter season (calving season), is pneumonia in its different clinical presentations that are not reported and are not scientifically validated in the etiological, symptomatologic and diagnostic perspective as well as its treatment and control (Cirilo et al., 2012; Guzmán et al., 2013), resulting in high rates of neonatal mortality in alpacas, causing economic and genetic losses (Cirilo et al., 2012; Morales et al., 2017), and in many cases breeders tend to abandon the activity because it results in low economic profitability (Sicha et al., 2020; Carhuapoma De la Cruz et al., 2020); the responsible pathogen being Pasteurellosis of clinical virulence (Zanabria et al., 2000; Cozens et al., 2019), but in alpacas this pathogen is not well studied scientifically as other domestic species (Rosadio et al., 2011; Ramírez et al., 2012), making its treatment and control very complex.

There are reports indicating that *Pasteurella multocida, Mannheimia haemolytica* and *Streptococcus pneumoniae* are usually agents responsible for pneumonic processes in domestic species, taking part of the oral tract, respiratory tract, and gastrointestinal system (Boukahil and Czuprynski, 2018), managing to colonize lungs and alveoli and affecting mainly immunocompromised animals (Cirilo et al., 2012), developing clinical signs of different pneumonic types and subtypes in cattle, sheep, swine, and poultry with high mortality rates (Carbonero et al., 2011).

Two sub-types of pneumonia were reported superficially in South American camelids, especially in alpacas, and *Pasteurella multocida* was observed as a bacterial agent involved in pneumonic processes (Rosadio et al., 2011; Cirilo et al., 2012; Rímac et al., 2017), and possibly as part of the nasal microbiota, as occurs in other domestic animals (Rosadio et al., 2012; Rímac et al., 2017). However, there are few studies on the characterization of pneumonic cases, types, subtypes and the identification of bacterial agents, causing high mortality in alpacas. The aim of this study is to characterize pneumonias and bacteria causing pneumonias in dead neonatal alpacas in high Andean communities of Huancavelica, Peru, to obtain more knowledge of pneumonias and their etiological agents, so that control and prevention strategies can be developed to minimize alpaca mortalities, improving the socioeconomic conditions of the breeder in the high Andean areas, since alpaca breeding is essential for their subsistence.

2 Materials y Methods

2.1 Study Area

The field studies were between January and March 2020 in the high Andean communities of Pastales Huando, Cachimayo, Sacsamarca, Astobamba, Pucapampa, Asociación Lachocc, Santa Barbara, Carhuancho, Choclococha and Matipaccana in Huancavelica-Peru, located between 4200 and 5200 masl, with annual average temperature of $5 - 8°C$. However, the first step to start the research was to obtain the communal authorization and the informed consent of the breeder. Bacteriological studies were carried out in the Animal Health laboratory, in the microbiology area of the Universidad Nacional Huancavelica-Peru.

2.2 Characterization of pneumonias

365 dead Huacaya alpaca pups were collected, who apparently died due to pneumonia with ages less than 45 days old and less than 12 hours *post mortem*,

without distinction of sex, excluding animals with pharmacological treatments.

An independent *in situ* necropsy was performed on the 364 dead alpaca pups, following the necropsy technique in domestic animals (Aldrete, 2002) under strict biosecurity measures. The characterization of pneumonia cases, pneumonic types and sub-types was performed by inspection and macroscopic visualization of lesions and anatopathological inflammatory processes of lung structure, considering alterations at the level of lobes, internal parenchyma, change of morphology (color and/or consistency, appearance and distribution of exudate), hemorrhagic foci through the use of the Photo Documentary Test of Pneumonic Pathologies (Pijoan et al., 1999), recording cards as appropriate: inflammatory reaction pneumonia (exudative, suppurative bronchopneumonia, fibrinous pneumonia, proliferative pneumonia) and lesion diffusion pneumonia (bronchopneumonia, lobar pneumonia, interstitial pneumonia, embolic pneumonia, granulomatous pneumonia), as shown in Figure 1: a, b and c.

