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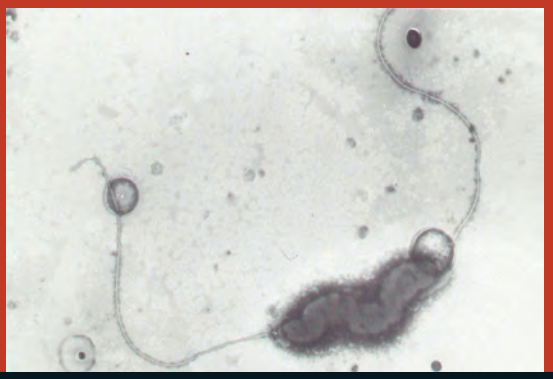

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Cover: Group of vicuñas (*Vicugna vicugna*) in the Parinacota region, included in the study by Norambuena *et al*. Image provided by Norambuena *et al*.

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Editorial

Methane production: Where should we focus our attention?

The UN recently called for reducing meat consumption due to its contribution to global warming. However, from my perspective as a researcher, the role of livestock greenhouse gas emissions is often exaggerated.

The annual reports of greenhouse gas (GHG) emissions from the EU[1] and the USA[2] reveal that the contribution of agriculture, particularly the livestock sector, is of little relevance compared to that of other industries. Thus, focusing attention and efforts to decrease emissions in those sectors would have a greater influence on GHG than the reduction of meat consumption.

The May 2019 EU report indicates that 4,330 tons of CO_2 equivalent were emitted in 2017, almost 80% of which were from the energy industry and only 10% (440ton) were from agriculture. Of those 440 tons, less than 40% corresponds to enteric fermentation methane. Furthermore, all GHG producing sectors have decreased their production of CO_2 equivalent since 1990, except for transportation and air conditioning, which together have increased by more than 260 tons in the same period. The equivalent CO2 production of cattle in Europe was 190 tons and has decreased by 50 tons in 27 years. Transport, on the other hand, produces 880 tons of CO2 equivalent (2017) and in the same 27 years has increased its production by 180 ton, which is roughly equivalent to emission from cattle in 2017.

The numbers are similar for the United States, however, GHG production has not decreased since 1990. Total GHG emissions in the USA in 2017 were 5700 tons of CO2 equivalent and, at 5400 tons of CO2 equivalent, the energy sector was the greatest contributor. Agriculture emitted 540 tons of CO2 equivalent, which represents 8.4% of the total production. Enteric fermentation by cattle was 175 tons. In contrast, from 1990-2017, fossil fuel combustion increased from 4740 tons to 4910 tons (170 tons), which is almost equivalent to all enteric fermentation. Although animal scientists should continue to search for ways to reduce GHG emissions, the facts prompt the question, where should we really focus our attention?

Editorial Committee
Austral Journal of Veterinary Sciences

[1] EU annual GHG inventory report May 2019.
[2] Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2017.

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ORIGINAL ARTICLE

Effect of test year, parity number and days in milk on somatic cell count in dairy cows of Los Ríos region in Chile

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ABSTRACT. In Chile, most dairy cattle are located in the southern region of the country, where the largest volume of milk is produced. This study aimed to quantify changes of somatic cell count (SCC) over 20 years in 11 herds of the Los Ríos region and to assess the effect of parity number, days in milk, milk yield, and milk fat and protein content on SCC. A database of approximately 277,709 observations based on 10,363 cows from 11 herds of the area was used. Data were gathered from 1998 to 2018. Outliers and inconsistent observations were eliminated. Multiple linear regression was used to model SCC on fixed variables such as test year, parity number, test season, and stage of lactation; covariables included in the model were milk yield, milk fat and protein yields, and milk urea concentration. There were statistically significant associations among SCC and all independent variables included in the model. Regarding parity number and stage of lactation, they increased along with SCC. SCC was higher in warmer months and, as SCC increased, the yield decreased. It was concluded that as test year increased, SCC decreased, indicating that the mammary gland health improved and the quality of the milk was better.

Key words: milk, test day, mastitis, microbiological quality of milk.

INTRODUCTION

Most of the milk produced in the southern regions of Chile is purchased by dairy companies and, since 1995, these companies have demanded higher quality standards to obtain uniform and top-quality dairy products, otherwise they would not be able to process such products (Carrillo and Vidal 2002). At farm level, to develop and keep plausible milk quality standards, it is necessary to use a comprehensive predictor of the mammary gland health status, as it is the case of somatic cell count (SCC) (McDougall *et al* 2009).

The cow mammary gland is a dynamic and complex organ composed of various cell types that act together aimed to milk synthesis and secretion, however, during bacterial or traumatic challenge, some of the endothelial cells divert their lactation function to protect the tissue from damage by initiating an inflammation process (Ryman *et al* 2015). Somatic cells (from the Greek "somatikós", meaning "from the body") contained in milk are mainly leucocytes and their movement towards the affected tissue, at the beginning of a mammary gland inflammation process, is regulated by endothelial cells (Ryman *et al* 2015).

In milk from a healthy mammary gland, leucocytes consist of several different cells, such as macrophages (60%), neutrophils (15%), and lymphocytes (25%) (Brito *et al* 1997). As well as leucocytes, milk somatic cells are also comprised by mammary gland endothelial cells; leucocytes move from the bloodstream toward the mammary gland as a response to a physical, chemical or infectious aggression (Ryman *et al* 2015). The inflammatory process resulting from bacteria or traumatic aggression attracts leucocytes, and when it is extremely intense there is an exaggerated presence of cells and other substances, also coming from the bloodstream, that move into the mammary gland and consequently into milk (Brito *et al* 1997).

For milk testing organizations, SCC recording is a standard procedure used to estimate mammary gland health status (Hernández and Bedolla 2008) because it provides information regarding the severity of the mammary tissue inflammation in each quarter, and milk samples can also come from the milk storage tank at the farm (Curbelo 2007).

Mastitis diagnosis based on SCC considers that a healthy mammary gland, producing normal milk, can contain up to 100,000 cell/mL, milk with 100,000 to 200,000 cell/mL is considered suspicious, while SCC over 200,000 cell/mL is an indication of subclinical mastitis presence and is considered abnormal milk (International Dairy Federation 1997, Hernández and Bedolla 2008). Several factors affect milk SCC, Hernández and Bedolla (2008) indicated that in cows that have never had a mammary gland infection their milk SCC varies from 20.000 to 50.000 cell/mL.

Higher environmental temperature and humidity are responsible for increasing bacteria content in manure; therefore, cows housed in dirty barns where the mammary gland is in direct contact with manure are more prone to acquire mastitis. Consequently, milk SCC is affected by the season, being lower in cold ($1.10x10^5$ cell/mL) and hot and dry weather ($1.11x10^5$ cell/mL) in contrast with climatic periods of high temperature and humidity ($2.14x10^5$ cell/mL) (Alvarado 2006).

A study conducted by Fox (2009), showed that initial milk SCC is low and subclinical mastitis and mammary gland infection severity is lower in heifers when compared

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to multiparous cows, and it also showed that SCC is low immediately after calving demonstrating a minimal level of subclinical mastitis incidence. According to Nyman *et al* (2009), cows showing clinical mastitis and high SCC during their first lactation are at higher risk of having both as recurrent problems in following lactations.

In countries where mastitis control programs are underway, results show that it is not impossible to keep SCC under control, for instance Germany, England and New Zealand reached an average SCC below 200,000 cell/mL (NMC 2013). Most of the Chilean milk is produced in the southern area of the country, with the most important dairy production regions being Araucanía, Los Lagos, and Los Ríos, where cow feeding is mainly based on pasture grazing and the production goal is switching from high yield per cow to high production of milk and milk solids per unit of land.

Chile does not have a SCC legal limit, however, dairy companies have adopted a range from 300,000 to 400,000 cell/mL as the maximum limit without economic penalty to dairy farmers. Consequently, dairy companies are demanding a milk quality in accordance with the requirements of the countries where some of the Chilean milk products are being exported (Butendieck 1997). According to Kruze (2000), from 1997 to 2000 the average SCC, in southern Chile, decreased from 460,000 to 330,000 cell/mL of milk. Agüero (2002) indicated that dairy farms that met sanitary standards and have in place official milk recording have made positive improvements, and their SCC average decreased from 471,000 cell/mL in 1994 to 256,000 cell/mL in 2001. Kruze (2005) indicated that Chilean SCC average was 311,000 cell/mL, while Werner (2014) reported a raw average SCC of 151,131 cell/mL of milk when analyzing 640,249 lactations from Malleco to Chiloé.

The objectives of this study were to quantify: a) SCC change across 20 years in dairy herds of Los Ríos region, b) SCC evolution across parity number, and c) SCC variation as lactation progresses.

MATERIAL AND METHODS

A database containing test day milk records of 11 herds from Los Ríos region, southern Chile was used which had 321,523 records gathered from 10,363 cows from 1998 to 2017. The records provided milk test day SCC and information on herd identification, days in milk, test day date, parity number, milk, fat and protein yield, and milk urea concentration. To remove outliers, the raw data was edited and records above and below three standard deviations from the mean were deleted. Records having SCC greater than 800,000 cell/mL of milk were deleted because it was assumed that those samples came from cows with clinical mastitis.

The analyses used linear regression to model milk SCC as a function of the fixed effects of parity number, herd, stage of lactation, test day season and year, and, as a covariate, milk, fat and protein yields and urea concentration. Stage of lactation had three levels; the first level included cows starting their lactation with increasing milk yield, from 6 to 100 days in milk; the second level included cows that reached their production peak and started to decline milk yield, from 101 to 200 days in milk; and the third level included cows reaching the end of lactation with decreasing milk yields, from 201 to 365 days in milk; test day records above 365 days in milk were not included in the analysis. Test day season also had three levels, the first season included the test day records made in April, May, June, and July (autumn, winter), second season test day records were made in August, September, October and November (spring), and the third level included records made in December, January, February, and March (summer). Parity number effect had six levels as cows having six or more lactations were grouped into a single level.

SCC is a variable that does not follow a normal distribution, to approximate normality, and fulfil the assumptions invocated in statistical hypothesis testing, it was transformed to Somatic Cell Score (SCS) as described by Ali and Shook (1980), SCS=Log2 (SCC/100)+3, where: Log2 = is the binary logarithm.

SCS least-square means for the year, season, parity number, and stage of lactation were estimated to quantify differences among them. The multiple regression model was:

$$y_{ijklmn} = \mu + Sea_i + H_j + Year_k + Par_l + SL_m + (b1)Milk_{ijklm} + (b_2)Prot_{ijklm} + (b_3)Fat_{ijklm} + (b_4)Urea_{ijklm} + e_{ijklmn}$$

Where:
y_{ijklmn} = is a SCS test day record
μ = intercept
Sea_i = fixed effect of the ith test day season (i =1, 2, 3)
H_j = fixed effect of the jth herd (j = 1, 2, ..., 11)
$Year_k$ = fixed effect of the kth test day year (k = 1, 2, …, 20)
Par_l = fixed effect of the lth parity number (l = 1, 2, ..., 6)
SL_m = fixed effect of the mth stage of lactation (m = 1, 2, 3)
b_1, b_2, b_3 y b_4 = regression coefficients of SCS test day on milk yield, and milk protein, fat and urea content, respectively.
e_{ijklmn} = random residual $\sim (0, \sigma_e^2)$

To conclude on the statistical significance of SCC trends across year and parity number, the estimated test day SCC least-square means were regressed on their respective levels.

Data editing and analyses were done using the Statistical Analysis System software (SAS 2000).

RESULTS

TEST YEAR

Table 1 shows the evolution of SCC from 1998 to 2017, the raw means ranged from 129,361 (1997) to 151,518 (2017) and presented rises and falls across the years.

Table 1. Number of observations (N), mean, standard deviation (SD), and minimum (Min) and maximum (Max) values for somatic cell count across years in dairy cattle of Los Ríos region, Chile.