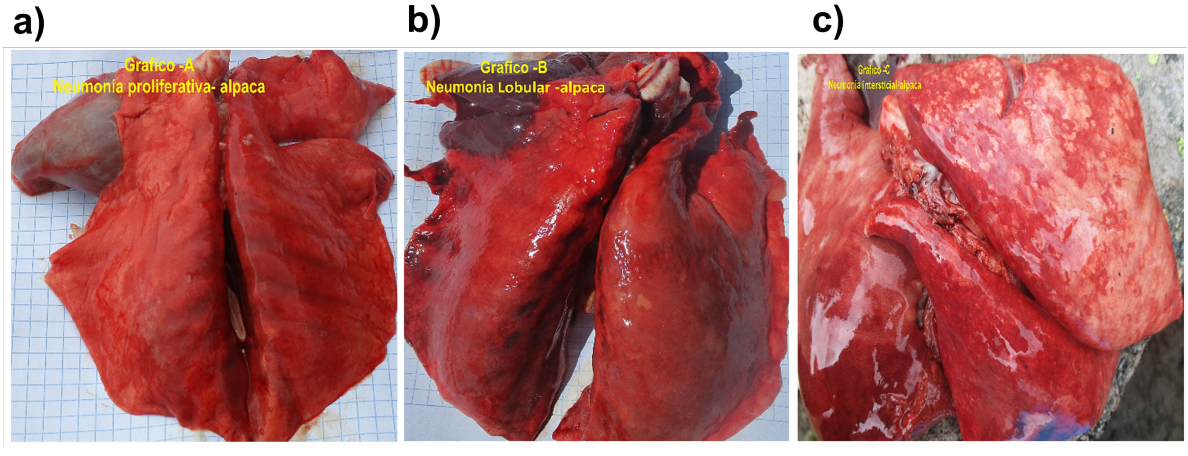

Figure 1. Characterization of pneumonia in alpaca pups. a) Proliferative pneumonia. b) Lobular pneumonia. c) Interstitial pneumonia.

2.3 Bacteriological studies

216 tracheal fluid samples were taken by tracheal swabbing (Schaefer et al., 2012) in sterile bottles with 5% peptonized buffer (10ml) from pneumonia-positive dead pups from 10 high Andean communities of Huancavelica-Peru (Figure 2: a, b and c, following biosafety measures. They were labeled and transported in a Vaccine Carrier Thermos at $8-10°C$ with biological ice (Gel Pack) to the Animal Health Laboratory: microbiology area-Universidad Nacional Huancavelica, for their microbiological study.

2.4 Isolation and bacterial identification

The 216 tracheal swab samples positive for pneumonia were inoculated independently in TSA broth (Tryptonga Soya Agar) and incubated at $37°C$ for 5 hours. Then, they were sown individually by exhaustion in Columbia Base Agar for *Pasteurella multocida, Mannheimia haemolytica* in Brain Heart Infusion Agar (BIH), both enriched with 5% sterile defibrinated alpaca blood (SAD), adding Gentamicin (0.75 μg/mL) as inhibitor of microorganisms and incubated at $37°C$ for 24 hours in aerobic processes (Avril et al., 1990; Moore et al., 1994). Brain Heart Infusion Agar (BIH) enriched with 5% sterile defibrinated alpaca blood (SAD) was used for the culture of *Streptococcus pneumoniae*, adding Gentamicin (0.75 μg/mL) and incubated at $37°C$ for 48 hours under anaerobic conditions in Gaspar Jar after adding Anaerocult® P (Moore et al., 1994).

Pasteurella multocida, Mannheimia haemolytica and *Streptococcus pneumoniae* strains were identified by

macroscopic (shape, color, border, elevation and consistency), microscopic (Gram staining), hemolytic (β hemolytic, α hemolytic, γ hemolytic), biochemical tests (TSI, LIA, SIMON, SIM), Catalase (Fegan et al., 1995) and optochin susceptibility test (Rosadio et al., 2012).

2.5 Statistical analysis

The research was descriptive with a prospective cross-sectional approach (Campbell Donald and Stanley Julian, 1995). The frequency and relationship of mortality cases in alpaca pups, types, subtypes of pneumonia and pneumonic bacteria, between the number of animals diagnosed positive against the total number of alpaca pups evaluated were determined by the Chi-square test ($p < 0{,}05$), using the statistical package SPSS Vers. 21.0 and expressing the results in percentage frequencies.

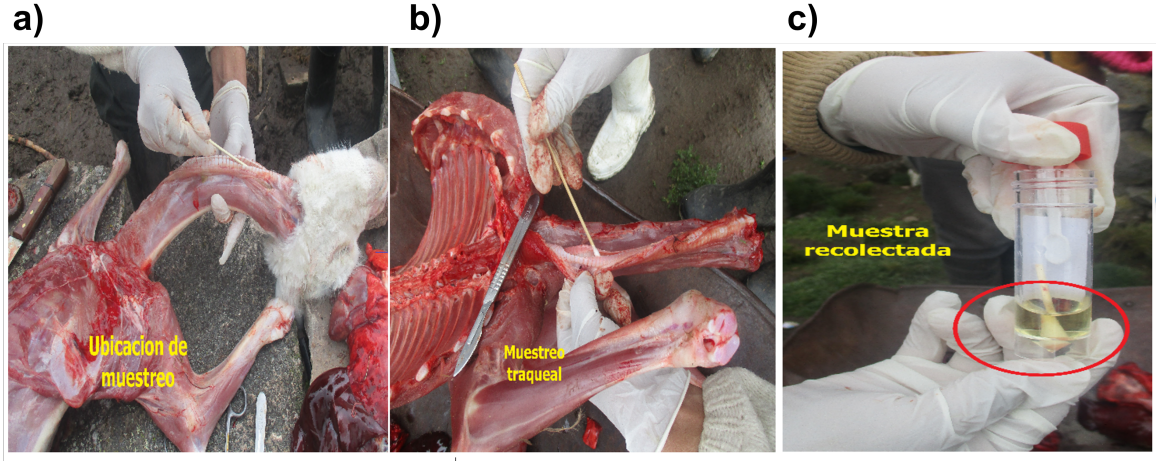

Figure 2. Sampling by tracheal swabbing in alpacas: a) Area of the collection. b) Tracheal sampling. c) Collected sample.

3 Results

A mortality rate of 59.8% was found for pneumonia among the 10 communities. Out of this total, 3 communities were found to have higher mortality due to pneumonia; Choclococha having 10.0%, Pucapampa 8.3% and Carhuancho 7.6% (Table 1), showing a relationship between the causes of mortality and the community ($p = ,000$) with a level of significance according to the Chi-square analysis ($p < 0{,}05$).

The most frequent type of pneumonia found was pneumonia due to inflammation (55.1%) and the least frequent was pneumonia due to lesion (44.9%), of which, the communities with higher cases of pneumonia due to inflammation and lesion were: Pastales de Huando (8.4%; 2.8%), Pucapampa (12.0%; 2.3%), Carhuancho (7.4%; 5.6%) and Choclococha (5.6%; 10.6%), while the rest of the communities resulted in lower proportion (Table 2), finding a relationship between types of pneumonia and community ($p = ,000$) according to Chi-square analysis ($p < 0{,}05$).

Table 1. Mortality frequency in alpaca (*Vicugna pacos*) pups by pneumonia in the high Andean communities of Huancavelica ($n = 365$).

COMMUNITY	Mortality by pneumonia		Mortality by other causes	
	F	%	F	%
Pastales de Huando	24	7.0	7	2.0
Pucapampa	31	8.2	5	1.4
As. Lachocc	21	6.1	17	4.5
Santa Bárbara	26	7.1	9	2.4
Sacsamarca	15	4.1	27	7.3
Astobamba	12	3.3	20	5.4
Cachimayo	14	3.7	20	5.4
Carhuancho	28	7.6	10	2.5
Choclococha	35	10.0	8	2.2
Matipaccana	10	2.7	26	7.1
TOTAL	216	59.8	149	40.2

Legend: F= Frequency of mortality;%= percentage of mortality.

Types of inflammatory pneumonia and subtypes by lesion were identified, being exudative 9.5%, suppurative 3.8%, proliferative 42.3% and lobar 17.8%, Interstitial 26.6%; evidenced in 3 communities (Pastales Huando, Carhuancho, Choclococha) with high rates of cases of 5 subtypes of pneumonia (Table 3), finding relationship between subtypes of pneumonia and community with significance level ($p = ,000$), according to Chi-square analysis ($p < 0{,}05$).