Year	N	Mean	SD	Min	Max
1998	4,362	113,453	146,720	50,000	800,000
1999	4,545	130,304	159,276	63,000	800,000
2000	5,116	145,489	165,059	76,000	799,000
2001	5,747	133,480	156,200	71,000	800,000
2002	6,304	142,667	166,403	71,000	800,000
2003	7,340	113,648	145,612	51,000	799,000
2004	9,122	121,336	146,651	59,500	796,000
2005	10,772	128,047	150,516	67,000	800,000
2006	11,765	126,822	150,768	65,000	800,000
2007	13,463	137,712	152,831	77,000	800,000
2008	14,710	135,611	151,281	74,000	800,000
2009	15,654	132,257	151,396	71,000	800,000
2010	16,716	139,458	147,923	84,000	800,000
2011	17,946	123,618	140,566	67,000	800,000
2012	23,090	112,917	136,005	57,000	798,000
2013	25,014	116,050	137,238	60,000	800,000
2014	26,417	118,138	137,200	64,000	800,000
2015	25,398	128,949	141,401	76,000	800,000
2016	24,188	130,214	147,423	72,000	800,000
2017	10,040	138,532	150,918	81,000	799,000

All fixed effects and covariables included in the model were statistically significant and the R-squared of the model was 0.24.

Figure 1 shows the least square means (LSM) of SCC across years, the highest SCC were seen in 2002, 2000, and 2001 while the lowest were observed in 2014 and 2016. Although the trend presented rises and falls there is a clear tendency to decrease SCC across years. The SCC LSM of the last observational years (2016 and 2017) are significantly lower than in previous years. Considering LSMs from the year 2002, when the highest LSM was estimated, they diminished from 111,228 to 70,360 cell/mL (figure 1). Across all study period, the average LSM was 90,794 cell/mL of milk. The estimated single lineal regression coefficient indicated a decreasing trend of 1,633 cell/mL per each study year.

PARITY NUMBER

Figure 2 shows LSM for SCC across parity number. There is a clear and significant trend toward an increment of SCC as parity number progresses. Parity LSM were, among them, all statistically different and ranged from 55,271 to 129,451 cell/mL, from first to sixth parity, respectively.

The estimated single lineal regression coefficient of LSMs on their parity number reveals that, at each increment of parity, the SCC increases by 15,605 cell/mL of milk.

STAGE OF LACTATION

As previously stated, days in milk were edited into three categories: 6 to 100, 101 to 200, and 201 to 365 days in milk for lactation stages I, II, and III, respectively. Table 2 shows LSM for SCS and SCC for all three lactation stages. LSMs were statistically different and as the stage of lactation progressed the number of somatic cells in milk increased, the increment in SCC from first to the third stage of lactation was 13% (table 2).

TEST DAY SEASON

There were significant differences among all three test day seasons, the third season, corresponding to test day observations made in the summer, had the highest SCC. On the contrary, test day observations gathered toward the end of fall to mid-winter time (first test day season) showed the lowest SCC. Least square means for first, second and third test day season were 81.4±12.5, 89.3±12.5 and 92±12.5 cells/mL, respectively.

MILK YIELD

The regression coefficient of SCS on milk yield was –0.049 ($P<0.05$), which indicates that as milk yield increased SCS decreased. Regression coefficients of

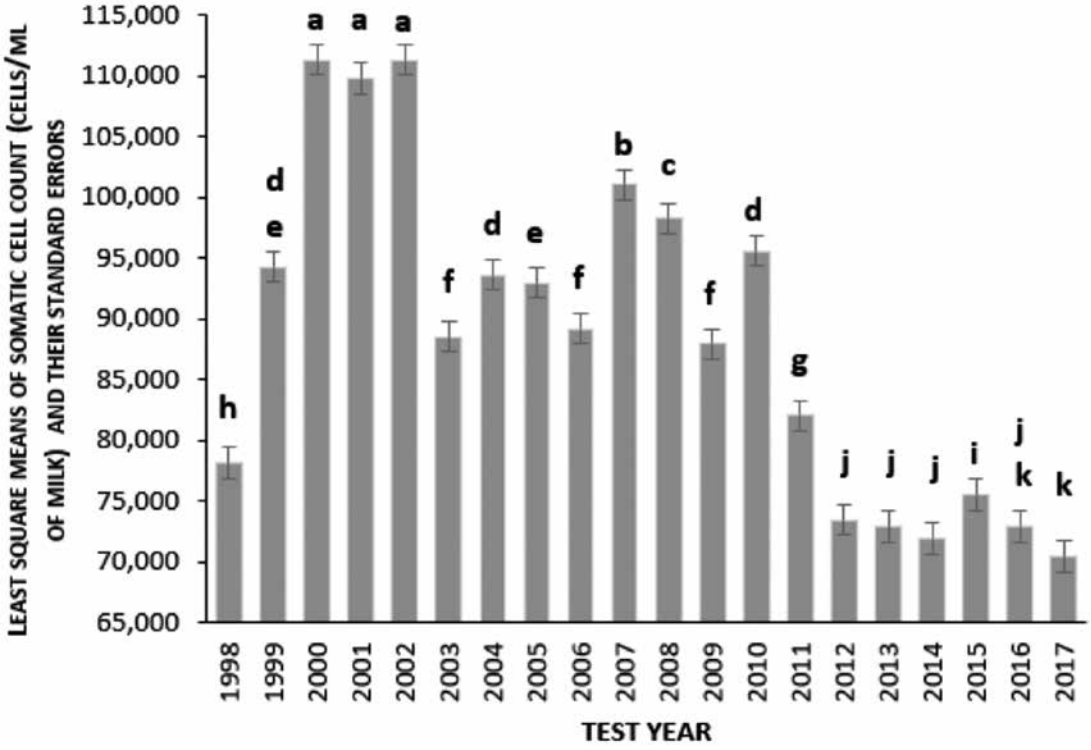

Figure 1. Least square means, and their standard errors, for somatic cell count versus test year. Means with the same letter are not significantly different (P>0.05).

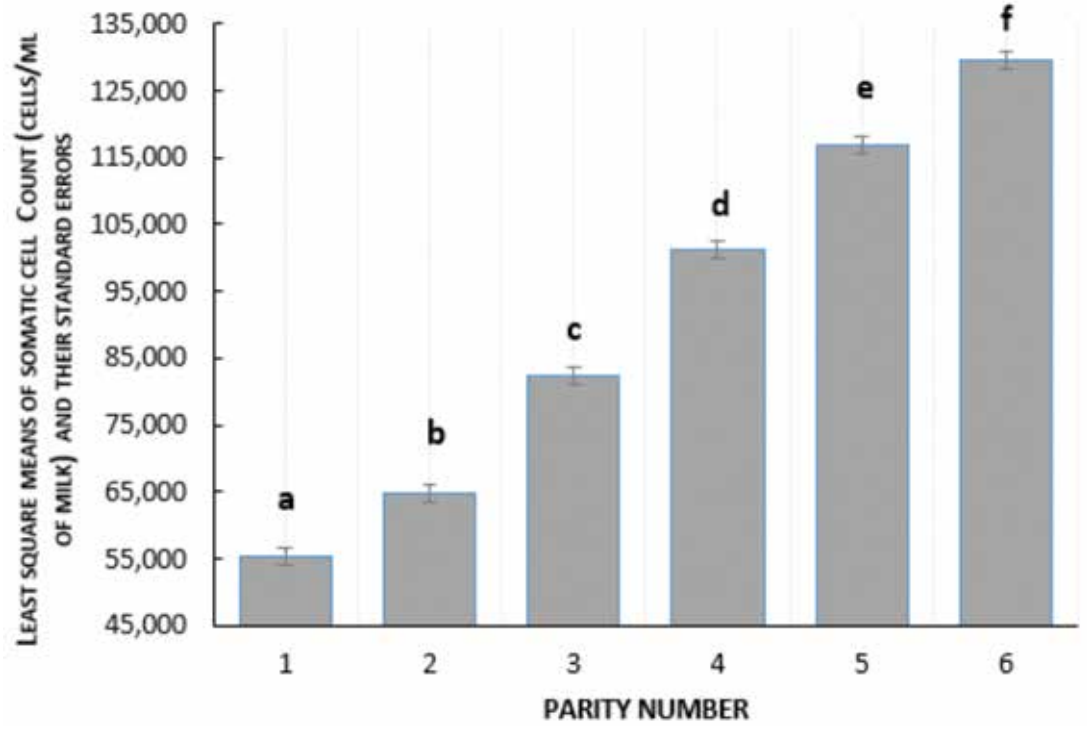

Figure 2. Least square means, and their standard errors, of somatic cell count versus parity number. Means with the same letter are not significantly different (P>0.05).

Table 2. Least square means (LSM) and standard error (SE) of somatic cell score (SCS) and somatic cell count (SCC) for lactation stage.

Lactation Stage	SCS	SE	SCC	SE
I[1]	12.551	0.007	75,013[a]	12.56
II[2]	12.767	0.006	87,128[b]	12.55
III[3]	13.003	0.006	102,613[c]	12.55

Rows with different superscript letter are statistically different ($P<0.05$).
[1] = 6 to 100 days. Before reaching lactation peak.
[2] = 101 to 200 days. Declining milk yield period.
[3] = 201 to 365 days. Period of lower milk yield.

SCS on milk fat and protein content were both positive and significant indicating that as fat and protein content increase SCS decreases.

DISCUSSION

The SCC trend showed in figure 1 significantly decreased across years, this result can be explained because SCC is a key component, national and internationally, to assess milk quality, mammary gland health and prevalence of clinical and subclinical mastitis. Also, implementation in Chile by dairy industries of milk payment schemes towards the end of 1995, where SCC was incorporated as an important variable of the final price paid to the farmer has led to raw milk reaching higher quality standards. Dairy industries offered attractive premium payment for superior quality raw milk and strong economic penalties for poor sanitary quality milk (Kruze 2003).

Other studies have also concluded that SCC has diminished across years after consistent actions in several aspects were taken, such as legislation, raw milk quality payment, education/training (Agüero 2002, Lukas *et al* 2005, Pinedo and Meléndez 2010). Statistics of some southern Chile dairy industries showed that average SCC diminished from 460,000 to 330,000 cell/mL from 1997 to 2000 (Kruze 2000). In the present study, the lowest SCC were recorded in the last 6 years (figure 1), this can be a consequence of the raw milk payment schemes imposed by diary industries in Chile where heavy economic penalties were given to milk that is below the minimum quality standards. Werner (2014), from a database of 640,249 lactations from 659 farms in southern Chile, from 2001 to 2011, reported that average SCC was 161,131 cell/mL of milk, this is similar to what is reported in this study for 2017 where the raw SCC average was 151,518 cell/mL (table 1). This study used test day records up to 2017 while the study of Werner (2014) included lactations records only up to 2011, other studies using Chilean dairy records (Pinedo and Meléndez 2010) are even older.

Ponce (2009) and Romero *et al* (2018) suggested that in order to improve milk quality and continuing lowering SCC, more consistent farmer training and use of information technology is needed and they must reach the dairy sector, mainly small-scale producers. Also, the infrastructure of dairy companies must improve and new ways to industrialize dairy products, that are consumer safe, must be developed.

In this study, including 11 herds and across 20 years, the recorded SCC was always below European Union quality parameters that stand below 400,000 cell/mL of milk. However, in the next years, this international parameter is expected to diminish because in Australia and Scandinavian countries the national SCC average varied from 200,000 to 250,000 cell/mL (More 2009). Also, SCC above 200,000 cell/mL is an indication of subclinical mastitis and consequently lower milk yield and quality and economic losses (Barbosa *et al* 2002, Coldebella *et al* 2004, Magalhães *et al* 2006). Philpot (1999) indicated that, above 200,000 cell/mL, for each 100,000 cell/mL of increment of SCC milk yield decreases by 2.5%.