Table 2. Frequency of types of pneumonia in alpaca (*Vicugna pacos*) pups in high Andean communities of Huancavelica-Peru ($n = 216$).

COMMUNITIES	Sub-types of pneumonia									
	Inflammatory pneumonia						Pneumonia by lesion			
	Exudative		Suppurative		Proliferative		Lobar		Interstitial	
	F	%	F	%	F	%	F	%	F	%
Pastales de Huando	4	1.8	2	0.9	12	5.6	2	0.9	4	1.8
Pucapampa	2	0.9	3	1.4	21	9.7	1	0.5	4	1.8
A. Lachocc	1	0.5	1	0.5	6	2.7	3	1.4	10	4.6
Santa Bárbara	1	0.5	-	0	9	4.2	8	3.6	8	3.6
Sacsamarca	3	1.4	1	0.5	8	3.6	3	1.4	-	-
Astobamba	-	-	1	0.5	7	3.2	4	1.8	-	-
Cachimayo	-	-	-	-	6	2.7	8	3.6	-	-
Carhuancho	4	1.8	-	-	12	5.6	3	1.4	9	4.2
Choclococha	4	1.8	-	-	8	3.6	5	2.3	18	8.3
Matipaccana	-	-	-	-	3	1.4	2	0.9	5	2.3
TOTAL	19	9.5	8	3.8	92	4.3	39	17.8	58	26.6

Legend: F= Frequency of cases;%= percentage of cases.

From a total of 216 cultured samples, strains of *Streptococcus pneumoniae* 43.1%, *Mannheimia Hemolitica* 14.7%, *Pasteurella Multocida* 20.5% and *Streptococcus pneumoniae - Pasteurella Multocida* (10.4%) with double associated predominance were isolated; observing high prevalence of independent and associated strains in the communities of Pucapampa, Pastales Huando and Choclococha, and low prevalence in the rest of the communities (Table 4; Figure 3: a, b and c), finding the relationship between bacterial agents and community ($p = ,000$) according to Chi-square analysis ($p < 0{,}05$).

Table 3. Frequency of pneumonia subtypes in alpaca (*Vicugna pacos*) pups in high Andean communities of Huancavelica-Peru ($n = 216$).

COMMUNITY	Types of pneumonia			
	Pneumonia by inflammation		Pneumonia by lesion	
	F	%	F	%
Pastales de Huando	18	8.4	6	2.8
Pucapampa	26	12.0	5	2.3
Lachocc	8	3.7	13	6.0
Santa Bárbara	10	4.6	16	7.4
Sacsamarca	12	5.6	3	1.4
Astobamba	8	3.7	4	1.9
Cachimayo	6	2.7	8	3.7
Carhuancho	16	7.4	12	5.6
Choclococha	12	5.6	23	10.6
Matipaccana	3	1.4	7	3.2
TOTAL	119	55.1	97	44.9

Legend: F= mortality rate in alpaca pups,%= mortality percentage of alpaca pups.

4 Discussion

The study reports 59.8% mortality of alpaca pups due to pneumonia in 10 high Andean communities of Huancavelica -Peru, resulting in 3 communities with high rates of pneumonic cases, which may be due to constant changes in extreme microclimates (prolonged snowfall, hailstorms, constant rainfall), long walks for grazing, inadequate management in livestock activities (Mamani et al., 2009), and aspiration of amniotic fluid at the time of delivery, or milk aspiration by false swallowing (Mamani et al., 2009), resulting in factors that are determinant for pneumonia in domestic animals (Guzmán et al., 2013).

Pneumonias are clinically important in the production of alpaca; however, there are few studies. Thus, Mamani et al. (2009) reported 31.12% of pneumonias in alpacas; Manchego et al. (1998) found mortalities due to pneumonia in alpaca pups with cyclical and irregular trends in rainy seasons in January (9.5%), February (17.5%), March (2.2%); Álvarez (2011) reported 48.1% mortality in pups due to CRB. The results of the study are usually slightly higher than the previous ones; therefore, apparently breeders do not practice adequate sanitary management strategies in alpaca pups.

Pneumonia types and subtypes are typical in cattle, swine and guinea pigs (Gonçalves et al., 2001; Boukahil and Czuprynski, 2018); reporting types of pneumonia: Inflammatory 55.1%, lesion 44.9%; subtypes of pneumonia: exudative 9.5%, suppurative 3.8%, proliferative 42.3%, lobar 17.8% and Interstitial 26.6%, observing high trends in 3 communities; these figures differ from those reported by Guzmán et al. (2013) who found 11 cases of multilobar pneumonia associated with fibrinous pleuritis and pulmonary edema in tuis alpacas. Cirilo et al. (2012) found 3 cases of suppurative fibrinnecrotizing bronchopneumonia, 10 cases of suppurative bronchopneumonia in neonatal alpacas; in other species Ramírez (2015) reported 8% of interstitial pneumonia, 6.3% of chronic suppurative bronchopneumonias, 12.2% of mucopurulent bronchitis in cattle. On the other hand, Guerrero (2011) found 48.6% of interstitial pneumonia in guinea pigs with predominance in adult males.

The high cases of types and subtypes of pneumonias found in neonatal alpacas would be linked to extreme changes of microclimates in high Andean communities (Rosadio et al., 2011; Rímac et al., 2017), complete or partial failure of transfer and low levels of immunoglobulins in colostrum (Rímac, 2016; Lucas et al., 2016), favoring massive proliferation of pneumonic bacteria (*M. Hemolytica, P. Multocida* and *S. pneumoniae*) that secrete Lipo poly saccharide (LPS), extracellular leukotoxin (Lkt) mediators of proinflammatory cytokines, hemorrhagic procoagulants and tumor necrosis alpha (TNF-α) in lungs of domestic animals (Kumar et al., 2009; Highlander, 2001), originating inflammatory tissue lesions in the lungs of alpaca pulps, making phar-

macological treatment complex and in many cases unsuccessful (Gonçalves et al., 2001; Singh et al., 2011).

Table 4. Frequency of pneumonic bacterial agents present in alpaca (*Vicugna pacos*) pups in the communities of Huancavelica - Peru ($n = 216$).

COMMUNITY	Pneumonic bacterial agents											
	Streptococcus pneumoniae		***Mannheimia-Hemolytica***		***Pasteurella Multocida***		***Streptococcus Pneumoniae-Mannheimia Hemolytica***		***Streptococcus pneumoniae-Pasteurella Multocida***		***Mannheimia Hemolytica-Pasteurella Multocida***	
	F	%	F	%	F	%	F	%	F	%	F	%
Pastales Huando	14	6.3	0	-	7	3.2	0	-	3	1.3	0	-
Pucapampa	15	6.9	0	-	11	5.0	0	-	5	2.3	0	-
A. Lachocc	8	3.7	9	4.2	0	-	4	1.9	0	-	0	-
Santa Bárbara	12	5.5	10	4.6	0	-	4	1.9	0	-	0	-
Sacsamarca	4	1.9	3	1.3	3	1.3	2	0.9	2	0.9	1	0.5
Astobamba	3	1.3	5	2.3	0	-	4	1.9	0	-	0	-
Cachimayo	7	3.2	0	-	5	2.3	0	-	2	0.9	0	-
Carhuancho	11	5.0	5	2.3	4	1.9	3	1.3	2	0.9	3	1.3
Choclococha	16	7.4	0	-	12	5.5	0	-	7	2.8	0	-
Matipaccana	4	1.9	0	-	3	1.3	0	-	3	1.3	0	-
TOTAL	94	43.7	32	14.9	45	20.9	17	7.9	23	10.7	4	1.9