Results shown in figure 2 indicate that SCC increases as parity number does, which is similar to that reported by Tineo and Andía (2017)[1], who studied the association between mastitis and parity number and found that higher SCCs were for cows in their third and above lactations. Also, Chacón *et al* (2006) concluded the same, stating that as parity numbers increase so does SCC. García (2004)[2] indicates that the association between parity number and SCC can be explained, from a productive point of view, because as productive life occurs there is an increment in mastitis incidence that in turns increases SCC, resulting in yield losses associated to both mastitis and SCC. In this study, the lowest SCC was observed in first lactation cows, parity number and age are highly correlated variables, therefore, first lactation cows are still growing and developing their mammary system, consequently have lower milk yield and SCC (Parra-Bracamonte *et al* 2005). Kirk (1984) indicates that older cows tend to have higher SCC, and age is not the only responsible factor, the prevalence of chronic udder infections is related to age and therefore the main cause of higher SCC.

Chacón *et al* (2006) and Castillo *et al* (2017) indicated that cows in first and second lactation have SCC below 200,000 cell/mL, however, there is an increment of SCC from the third lactation, which matches productive peak yield. Ahmadzadeh *et al* (2009) reported similar results than those presented in this study suggesting that, as parity number increases, there is greater exposure to pathogens

[1] Tineo J, Andía V. 2017. Mastitis bovina por recuento de células somáticas con PortaSCC® y Test de California en el fundo de Allpachaca. *Revista Electrónica de Veterinaria* 18, 1-13. https://www.redalyc.org/articulo.oa?id=63652580009. Accessed February 2019.

[2] García AD. 2004. Células somáticas y alto recuento bacteriano ¿cómo controlarlos? http://openprairie.sdstate.edu/extension_extra/537. Accessed May 2019.

due to the interaction among healthy and diseased cows, as herd life progresses.

SCC increased with days in milk (table 2) similar to that reported by Alvarado (2006) who observed that SCC was low before 100 days in milk, this coincides with lactation Stage I of this study and, as days in milk progressed SCC increased, same as reported in this work. Stage III of lactation had the highest SCC similar to the results of Butendieck (1997), who reported higher SCC toward the end of the lactation, however, the same author also found high SCC at the very beginning of the lactation, and explaining this fact as a biological immunological preparation of the cow for calving.

The increase of SCC toward the end of the lactation is associated to a greater pathogen exposure due to a more frequent interaction among healthy and diseased cows, in addition, as days in milk progress, udder manipulation and opening of the teat duct may facilitate bacteria entrance and multiplication that leads to higher SCC (Ruegg and Pantoja 2013).

Results of this study show that the time of the year, when a test day record is gathered, has a significant effect on SCC. The coldest season recorded lower SCC, and this is in agreement with Correa-Calderón *et al* (2002), Alvarado (2006) and La Manna *et al* (2014) who found that cows exposed to low environmental temperature and/or ventilation had lower SCC and higher milk yields as compared to cows exposed to high environmental temperature and/or humidity. The increment of SCC observed in the summer months can be explained by the fact that environmental temperature and humidity are ideal conditions for bacterial growth and these, added to cow heat stress, may end up in cows suffering subclinical mastitis.

Milk yield as a covariable for SCS was statistically significant, the association was negative and, per each additional kg of milk the SCS decreased in 0.049. Similar conclusion was reached by Werner (2014) and Pinedo and Meléndez (2010) who found a negative association between SCS and milk yield also, Hagnestam et al (2007) described that cows, in their first lactation, with higher RCS had up to 9% lower milk yield as compared to low RCS cows. These results agree with the fact that if SCC increases, it is mainly due to an udder injury, a possible subclinical mastitis, which immediately decreases milk yield. Another reason for this negative association is due to that after lactation peak, milk yield declines and somatic cell production can remain constant then a larger SCC concentration can be detected toward the end of the lactation. Sneddon *et al* (2015) estimated genetic parameters for milk components in New Zealand dairy cows and reported an estimated genetic correlation, between milk yield and SCS, equal to cero (–0.16±0.15).

The association described in this study between milk solids (fat and protein) and SCS reveals that as fat and/or protein content increases SCS also does, regression coefficients of SCS on fat and protein percentage were 0.067±0.004 and 0.419±0.010, respectively. Werner (2014) researched the association between SCS and milk fat and protein yield and concluded that they were not significantly associated. However, at the genetic level, Sneddon *et al* (2015) reported a genetic correlation of 0.22±0.14 between SCS and milk fat percentage.

It is concluded that there is a significant and positive effect of test year on SCC; as years went by the SCC decreased, consequently raw milk quality in Los Ríos region, as a result of hygienic and sanitary norms being met, improved in the last 20 years. Older cows are prone to higher SCC as well as cows in their late days in milk. Test day records gathered in summer months are likely to have higher SCC.

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REFERENCES

Agüero H. 2002. Calidad de leche en la X Región. In: Agüero H (ed). *Seminario Internacional Avances en Control de Mastitis y Mejoramiento de la Calidad de Leche*. Universidad de Chile y Cooprinsem, Santiago, Chile.

Ahmadzadeh A, Frago F, Shafii B, Dalton DC, Price WJ, *et al*. 2009. Effect of clinical mastitis and other diseases on reproductive performance of Holstein cows. *Anim Reprod Sci* 112, 273-282.

Ali AKA, Shook GE. 1980. An optimum transformation for somatic cell concentration in milk. *J Dairy Sci* 63, 487-490.

Alvarado D. 2006. Algunas variables climatológicas y ambientales y su relación con el recuento de células somáticas en la leche de estanques prediales en la provincia de Bío Bío, Chile. *Tesis*, Universidad de Concepción, Chillán, Chile.

Barbosa CP, Benedetti E, Ribeiro SCA, Guimaraes EC. 2002. Relação entre contagem de células somáticas (CCS. e os resultados do "California Mastitis Test"(CMT), no diagnóstico de mastite bovina. *Bioscience Journal* 18, 93-102.

Brito JFF, Caldeira GAV, Verneque RS, Brito MAVP. 1997. Sensibilidade e especificidade do "California Mastitis Test" como recurso diagnóstico da mastite subclínica em relação à contagem de células somáticas. *Pesquisa Veterinária Brasileira* 17, 49-53.

Butendieck N. 1997. Células somáticas, mastitis y calidad de leche. *In*: Calidad de leche e interpretación de resultados de laboratorio. Curso - Taller. *Serie Carillanca Nº 62*. INIA, CRI Carillanca, Temuco, Chile, Pp 15-32.

Carrillo B, Vidal C. 2002. Los esquemas de pago de leche en Chile. Centro de Economía Rural de Frutillar (CER) y Fundación Chile, Puerto Varas, Chile, Pp 1-14.

Castillo-Umaña AM, Alpizar-Naranjo A, Padilla-Fallas J, Keim J. 2017. Efecto de la edad a primer servicio, número y época de parto sobre el comportamiento de la curva de lactancia en vacas jersey. *Nutrición Animal Tropical* 11, 1-22.

Chacón A, Jiménez R, Vargas RC. 2006. Incidencia en el conteo de células somáticas de un sellador de barrera (yodo-povidona 0,26%) y un sellador convencional (yoduro 0,44%). *Agronomía Mesoamericana* 17, 207-212.

Coldebella A, Machado PF, Demétrio CGB, Ribeiro-Júnior PJ, Meyer PM, *et al.* 2004. Contagem de células somáticas e produção de leite em vacas holandesas confinadas. *Revista Brasileira de Zootecnia* 33, 623-634.

Correa-Calderón A, Avendaño-Reyes L, Rubio-Villanueva A, Dennis V, Armstrong J, *et al.* 2002. Effect of a cooling system on productivity of Holstein cows under heat stress. *Agrociencia* 36, 531-539.

Costa HN, Molina LR, Lage CFA, Malacco VMR, Facury Filho EJ, *et al*. 2017. Estimativa das perdas de produção leiteira em vacas mestiças Holandês x Zebu com mastite subclínica baseada em duas metodologias de análise. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 69, 579-586.

Curbelo R. 2007. Relación entre los recuentos de células somáticas, prácticas de manejo y patógenos causantes de mastitis en rebaños lecheros de Puerto Rico. *Tesis*, Universidad de Puerto Rico, Puerto Rico.

Fox L. 2009. Prevalence, incidence and risk factors of heifer mastitis. *Vet Microbiol* 134, 82-88.

Hagnestam C, Emanuelson U, Berglund B. 2007. Yield losses associated with clinical mastitis occurring in different weeks of lactation. *J Dairy Sci.* 90, 2260-2270.

Hernández R, Bedolla C. 2008. Importancia del recuento de células somáticas en la calidad de la leche. *Revista Electrónica de Veterinaria* 9, 1-34.

International Dairy Federation. 1997. Recommendations for presenting of mastitis related data. *IDF Bulletin 321*, Brussels, Belgium, Pp 7-25.

Kirk JH. 1984. Programmable calculator program for linear somatic cell scores to estimate mastitis yield losses. *J Dairy Sci* 67, 441-443.

Kruze J. 2000. Milk quality in Chile: Progress towards reducing SCC and TBC in raw milk during the last twenty years. *Pacific Congress on Milk Quality and Mastitis Control*, 13-16 November, Nagano, Japan., Pp 113-118.

Kruze J. 2003. Evolución de la calidad higiénica de la leche cruda en los últimos 10 Años. *III Seminario: Calidad de leche*, Osorno, Chile, Pp 11-17.

Kruze J. 2005. Milk production and mastitis control in emerging dairy countries: The experience in Chile. In: Hogeveen H (ed). *Mastitis in dairy production*. Wageningen Academic Publishers, Wageningen, The Netherlands. Pp 123-128.

La Manna A, Román L, Bravo R, Aguilar I. 2014. Estrés térmico en vacas lecheras: con sombra y bienestar las vacas producen más. *Revista INIA* 39, 34-39.

Lukas JM, Hawkins DM, Kinsel ML, Reneau JK. 2005. Bulk tank somatic cell counts analysed by statistical process control tools to identify and monitor subclinical mastitis incidence. *J Dairy Sci* 88, 3944-3952.

Magalhães HR, El Faro L, Cardoso VL, Paz CCP, Cassoli LD, *et al*. 2006. Influência de fatores de ambiente sobre a contagem de células somáticas e sua relação com perdas na produção de leite de vacas da raça Holandesa. *Revista Brasileira de Zootecnia* 35, 415-421.

McDougall S, Parker KI, Heuer C, Compton CWR. 2009. A review of prevention and control of heifer mastitis via non-antibiotic strategies. *Veterinary Microbiology* 134, 177-185.

More SJ. 2009. Global trends in milk quality: implications for the Irish dairy industry. *Irish Vet J* 62, 5-14.

National Mastitis Council. 2013. *52nd Annual Meeting*. NMC, San Diego, California.

Nyman AK, Emanuelson U, Gustafsson AH, Persson KW. 2009. Management practices associated with udder health of first-parity dairy cows in early lactation. *Prev Vet Med* 88, 138-149.

Parra-Bracamonte G, Magaña J, Delgado R, Osorio-Arce M, Segura-Correa J. 2005. Genetic and non-genetic effects on productive and reproductive traits of cows in dual- purpose herds in south eastern Mexico. *Genet Mol Res* 4, 482-490.

Philpot N. 1999. Aumento de la rentabilidad mediante el mejoramiento de la calidad de leche y la reducción de la mastitis. *Curso de Perfeccionamiento Mejoramiento de la Calidad Higiénica de Leche de Pequeños Productores*, 6-8 Diciembre, Osorno, Chile.

Pinedo PJ, Meléndez P. 2010. Patrones temporales de recuento de células somáticas, grasa, proteína y nitrógeno ureico en leche de estanque y su asociación con fertilidad en ganado lechero en la zona centro-sur de Chile. *Arch Med Vet* 42, 41-48.

Ponce P. 2009. Un enfoque crítico de la lechería internacional y cubana. *Rev Salud Anim* 31, 77-85.

Romero PA, Calderón AR, Rodríguez VR. 2018. Evaluación De La Calidad De Leches Crudas En Tres Subregiones Del Departamento De Sucre, Colombia. *Revista Colombiana De Ciencia Animal* 10, 43-50.

Ryman VE, Packiriswamy N, Sordillo L M. 2015. Role of endothelial cells in bovine mammary gland health and disease. *Anim Health Res Rev* 16, 135-149.

Ruegg PL, Pantoja JCF. 2013. Understanding and using somatic cell counts to improve milk quality. *Irish J Agric Food Res* 52, 101-117.

Santos MV. 2006. O uso da CCS em diferentes países *In*: Mesquita AJ, Durr JW, Coelho KO (eds). *Perspectivas e avanços da qualidade do leite no Brasil*. Editora Talento, Goiânia, Brazil, Pp 181-197.

SAS, Statistical Analysis System. 2000. *SAS version 6.0*. SAS Institute Inc., Cary, NC, USA.

Sneddon NW, Lopez-Villalobos N, Davis SR, Hickson RE, Shalloo L. 2015 Genetic parameters for milk components including lactose from test day records in the New Zealand dairy herd. *New Zeal J Agric Res* 58, 97-107.

Werner EG. 2014. Relación de la producción de leche y calidad sobre el recuento de células somáticas en rebaños del sur de Chile. *Tesis*, Universidad Austral de Chile, Valdivia, Chile.

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ORIGINAL ARTICLE

Comparison of two phenotypical methods to segregate resistant and susceptible lambs to parasitic nematodes

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ABSTRACT. The objective of this study was to compare two segregation methods to select resistant and susceptible female Pelibuey lambs infected naturally with gastrointestinal nematodes (GINs) in relation to their haematological and immunological response. For 6 months, faeces and blood samples were taken fortnightly from 40 grazing 5-month-old female lambs. The lambs were classified according to two methods using faecal egg count (FEC) as a phenotypical trait. In the first (reference) method (M3SE, n = 22), resistant (RES) lambs had FEC lower than the mean – 3 standard errors, the susceptible (SUS) lambs levels higher than + 3 standard errors (n = 10) and the intermediate (INT) lambs (n = 8) were categorised by having FECs between the two values. The second method (QUM) divided the population, using quartiles, into resistant (RES; 25%), intermediate (INT; 50%), and susceptible (SUS; 25%) lambs. The agreement between both methods was estimated using the Kappa index. The packed cell volume (PCV), total plasma protein (TPP) and peripheral eosinophils (EOS) were determined for each group. Serum was used to evaluate the IgA levels. PCV and TPP values were higher (P<0.01) in the RES lambs (31.5 ± 3.4 and 6.16 ± 0.5 g/dL by QUM, respectively, and 31.5 ± 3.9 and 6.24 ± 0.49 g/dL by M3SE, respectively) than the SUS lambs (28.1 ± 4.7 and 5.94 ± 0.5 g/d, respectively, by both methods). The EOS and IgA values increased with age. M3SE and QUM were in moderate agreement (Kappa = 0.43). We concluded that the two segregation methods allowed for the identification of the same female SUS lambs, but a greater number of animals were categorised phenotypically as resistant using the M3SE method. PCV and TPP can help to identify phenotypically resistant animals.

Key words: eosinophils, selection, IgA, plasma protein.

INTRODUCTION

Gastrointestinal nematodes (GINs) are responsible for the deterioration in sheep health, especially in tropical areas, where climatic conditions favour their development and propagation throughout the year. GINs infections limit the productivity of sheep, causing economic losses due to low weight gain and increased mortality in the most susceptible animals. Among parasitic nematode infections, the most important is that caused by *Haemonchus contortus* because, in addition to its high prevalence and pathogenicity, it is a hematophagous species, making it a risk for animal health (Mavrot *et al* 2015).

Nematode infections have been mainly controlled using anthelmintics. The use of these products has helped to control the effects of parasitism, but their frequent use to eliminate susceptible nematodes has led to the selection of populations with anthelmintic resistance (AR). For this reason, these drugs have lost their effectiveness against several GINs species (Rose *et al* 2015). Alternative methods to control the effects of GINs, and combinations of these methods, have been widely used to avoid AR problems (McMahon *et al* 2013).

The host resistance is the ability to control the infection of endo-parasitic stages, contrary to susceptible ones that allow the infection with acute clinical signs. Parasitic infections with GINs consider the faecal egg count (FEC) as the main indicator to identify phenotypically resistant hosts. Selecting for increased resistance leads to decreased FEC resulting from ever-decreasing pasture contamination and hence decreased infectious challenge (Bishop 2012). The search for natural genetic resistance in sheep as the main objective implies the selection of several generations of sheep because genetic resistance against GINs is a moderately heritable characteristic (h^2 = 0.11 to 0.40, Gauly and Erhardt 2001). To demonstrate this genetic resistance, phenotypic, histological, immunological and molecular indicators have been studied (Sweeney *et al* 2016). Despite its limitations, FEC is the phenotypic indicator that is most frequently used to assess the level of parasitic infection because is an estimator of parasitic burden (Morris *et al* 2000). However, identifying the most heavily infected individuals requires several samples due to variability in FEC. For this reason, several pathophysiological indicators have since been developed to indicate the presence and intensity of a GINs infection, such as the packed cell volume (PCV) to indicate anaemia. Similarly, the dag score and body condition has also been used to

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evaluate the health deterioration caused by nematode infection (Bentounsi *et al* 2012).

To detect animals with resistance, several segregation methods have been used. One of these is the arithmetic mean of FEC along with the standard error (Morteo-Gómez *et al* 2004). The other method uses practical rules and categorises animals as resistant when they have a FEC <1000 eggs per gram of faeces (EPG), intermediate when the FEC is between 1000 and 2500 EPG and susceptible if it is >2500 EPG (Fakae *et al* 2004). In recent years, hair sheep have been segregated using the quartile method (Palomo-Couoh *et al* 2016, Zaragoza-Vera *et al* 2019). Others studied indicators include PCV, total plasma protein (TPP), pepsinogen, blood cell count and immunoglobulin, mainly IgA and IgE. These have been explored for the effective identification of natural resistance to GINs in sheep (Preston *et al* 2014, Zaros *et al* 2014).

The main host defence mechanism against GINs is acquired immunity, which develops over time in response to reinfection and depends on age, nutritional status and genotype (McRae *et al* 2015). A current challenge for sheep breeders is allowing sufficient GINs exposure to develop immunity without impairing growth, and hence herd productivity, and maintaining anthelmintic efficacy on farms (McMahon *et al* 2013). Due to this situation, the proposed hypothesis was that the lambs will express acquired resistance against GINs according to their segregation, which implies that resistant female lambs will have more eosinophils and better haematological values than susceptible sheep. The objective of this study was to compare two segregation methods to select resistant and susceptible female Pelibuey lambs naturally infected with GINs in relation to their haematological and immunological response.

MATERIAL AND METHODS

STUDY AREA LOCATION

The study was conducted at the Centre for Training and Reproduction of Small Species (CECAREM) in Tabasco, México, located in Villahermosa (17°92' N, 93°00' W). The climate of the region is hot humid with rain throughout the year[1]. The mean temperature is 27.1 °C and annual rainfall is 1958 mm (figure 1).

ANIMAL MANAGEMENT

From a group of 300 contemporary Pelibuey lambs born in a controlled mating, 40 lambs aged 5 months with age differences not exceeding 15 days were selected.

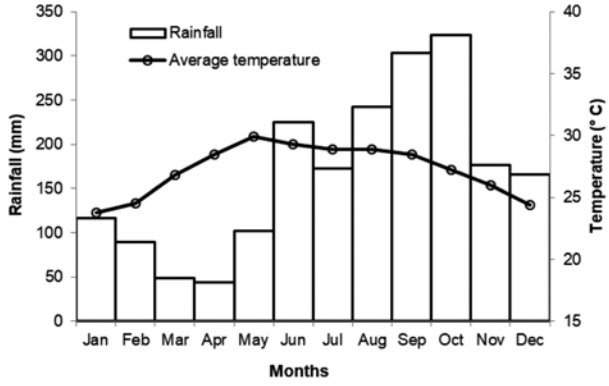

Figure 1. Mean monthly temperature (lines) and monthly distribution of rainfall (columns) throughout the year in Villahermosa, Tabasco, México. Obtained from the National Meteorological Service. Climatological norms of the Tabasco state.

https://smn.cna.gob.mx/es/informacion-climatologica-por-estado?estado=tab

Additionally, all selected lambs showed similar body condition and were kept in a group that received similar food and sanitary handling. During the trial, all the lambs grazed on the same paddocks of Star grass pasture (*Cynodon nlemfuensis*) and were naturally infected with GINs for 6 months from May to October.

The lambs were protected in galleys. They were supplemented with 200 g of food (14% crude protein) and received water *ad libitum*. The procedures were performed following the Mexican Official Standard guideline 051-ZOO-1995 and the Mexican Official Standard of technical specifications for production, care and use of experimental animals.

PARASITOLOGICAL AND IMMUNOLOGICAL METHODS

The group of female lambs remained in-house from birth to 5 months old, at which time the natural infection began during grazing. Blood and faecal samples were collected every 14 days over the 6 months from April to October. The faecal samples were collected directly from the rectum and the FEC was determined according to the McMaster method (Cringoli *et al* 2004) with a sensitivity of 50 EPG. Also, faecal cultures were processed to collect infective larvae (L3) at the beginning, middle and end of the study period and morphological identification was made using the practical laboratory guide of van Wyk and Mayhew (2013). Blood samples were obtained from the jugular vein into EDTA tubes and other samples were collected in tubes with a coagulation accelerator (Vacutainer, Becton Dickinson, New York, USA). PCV was determined by the microhaematocrit technique, and peripheral eosinophils (EOS) were counted in a Neubauer chamber after staining the cells with Carpentier solution (Dawkins *et al* 1989). TPP was quantified in a refractometer (Atago, Japan). Serum samples were centrifuged at 2000*g*, then stored at –20 °C

[1] SMN, Servicio Meteorológico Nacional. 2019. Normales Climatológicas del estado de Tabasco. https://smn.cna.gob.mx/es/informacion-climatologica-por-estado?estado=tab. Consulted 11.03.2019.

until use. An indirect ELISA was used to determine the IgA level (% OD with respect to positive control), using *Haemonchus contortus* and *Trichostrongylus colubriformis* crude extract antigen, according to the technique of González-Garduño *et al* (2017).

CRITERIA FOR THE SELECTION OF RESISTANT AND SUSCEPTIBLE INFECTED HOSTS

The arithmetic mean FEC of the whole trial, which consisted of 11 biweekly samples, was used to classify the host response against GINs as resistant (RES), susceptible (SUS) or intermediate (INT), using two methods. In the first, the lambs were classified using the mean ± 3 standard errors (M3SE) as follows: RES female lambs were chosen as those with FECs less than the mean – 3SE; SUS lambs were those with FECs greater than the mean + 3SE; the INT group was defined by FECs between the two ranges (Morteo-Gómez *et al* 2004).

In the second method, the quartile method (QUM), as previously described by Palomo-Couoh *et al* (2016), was used. Briefly, the lambs were divided as follows: the first quartile (Q1) of infected lambs with low FEC were identified as RES hosts (25%); the lambs in the highest FEC and third quartile (Q3) were identified as SUS (25%); and a third group, considered INT, had a FEC between Q1 and Q3 and represented 50% of the total population.

STATISTICAL ANALYSIS

To compare the two methods, the Kappa index (Landis and Koch 1977) was calculated with the formula Po - Pe/ (1 - Pe). Where Po = total proportion of concordance observed and Pe = expected random proportion. The concordance value (Youden's J), sensitivity, specificity, predicted positive and negative values, the proportion of false positives and false negatives, and the accuracy were calculated according to Palomo-Couoh *et al* (2016) relative to an assumed reference method (M3SE). To perform the validity test, the data were grouped in 2 × 2 contingency tables, considering as reference the method of M3SE, because the mean values together with the standard deviation are part of the theory of the selection of animals used to calculate the genetic progress of phenotypic characteristics (Morris *et al* 2000, McRae *et al* 2014).