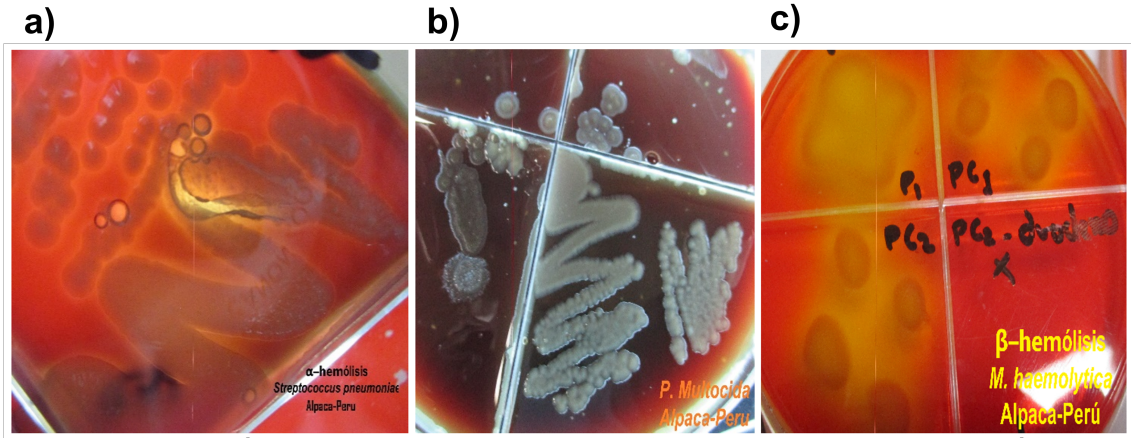

Figure 3. Isolated bacteria from alpaca pups with pneumonia (S-1, P-1 and M-1): a) Cepa S-1: α-haemolysis. b) Cepa P-1: *P. Multocida*. c) M-1: β-haemolysis.

The study reported *S. pneumoniae* 43.7%, *M. Hemolitica* 14.9%, *P. Multocida* 20.9%, double associates between 10.7% and 1.9%; these results differ from those of Rímac et al. (2017), who isolated 24 strains of *P. Multocida* from 46 lungs of neonatal alpacas aged 1-2 months with pneumonia. Similarly, Cirilo et al. (2012) in 22 samples isolated 11 positive cases of *P. Multocida*, 7 of *M. Hemolitica* in alpaca neonates with acute pneumonias, results that differ from Pijoan et al. (1999), who isolated *P. Multocida* in 34 cases, *Salmonella* spp. in 12 cases and *Staphylococcus* spp. in 7 cases in calves with pneumonia. Tocqueville et al. (2017) found *P. Multocida* with a load 10^5, 10^7 and 10^8 in pneumonic swine; Gamal et al. (2016) found 5 isolates of *P. Multocida* KMT1 in sheep with 94% homology to buffalo; regarding *S. pneumoniae*

we differ to all reported, resulting as the first case reported in alpacas whose virulence would be originated by genes PM 2.5, luxA-E,ST615 (Herbert et al., 2018; Chen et al., 2020; Panagiotou et al., 2020).

P. multocida, M. haemolytica and *S. pneumoniae* are part of the microbiota of oral tract, respiratory tract, gastrointestinal system in domestic and wild animals (Boukahil and Czuprynski, 2018), being the first two bacteria frequent in alpacas (Cirilo et al., 2012; Rímac et al., 2017). However, *S. pneumoniae* is frequent in humans and rodents (Sandgren et al., 2005), justifying the presence of *P. multocida, M. haemolytica* in the study and its virulence would be influenced by serovars 10, 11, 12 ,15 of toxA, tbpA, pfhA genes of low genetic diversity (Rímac, 2016; Rímac et al., 2017), and by the excessive and improper use of growth-promoting antimicrobials to antibacterial resistance possibilities (Carhuapoma et al., 2018; Carhuapoma De la Cruz et al., 2020), promoting colonization processes and cross-reactivity to subclinical infections in the animal (Díaz et al., 2017), leading to respiratory failures and progressive death in alpaca pups.

The study shows that literature to characterize pneumonic conditions and their etiology is necessary to understand the clinical significance and to achieve an effective and timely therapeutic intervention for a sustainable production of alpacas and other domestic animals.

5 Conclusions

High rates of pneumonia of different types and subtypes associated with pneumonic bacteria such as *Mannheimia Hemolitica, Pasteurella Multocida* and *Streptococcus pneumoniae* were observed in dead alpaca neonates. Similarly, it is observed that three high Andean communities present high trends of pneumonias associated with Gram $(+)$ bacteria.

Data confidentiality

The authors declare that they have followed a strict methodological approach to obtain the bases and adequate statistical model and program for data processing.

Right to privacy and informed consent

The authors declare that no patient or animal data appear in this article and that communal authorization and informed consent from the breeder were required.

Source of funding

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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