To compare the response of each segregated group (RES, INT and SUS) a multivariate linear mixed-effect regression model was used under a design of repeated measures over time, by which different covariance structures were tested for each study variable. The autoregressive structure (AR1) was selected and adjusted to each model by presenting lower AIC and BIC values. The analysis was performed with the MIXED procedure of SAS software (SAS, 2004). Two segregation methods were considered (M3SE and QUM) and one statistical analysis was performed per variable in each method (10 runs in total). Untransformed FEC data and other response variables (PCV, TPP, EOS and IgA) were analysed (Peña-Espinoza *et al* 2016) following the statistical model:

$$Y_{ijkl} = \mu + \rho_i + \tau_j + \rho * \tau_{ij} + \zeta(\rho)_{ik} + \varepsilon_{ijkl}$$

where Y_{ijkl} = response variable (FEC, PCV, TPP, EOS, IgA); μ = general mean; ρ_i = fixed effect of the treatment (i = RES, INT, SUS); τ_j = fixed effect of time (j = 1, 2, 3…11 samplings); $\rho * \tau_{ij}$ = joint effect of treatment and time; $\zeta(\rho)_{ik}$ = random effect of the animal nested in each treatment; and ε_{ijkl} = experimental error. Comparisons of RES, INT and SUS and differences in time of sampling were observed with Tukey's tests (SAS 2004). Pearson correlations were calculated to determine the relationship between the response variables (FEC, PCV, TPP, IgA) in each of the segregated groups (RES and SUS) per method. Spearman correlations were made for age and countable variables. With regard to the interaction between time and treatment (dynamics), the study focused only on the divergent performance of RES and SUS groups.

RESULTS

CLASSIFICATION OF LAMBS ACCORDING TO NEMATODE INFECTION

Table 1 shows the parameters FEC, TPP, TCV and EOS according to the classification of the female Pelibuey lambs. In the first parasitic classification method (M3SE), 22 infected lambs were identified as RES (55%), 8 as INT (20%) and 10 as SUS (25%). Using the second method (QUM), 10 infected lambs (25%) were identified as RES, 20 (50%) as INT and 10 as SUS (25%). The SUS lambs corresponded to the same animals under the two methods, so the study variables were similar. The arithmetic mean of FEC during the study period for the SUS group was higher (672 EPG) than that of the RES (171 EPG and 288 EPG) and INT groups (39 EPG and 67 EPG) from QUM and M3SE methods, respectively (P<0.05). The mean FEC in the RES group was similar in the two methods. For TPP and PCV analysis, there was an increased response in the resistant group, and the lowest TPP and PCV values were obtained for the susceptible group (P<0.05) in both methods.

The Kappa index between the two methods was moderate (0.43). When comparing the QUM with the M3SE as a standard method, it was observed that QUM showed low sensitivity and a low percentage of negative predictive values, where only 10 of the 22 resistant lambs were detected, so the accuracy was 70% and the Youden index was 0.5 (table 2).

DYNAMICS OF FAECAL EGG COUNT

The same lambs were categorised as SUS lambs by both QUM and M3SE methods, so the performance was

Table 1. Arithmetic mean of faecal egg count, packed cell volume and total plasma protein throughout the study period in female Pelibuey lambs classified as resistant, intermediate and susceptible according to segregation method.

Variable and method	Female lamb phenotype classification								
	Resistant			Intermediate			Susceptible		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Faecal egg count (EPG)									
Method 1. M3SE	22	67 ±	123[a]	8	288 ±	532[b]	10	672 ±	1068[c]
Method 2. QUM	10	39 ±	65[a]	20	171 ±	372[b]	10	672 ±	1068[c]
Packed cell volume (%)									
Method 1. M3SE	22	31.5 ±	3.9[a]	8	32.3 ±	3.7[a]	10	28.1 ±	4.7[b]
Method 2. QUM	10	31.5 ±	3.4[a]	20	31.9 ±	4.0[a]	10	28.1 ±	4.7[b]
Total plasma protein (g/dL)									
Method 1. M3SE	22	6.24 ±	0.49[a]	8	6.02 ±	0.44[b]	10	5.94 ±	0.50[b]
Method 2. QUM	10	6.16 ±	0.50[a]	20	6.19 ±	0.48[a]	10	5.94 ±	0.50[b]
Peripheral eosinophils (cells/µL)									
Method 1. M3SE	22	460 ±	633[a]	8	407 ±	463[a]	10	363 ±	380[a]
Method 2. QUM	10	377 ±	376[a]	20	481 ±	673[a]	10	363 ±	380[a]

EPG: Eggs per gram of faeces. QUM: Quartile method. M3SE: Means-3 standard error method. Different lowercase letters in the same row indicate significant differences (P<0.05). SD, Standard deviation. SE, Standard error.

Table 2. Evaluation of the concordance between two segregation methods to detect resistant Pelibuey female lambs against gastrointestinal nematodes.

Item	Segregation method			
	Mean-3 Standard Error		Quartile	
	Value	CI 95%	Value	CI 95%
Sensitivity (%)	100.0	85.1-100.0	45.5	26.9-65.3
Specificity (%)	100.0	82.4-100.0	100.0	82.4-100.0
Positive predictive value (%)	100.0	85.1-100	100.0	72.2-100.0
Negative predictive value (%)	100.0	82.4-100	60.0	42.3-75.4
False positives proportion (%)	0.0	0.0-17.6	0.0	0.0-17.6
False negative proportion (%)	0.0	0.0-14.9	54.5	34.7-73.1
Accuracy (%)	100.0	91.2-100	70.0	54.6-81.9
Youden J index (%)	1.0		0.5	

similar. However, for RES lambs, there were a greater number of animals categorised by M3SE (n = 22), while only 10 lambs were selected by QUM. An increase in FEC was shown from August to October, especially in SUS female lambs (figure 2), which was attributable to the increase in rainfall during the months of August to October (figure 1). The nematode species recovered from the faecal cultures at the beginning of the experiment were *Haemonchus* spp. (77%), *Trichostrongylus* spp. (16%) and *Oesophagostomum* spp. (7%). These species remained in similar proportions until the end of the study (65.64%, 25.04% and 9.32%, respectively).

DYNAMICS OF PACKED CELL VOLUME AND TOTAL PLASMA PROTEIN

The PCV percentage in RES lambs showed significant differences (P<0.01) with respect to SUS lambs (table 1). From April to June, the PCV value remained normal. However, after the rainy season at the end of July, the FEC increased and the PCV percentage decreased (figure 3) due to the presence of blood-feeding nematodes such as *H. contortus*. In August, the PCV increased when the FEC decreased slightly, as suggested by the correlation coefficient (CORR; r = –0.23). The lowest PCV value

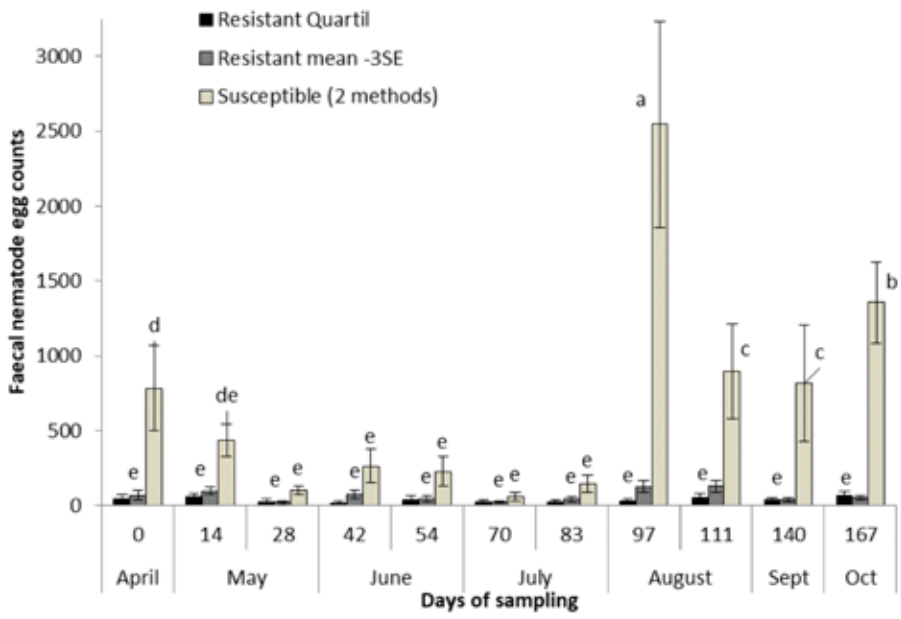

Figure 2. Divergent behaviour of nematode eggs per gram of faeces determined in resistant lambs (n = 10 in quartile method, n = 22 in mean - 3SE method) and susceptible lambs (n = 10; both methods were similar) in Pelibuey breed by month and sampling day. The bars represent the SE in each group. The different letters of each group represent statistical differences (P<0.01).

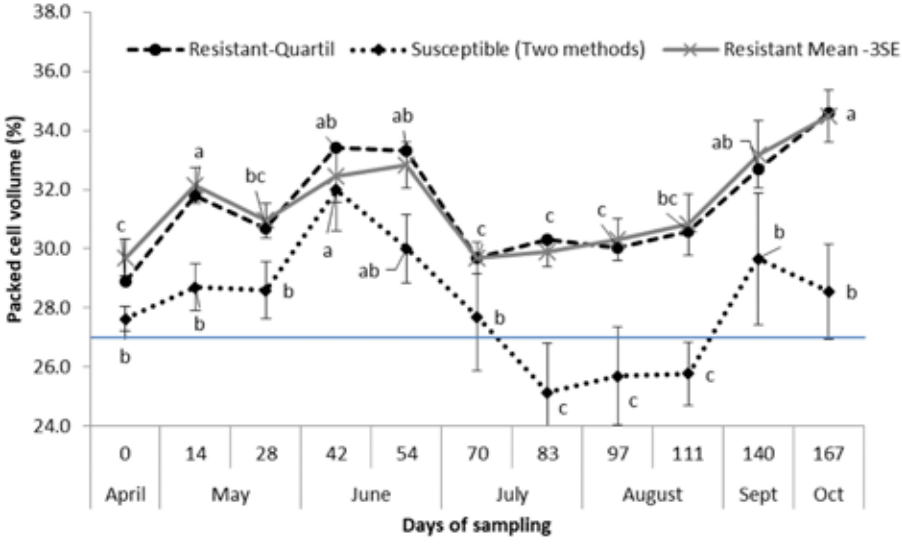

Figure 3. Divergent behaviour of PCV determined in resistant lambs (n = 10 in quartile method, n = 22 in mean - 3SE method) and susceptible lambs (n = 10; both methods were similar) in Pelibuey breed by month and sampling day. The bars represent the SE in each group. The different letters of each group represent statistical differences (P<0.01). The solid line represents the threshold physiological level.

(26%) was observed between days 83 and 97 in SUS lambs.

For the TPP analysis, there was a significant reduction (P<0.01) between days 28 and 42 and a tendency towards a reduction in the last three samplings for RES lambs. The SUS lambs had values below 6 g/dL, the physiological threshold, in 54% of the samples (figure 4). Also, TPP values decreased when the FEC increased, which was shown by the CORR (table 3).

PERIPHERAL EOSINOPHIL DYNAMICS

In this analysis, there were no differences in EOS counts between the RES and SUS lambs segregated according to either method. There was also a progressively increased number of eosinophils as female lambs grew older. Figure 5 shows the change in EOS count with time. The CORR between age and the number of eosinophils was high (ρ = 0.43), and remained similar in both RES and SUS animals regardless of the segregation method.

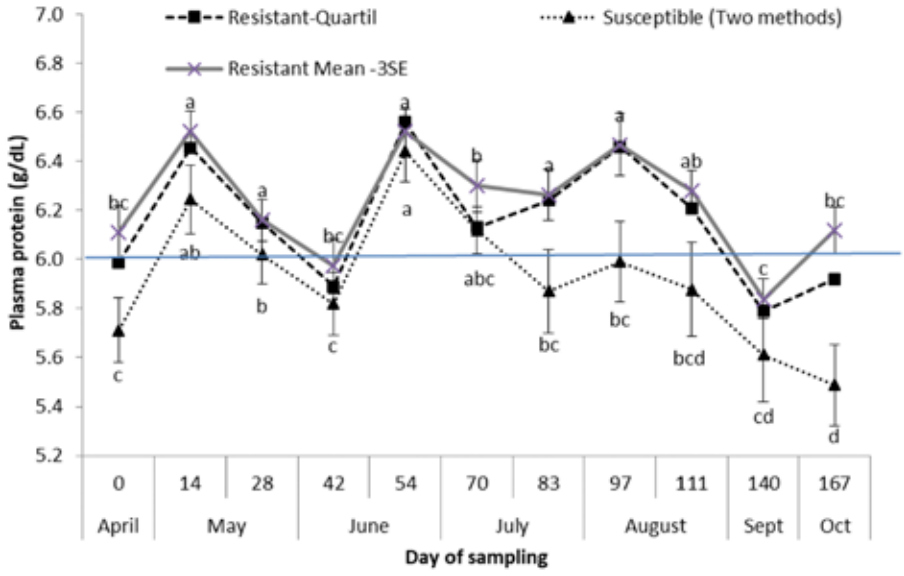

Figure 4. Divergent behaviour of TPP determined in resistant lambs (n = 10 in quartile method, n = 22 in mean - 3SE method) and susceptible lambs (n = 10; both methods were similar) in Pelibuey breed by month and sampling day. The bars represent the SE in each group. The different letters of each group represent statistical differences (P<0.01). The solid line represents the threshold physiological level.

Table 3. Correlation coefficients between the studied variables in Pelibuey female lambs classified as resistant and susceptible according to segregation methods.

Variable	Resistant		Susceptible
	Method -3 SE	Quartile	Two methods
Age (days)			
Faecal egg counts (EPG)	0.01^{ns}	0.06^{ns}	0.29**
Packed cell volume (%)	0.17**	0.21*	–0.09^{ns}
Total plasma protein (g/dL)	–0.12^{ns}	–0.15^{ns}	–0.27**
Peripheral eosinophils (cells/µL)	0.47**	0.45**	0.44**
IgA*-*H. contortus* antigen	0.47**	0.47**	0.50**
IgA*-*T.colubriformis* antigen	0.44**	0.34**	0.42**
PCV (%)			
Total plasma protein (g/dL)	0.147*	–0.15^{ns}	0.12^{ns}
Peripheral eosinophils (cells/µL)	0.30**	0.45**	0.13^{ns}
IgA*-*H. contortus* antigen	0.35**	0.47**	0.26*
IgA*-*T.colubriformis* antigen	0.32**	0.34**	0.24^{ns}
FEC			
Packed cell volume (%)	–0.04^{ns}	0.03^{ns}	–0.22*
Total plasma protein (g/dL)	–0.02^{ns}	–0.02^{ns}	–0.26**
TPP (g/dL)			
Peripheral eosinophils (cells/µL)	–0.06^{ns}	–0.10^{ns}	–0.23*
IgA*-*T.colubriformis* antigen	0.19*	0.22^{ns}	–0.04^{ns}
IgA*-*H. contortus* antigen			
IgA*-*T.colubriformis* antigen	0.88**	0.91**	0.95**

IgA*: % OD respect to the positive control. FEC: Faecal egg count. TPP: Total plasma protein. PCV: Packed cell volume. ** Highly significant differences (P<0.01). * Significant differences (P<0.05). ns= Not significant differences (P>0.05).

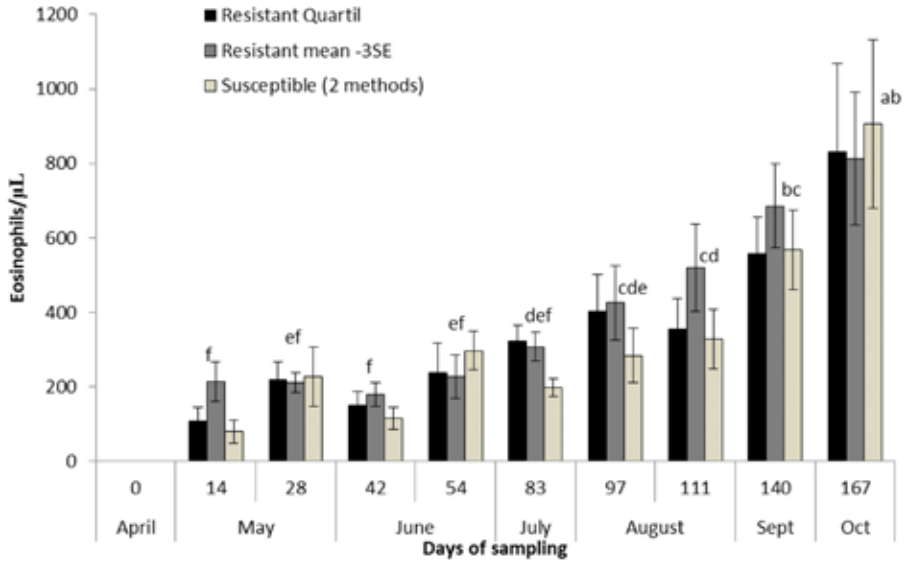

Figure 5. Simple effect of peripheral eosinophil counts in contemporary female Pelibuey lambs (n = 40) by sampled day and age. The bars represent the SE in each group and different letters represent significant statistical differences (P<0.01). The different letters by the arithmetic mean for all groups represent statistical differences (P<0.01).

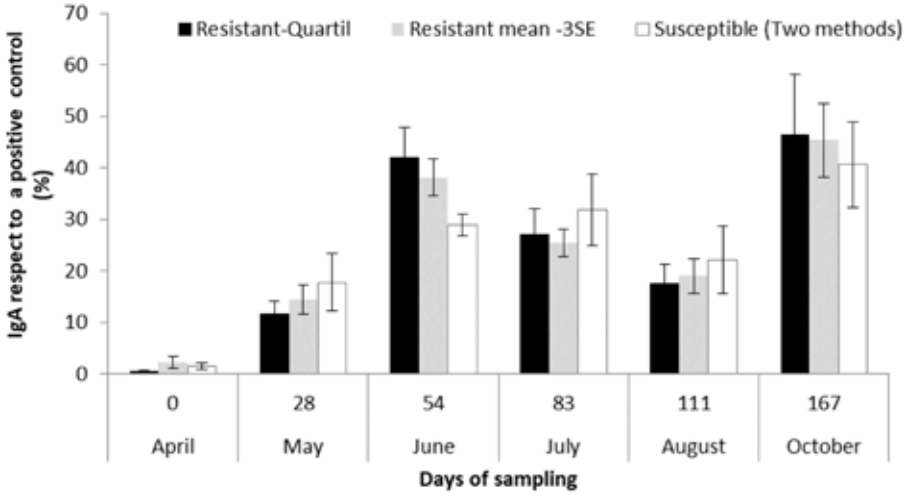

Figure 6. IgA levels respect to a positive standard control using *H. contortus* somatic antigen in resistant lambs (n = 10 in quartile method, n = 22 in mean - 3SE method) and susceptible lambs (n = 10; both methods were similar) in Pelibuey breed by month and sampling day. The bars represent the SE in each group. A similar response was seen with *T. colubriformis*.

DYNAMICS OF IMMUNOGLOBULIN A

The female lambs showed low levels of serum IgA (2% with respect to the control) from initial sampling (day 0), increasing gradually with age (P<0.05). Lambs showed increased levels of IgA after 7 months of age (50% with respect to the positive control); these results show that IgA tended to increase with animal age, in association with FEC (figure 6); this was also seen in the CORR values, which were high (ρ = 0.44 to 0.47). The IgA response in RES lambs was similar to that in SUS lambs. Also, the immune response with IgA was similar between the two species of nematodes used (*H. contortus* and *T. colubriformis*).

CORRELATION COEFFICIENTS

The FEC, PCV, number of eosinophils and IgA levels increased with age (P<0.05). TPP had a negative coefficient; therefore, as age increased, the TPP values decreased (ρ = –0.14, P<0.01). In the RES lambs, the FEC had no

significant correlation (P>0.05) with PCV or TPP, whereas in the SUS lambs there was no relationship of PCV with TPP, eosinophils or IgA levels (table 3). Other important correlations occurred between FEC and TPP, with negative values (r = –0.23) in SUS lambs. The correlation coefficients between the crude worm antigen of *H. contortus* and *T. colubriformis* were very high (r = 0.90).

DISCUSSION

FAECAL EGG COUNT

In the grazing lambs, the ingestion of infective larvae will tend to homogenise over longer periods (Stear *et al* 2007). Under this scenario, the RES, SUS and INT lambs would have the same chance of becoming infected and the difference between the methods would be the choice of resistant animals. The use of the QUM and M3SE allowed for the classification of the same SUS lambs. However, in RES lambs, M3SE allowed for the selection of 22 individuals, whereas with QUM only 10 were categorised as RES. In our study, in 70% of samplings (7 out of 11 samplings), more than 25% of lambs showed 0 EPG, and the complication of QUM was to detect RES lambs when more than 25% showed resistance, as occurred in the flock. The objective of any segregation method is to select lambs for phenotypical resistance or tolerance to GIN infection that will result in a reduced reliance on anthelmintics to control parasitic nematodes, with the associated economic advantages (Cloete *et al* 2007). This is especially important when many animals exhibit resistance in at least at some of their physiological stages, as is the case during female lamb growth. This situation requires either many samples to detect differences between the RES animals, or the inclusion of another variable to select lambs with GIN resistance. For this reason, M3SE was considered the method of reference.

The M3SE method allowed to select at least all lambs with 0 EPG, therefore, the lambs not selected by the QUM method were considered as false negatives. For this reason, QUM showed low sensitivity.

When selecting for resistance to GINs, it is important to consider that FEC has a very small heritability (0.16 to 0.22; Morris *et al* 2005). Also, environmental conditions (feeding, facilities, pasture management), together with animal variables (such as age, development of immunity and physiological stage; González-Garduño *et al* 2014) and parasite variables (species of nematode, length of survival of parasite in the host, arrested development of infections, etc), lead to differences in the faecal egg output per month, as indicated in other studies (Amarante *et al* 2009). The classified groups showed significant differences (P<0.05) between RES and SUS lambs in both the FEC and PCV. The RES lambs showed lower FEC and higher PCV than SUS lambs, as indicated in another study with Pelibuey ewes during lactation (Palomo-Couoh *et al* 2016).

PACKED CELL VOLUME AND TOTAL PLASMA PROTEIN

PCV and TPP are used to evaluate the degree of resistance or resilience to GIN infections, mainly when *H. contortus* is the most prevalent GIN. In this study, the values of both parameters remained within the physiological threshold for sheep (PCV from 27 to 45% and TPP from 6 to 7.5 g/dL; Byers and Kramer, 2010). The PCV values were lower than the physiological threshold during the rainy months (July and August), in response to an increase in the FEC associated with the high prevalence of *H. contortus*. This situation has been widely reported, and several studies indicate a very high negative correlation coefficient between FEC and PCV (r = –0.7) when this nematode is present (Amarante *et al* 2009, Emery *et al* 2016). However, the correlation coefficient found in our study was only –0.23. TPP also had a similar magnitude of correlation with FEC (r = –0.20), so both variables can be used as indicators of resistance to GIN, since the values were higher in resistant lambs. As indicated in another study, TPP can be effective in selecting animals with greater resistance to GINs (Zaros *et al* 2014). The slightly diminished TPP values in susceptible lambs could be an important indication of the reduction in ghrelin gene expression associated with the suppression of intake and malnutrition in animals infected with GINs and a decreased inflammatory response in susceptible lambs (Alba-Hurtado and Muñoz 2012).

PERIPHERAL EOSINOPHILS

Eosinophils are an important element in the inflammatory response against GINs. The association of eosinophils with helminths shows significant correlations between the susceptibility or resistance to infection and the magnitude of the EOS response, which suggests that eosinophils have a role in resistance to helminth infection (Balic *et al* 2000, Yacob *et al* 2009). However, in this study, there were no differences between RES and SUS lambs regardless of the method of segregation, which suggests that the immune system is immature at this age; therefore, this parameter cannot be used for the selection of resistance to GINs in young animals (Preston *et al* 2014). There was also no relationship between FEC and EOS, probably due to the age of the animals. Similar to other studies (Amarante *et al* 2009), in this study there was a high Spearman correlation between the age of the lambs and EOS. In both RES and SUS lambs, the correlation was between 0.44 and 0.47 (P<0.01). This indicates the maturation of the animals' immune system over the first 10 months of life, unlike another study that indicates that EOS increased from birth to 5 months of age, associated with a subsequent ability to reduce parasite fecundity (Greer and Hamie 2016).

IMMUNOGLOBULIN A

Secretory IgA is the main immunoglobulin secreted actively through the epithelium of the mucosa of the

abomasum (Macpherson *et al* 2008). IgA response is associated with protective immunity to nematode infections in sheep. However, in our study the low association between the IgA level and the FEC at the beginning was due to the IgA level being dependent on age: after 6 months the FEC fluctuations were related to environmental conditions. For this reason, from July to October (characterised by high humidity), there was an increased FEC, but the IgA levels were relatively low in growing lambs.

The low immunological response to GINs in the young sheep in this study has also been reported in other studies and has been attributed to the low proportion of CD4+ and CD8+ lymphocytes in young sheep compared to adults. Salivary IgA levels in grazing lambs, determined with a larval antigen (CarLA), indicated that the development of immunity occurs in most animals after 6 months of age (Shaw *et al* 2013). Also, Smith *et al* (1985) reported that 4-month-old lambs had a lower response in terms of IgA levels than their 10-month-old counterparts after a challenge with GINs. The negative correlation between IgA levels and infection parameters such as FEC and the number of adult nematodes (Beraldi *et al* 2008) suggests that it can be used as a marker for resistance selection (Shaw *et al* 2013). However, in the present study, IgA showed no differences between RES and SUS in all samples. The differences occurred only as a result of age.

Female Pelibuey lambs show high variability in their FEC. The two evaluated methods of segregation allow for the identification of susceptible lambs to a similar extent, but a large number of lambs showed a high degree of infection resistance, as was detected by the M3SE method of segregation, while the method of segregation by QUM selected only 25% of resistant ewe lambs; for this reason the QUM resulted in low sensitivity and low accuracy. The high PCV and high TPP in resistant Pelibuey lambs can be considered in the phenotypic selection of individuals resistant to GIN, particularly to infections with the hematophagous *H. contortus*. It can also be concluded that the eosinophil count reveals the immaturity of the immune system of 6-month-old in primo-infected lambs and is not an important element in the selection of resistant animals of this age.

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REFERENCES

Alba-Hurtado F, Muñoz-Guzmán MA. 2012. Immune responses associated with resistance to haemonchosis in sheep. *BioMed Res Intern* 2013, article ID 162158.

Amarante AFTD, Susin I, Rocha RA, Silva MB, Mendes CQ, *et al.* 2009. Resistance of Santa Ines and crossbred ewes to naturally acquired gastrointestinal nematode infections. *Vet Parasitol* 165, 273-280.

Balic A, Bowles VM, Meeusen EN. 2000. The immunobiology of gastrointestinal nematode infections in ruminants. *Adv Parasitol* 45, 181-241.

Bentounsi B, Meradi S, Cabaret J. 2012. Towards finding effective indicators (diarrhoea and anaemia scores and weight gains) for the implementation of targeted selective treatment against the gastro-intestinal nematodes in lambs in a steppic environment. *Vet Parasitol* 187, 275-279.

Beraldi D, Craig BH, Bishop SC, Hopkins J, Pemberton JM. 2008. Phenotypic analysis of host-parasite interactions in lambs infected with *Teladorsagia circumcincta*. *Int J Parasitol* 38, 1567-1577.

Bishop S. 2012. A consideration of resistance and tolerance for ruminant nematode infections. *Frontiers in Genetics* 3, 168.

Byers SR, Kramer JW. 2010. Normal hematology of sheep and goats. In: Weiss DJ, KJ Wardrop (eds). *Schalm's veterinary hematology*. 6th ed. Wiley-Blackwell, Ames, USA, Pp 836-842.

Cloete SWP, Olivier JJ, Du Toit E, Dreyer FH. 2007. Genetic analysis of faecal worm egg count in South African Merinos under natural challenge. *South African J Animal Sci* 37, 237-247.

Cringoli G, Rinaldi L, Veneziano V, Capelli G, Scala A. 2004. The influence of flotation solution, sample dilution and the choice of the McMaster technique in estimating the faecal egg count of gastrointestinal strongyles and *Dicrocoelium dentriticum* in sheep. *Vet Parasitol* 123, 121-131.

Dawkins HJS, Windon RG, Eagleson GK. 1989. Eosinophil responses in sheep selected for high and low responsiveness to *Trichostrongylus colubriformis*. *Intern J Parasitol* 19, 199-205.

Emery DL, Hunt PW, Le Jambre LF. 2016. *Haemonchus contortus*: the then and now, and where to from here? *Intern J Parasitol* 46, 755-769.

Fakae BB, Musongong GA, Chiejina SN, Behnke JM, Ngongeh LA, *et al*. 2004. Variability in the resistance of the Nigerian West African Dwarf goat to abbreviated escalating trickle and challenge infections with *Haemonchus contortus*. *Vet Parasitol* 122, 51-65.

Gauly M, Erhardt G. 2001. Genetic resistance to gastrointestinal nematode parasites in Rhön sheep following natural infection. *Vet Parasitol* 102, 253-259.

González-Garduño R, Torres-Acosta JFJ, Chay-Canul AJ. 2014. Susceptibility of hair sheep ewes to nematode parasitism during pregnancy and lactation in a selective anthelmintic treatment scheme under tropical conditions. *Res Vet Sci* 96, 487-492.

González-Garduño R, López-Arellano ME, Conde-Felipe MM, Mendoza-de Gives P, Aguilar-Marcelino L, *et al.* 2017. Immune and haematological parameters of Blackbelly ewes infected with gastrointestinal nematodes. *Rev Colomb Cienc Pecu* 30, 219-230.

Greer AW, Hamie JC. 2016. Relative maturity and the development of immunity to gastrointestinal nematodes in sheep: an overlooked paradigm? *Parasite Immunol* 38, 263-272.

Landis JR, Koch GG. 1977. The measurement of observer agreement for categorical data. *Biometrics*, 33,159-174.

Macpherson AJ, McCoy KD, Johansen FE, Brandtzaeg P. 2008. The immune geography of IgA induction and function. *Mucosal Immunol* 1, 11.

Mavrot F, Hertzberg H, Torgerson P. 2015. Effect of gastro-intestinal nematode infection on sheep performance: a systematic review and meta-analysis. *Parasites & Vectors* 8, 557-568.

McMahon C, McCoy M, Ellison SE, Barley JP, Edgar HWJ, *et al.* 2013. Anthelmintic resistance in Northern Ireland (III): Uptake of 'SCOPS' (Sustainable Control of Parasites in Sheep) recommendations by sheep farmers. *Vet Parasitol* 193, 179-184.

McRae KM, McEwan JC, Dodds KG, Gemmell NJ. 2014. Signatures of selection in sheep bred for resistance or susceptibility to gastrointestinal nematodes. *BMC genomics* 15, 637.

McRae KM, Stear MJ, Good B, Keane OM. 2015. The host immune response to gastrointestinal nematode infection in sheep. *Parasite Immunol* 37, 605-613.

Morris CA, Vlassoff A, Bisset SA, Baker RL, Watson TG, *et al.* 2000. Continued selection of Romney sheep for resistance or susceptibility to nematode infection: estimates of direct and correlated responses. *Animal Sci* 70, 17-27.

Morris CA, Wheeler M, Watson TG, Hosking BC, Leathwick DM. 2005. Direct and correlated responses to selection for high or low faecal nematode egg count in Perendale sheep. *New Zealand J Agricul Res* 48, 1-10.

Morteo-Gómez R, González-Garduño R, Torres-Hernández G, Nuncio-Ochoa G, Becerril-Pérez C, *et al.* 2004. Efecto de la variación fenotípica en la resistencia de corderos Pelibuey a la infestación con nematodos gastrointestinales. *Agrociencia* 38, 395-404.

Palomo-Couoh JG, Aguilar-Caballero AJ, Torres-Acosta JF, Magaña-Monforte JG. 2016. Evaluation of different models to segregate Pelibuey and Katahdin ewes into resistant or susceptible to gastrointestinal nematodes. *Trop Animal Health Prod* 48, 1517-1524.

Peña-Espinoza M, Thamsborg SM, Desrues O, Hansen TV, Enemark HL. 2016. Anthelmintic effects of forage chicory (*Cichorium intybus*) against gastrointestinal nematode parasites in experimentally infected cattle. *Parasitology* 143, 1279-1293.

Preston SJM, Sandeman M, González J, Piedrafita D. 2014. Current status for gastrointestinal nematode diagnosis in small ruminants: where are we and where are we going? *J Immunol Res* 1-12.

Rose H, Rinaldi L, Bosco A, Mavrot F, De Waal T, *et al.* 2015. Widespread anthelmintic resistance in European farmed ruminants: a systematic review. *Vet Record* 176, 546-546.

SAS, Statistical Analysis System. 2004. *SAS Version 9.2*. SAS Institute Inc., Cary, NC, USA.

Shaw RJ, Morris CA, Wheeler M. 2013. Genetic and phenotypic relationships between carbohydrate larval antigen (CarLA) IgA, parasite resistance and productivity in serial samples taken from lambs after weaning. *Intern J Parasitol* 43, 661-667.

Smith WD, Jackson F, Jackson E, Williams J. 1985. Age immunity to *Ostertagia circumcincta*: comparison of the local immune responses of 4½ and 10-month-old lambs. *J Comparative Pathol* 95, 235-245.

Stear MJ, Fitton L, Innocent GT, Murphy L, Rennie K, *et al*. 2007. The dynamic influence of genetic variation on the susceptibility of sheep to gastrointestinal nematode infection. *J Roy Soc Interface* 4, 767-776.

Sweeney T, Hanrahan JP, Ryan MT, Good B. 2016. Immunogenomics of gastrointestinal nematode infection in ruminants-breeding for resistance to produce food sustainably and safely. *Parasite Immunol* 38, 569-586.

van Wyk JA, Mayhew E. 2013. Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: a practical lab guide. *The Onderstepoort J Vet Res* 80, 539.

Yacob HT, Mistre C, Adem AH, Basu AK. 2009. Parasitological and clinical responses of lambs experimentally infected with *Haemonchus contortus* (L3) with and without ivermectin treatment. *Vet Parasitol* 166, 119-123.

Zaragoza-Vera CV, Aguilar-Caballero AJ, González-Garduño R, Arjona-Jiménez G, Zaragoza-Vera M, *et al.* 2019. Variation in phenotypic resistance to gastrointestinal nematodes in hair sheep in the humid tropics of Mexico. *Parasitol Res* 118, 567-573.

Zaros LG, Neves MRM, Benvenuti CL, Navarro AMC, Sider LH, *et al.* 2014. Response of resistant and susceptible Brazilian Somalis crossbreed sheep naturally infected by *Haemonchus contortus*. *Parasitol Res* 113, 1155-1161.

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SHORT COMMUNICATION

Histopathological lesions compatible with nymphs of *Linguatula serrata* in bovine liver

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ABSTRACT. *Linguatula serrata* is the causative parasite of Linguatulosis, a disease that not only produces economic losses in cattle but also represents a public health risk due to its zoonotic nature. This study aimed to microscopically characterise the compatible lesions produced by this parasite in bovine liver collected at a slaughterhouse in the city of Curicó, Chile. Histologic compatible lesions with Linguatulosis were observed in 18 out of 269 livers. Furthermore, nymphs were visualised in 3 cases, allowing an etiologic diagnosis. Microscopic lesions containing nymphs demonstrated two patterns of inflammatory reactions, one pattern with a predominance of eosinophils, accompanied by lymphocytes and some macrophages, and another pattern with a predominance of lymphocytes and plasma cells with some macrophages. A microscopic characterisation was performed in compatible lesions without nymphs (n=15), defining 4 types of granulomas attributable to visceral Linguatulosis, according to the presence and features of cells at the centre of the lesion, the presence of presumably degenerated nymphal structures, location of inflammatory infiltrate, as well as location and amount of surrounding fibrous tissue. These lesions can concede a presumptive microscopic diagnosis. Also, different microscopic features of granulomas compatible with Linguatulosis analysed in this study suggest a temporal evolution of the lesions. The description of lesions generates a better understanding of the host-parasite interaction of this agent which has importance in both animal productivity and public health.

Key words: linguatulosis, microscopic diagnosis.

INTRODUCTION

Linguatulosis is a disease caused by the pentastomid parasite *Linguatula serrata*. This disease has a zoonotic character, therefore, it represents a risk to public health and produces important economic losses in livestock production (Taylor *et al* 2016).

L. serrata mainly infests mammals including humans and has an indirect life cycle in which the definite host are domestic or wild carnivores, while the intermediate host are herbivores, especially ruminants. The disease has two presentations: visceral and nasopharyngeal. The visceral form is presented by the intermediate hosts while the nasopharyngeal form is developed by the definitive host. Humans are considered an accidental host able to develop both forms of the disease (Machado *et al* 2006, Gunn and Pitt 2012). The larva hatches in the intestine and migrates to the liver and mesenteric, hepatic, and mediastinal lymph nodes, which are the target organs of the parasite. At this point the larva becomes encysted and after several moults, reaches its infective form called nymph (Azizi *et al* 2015).

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The diagnosis of visceral Linguatulosis is performed by visual identification of macroscopically compatible lesions and the detection of the nymph. Thus, the main affected organs are liver and mesenteric lymph nodes (Yakhchali and Tehrani 2013, Azizi *et al* 2015, Hajipour and Tavassoli 2019). The macroscopic lesions compatible correspond to well delimited, flat or slightly elevated, usually solitary greyish-white nodules of 2 to 4 mm diameter, located in the subcapsular area of the liver. Due to this feature and small size they can often go unnoticed, therefore the frequency of the diagnosis in animals could be underestimated (Valenzuela *et al* 1995, Castro *et al* 2015, Carrillo *et al* 2017).

The nymph of *L. serrata* is characterised by the possession of a saw-like cuticle with multiple transverse grooves, a ventral buccal apparatus with two pairs of peribuccal hooks and acidophilic glands, a complete digestive system and primordial genital organs. Moreover, in degraded nymphs, the hooks and remnants of the cuticle are often the only findings that allow the diagnosis of the infestation (Tappe and Büttner 2009, Rabeeh *et al* 2017).

In animals, few studies describe and characterise the inflammatory reaction triggered by *L. serrata* (Farjanikish and Shokrani 2016, Paredes and Muñoz 2016, Rabeeh *et al* 2017). In contrast, microscopic patterns of tissue reactions for each type of lesion have been described for humans, concerning the intensity of the inflammatory response, cell populations and the repair process of the surrounding tissue (Tappe *et al* 2006). This type of studies has allowed describing three types of diagnoses: etiopathological, sub-etiopathological and presumptive (Ma *et al* 2002).

The etiopathological diagnosis is based on the stereomicroscopic visualization of the intact nymph surrounded

by a capsule with little or no adjacent cellular infiltration (Tappe and Büttner 2009). The sub-etiopathological diagnosis is conferred by the presence of at least one characteristic structure of the pentastomids or parts of the nymph within a macroscopically and microscopically compatible lesion (Tappe *et al* 2006). Finally, the presumptive diagnosis is based on the description of macroscopic or microscopic compatible lesions that could have been produced by *L. serrata* or another agent (Ma *et al* 2002).

Hajipour *et al* (2019) reported that *L. serrata* infestation in the mesenteric lymph nodes of the goats showed that the rate and intensity of infestation were greater in lymph nodes with altered colour and consistency. It was also revealed that the infestation rate was age-dependent, showing that the infestation increases as goats grow older. Moreover, the prevalence of infestation rate in female goats was significantly higher than that of male goats. The prevalence differs among animals depending on the location, probably climatic factors affect the survival of the eggs, temperature and humidity can play important roles in the epidemiology. The prevalence of *L. serrata* is higher in goats in comparison to other animals in Iran, which can be an important risk factor for human infestation (Hajipour and Tavassoli 2019).

Studies of the histologic lesions caused by *L. serrata* in animals would represent a contribution to improve the current understanding of parasite-derived pathogenic mechanisms, therefore, the objective of this study is to microscopically characterise the lesions produced by the parasite in the liver of cattle obtained from a slaughterhouse in Curico, Chile.

MATERIAL AND METHODS

In the city of Curicó, Chile, 269 bovine livers collected at a slaughterhouse were examined. The liver capsule was inspected, and several cuts were made in the visceral side of the organ. The analysis was carried between June and August 2018. The age and gender of each analysed animal were registered.

The number of animals was determined with a formula for finite populations (Aguilar-Barojas, 2005), where an N of 1,600 animals slaughtered monthly was considered, with a confidence of 95% and a precision of 0.05.

For the presumptive macroscopic diagnosis of visceral Linguatulosis, the liver capsule was inspected, and 15 longitudinal cuts were made in search of compatible lesions (Valenzuela *et al* 1995, Castro *et al* 2015, Carrillo *et al* 2017). The detected compatible lesions were extracted with a margin of surrounding normal hepatic tissue of approximately 0.5 cm, immersed in 10% formalin (v/v), labelled and sent to the laboratory for routine histological processing.

After 24 hours of fixation, all compatible lesions were cross-sectioned and analysed with a light stereomicroscope (Kyoto model Optical XTJ-4400) for the recognition of nymphs of *L. serrata*. The differential morphological characteristics described by Hamid *et al* (2012) were considered.

Samples with compatible lesions were subjected to routine histological processing and staining with Hematoxylin-Eosin (H-E). Subsequently, the slides were observed with a light optical microscope (Olympus CX22). A descriptive comparison was made with the presence and absence of nymphs, as well as the presence and predominance of inflammatory cell population based on some parameters of microscopic features of lesions proposed by Gibson-Corley (2013). Accordingly, lesions without nymphs, attributable to Linguatulosis, were classified as pentastomid granulomas type 1, 2, 3, and 4 according to the presence and features of the cells at the centre of the lesion, the presence of parasitic structures, the location of the inflammatory infiltrate, and the amount of the surrounding fibrous tissue.

STATISTICAL ANALYSIS

The present study corresponds to an observational and descriptive investigation.

RESULTS AND DISCUSSION

From the 269 livers examined, 18 (6.6%) showed compatible macroscopic lesions and, therefore, a presumptive diagnosis of Linguatulosis. No nymphs were identified in any cross-section of the lesion with a light stereomicroscope. However, in 3 out of the 18 lesions, the whole nymph or fragments were histologically identified, establishing an etiopathological diagnosis of Linguatulosis. A fine continuous capsule was observed in one of the cases and an apparently intact nymph was visualised in its central content (figure 1). The infiltrate was composed predominantly of eosinophils, accompanied by lymphocytes and some macrophages. In two cases, a well-defined inflammatory infiltrate was observed, in close contact with nymphal structures such as spicules and hooks, surrounded by the remains of a thin discontinuous capsule (figure 1). The predominant inflammatory infiltrate was composed of lymphocytes and plasma cells together with some macrophages.

In Talca, Chile, Carrillo *et al* (2017) analysed bovine liver obtained at a slaughterhouse and described the macroscopic lesions compatible with Linguatulosis, which were afterwards etiologically confirmed by stereoscopy, obtaining a prevalence of 13.8% and 6,7% respectively. Castro *et al* (2015) indicated a 5.97% of prevalence in bovine lymph nodes. The diagnostic method of Linguatulosis in slaughterhouses relies on the macroscopic visualisation of compatible lesions with occasional identification of nymphs. Is important to consider the low size of the lesion and nymphs, this infection is hard to detect during the routine examination, especially when this is not a mandatory report disease (Ministerio de Salud 2002, Carrillo *et al* 2017, Hajipour and Tavassoli 2019).

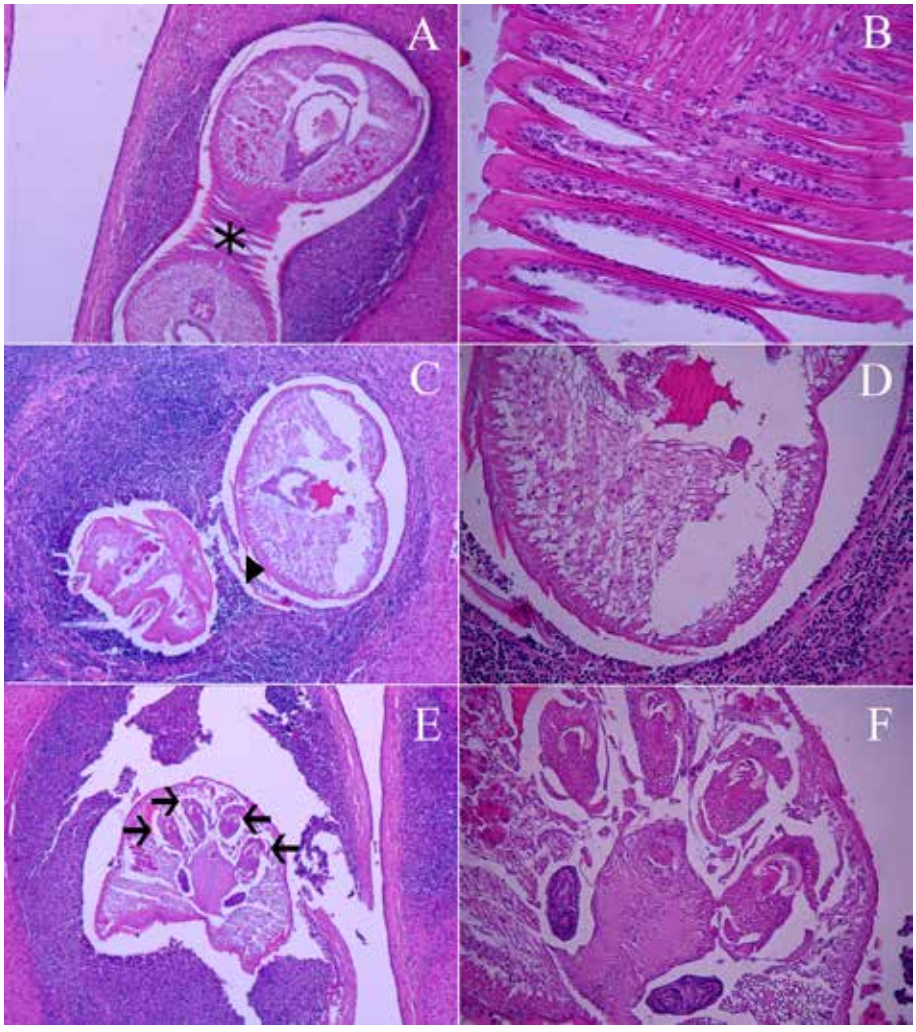

Figure 1. Pentastomid cyst in liver compatible with *L. serrata*, Hematoxylin-Eosin stain. A. longitudinal section of a nymph (10X). B. Transversal grooves detail (asterisk) (40X). C. Transversal section of a nymph with a peripheral inflammatory infiltrate (10X), the arrow indicates the spicules. D. Spicules (arrowhead) (40X). E. Transversal section of nymph head with peripheral inflammatory infiltrate (10X). F. Peribuccal hooks and acidophilic glands (arrows) (40X).

The lesions without the presence of nymphs (n=15) were described and classified in 4 groups of pentastomid granuloma. Pentastomid Granuloma Type 1 (figure 2) (n=1; 8.33%) were formed by a dense, predominantly eosinophilic inflammatory core, accompanied by lymphocytes and macrophages, all circumscribed by a thin band of connective tissue and fibroblasts. In the case of Pentastomid Granuloma Type 2 (figure 2) (n=7; 58.33%), they presented a cavity in the middle of the lesion with extensive loss of tissue. The inflammatory infiltrate was arranged in concentric layers of macrophages, followed by lymphocytes and lesser eosinophils, all circumscribed by a band of fibroblasts and fibrous tissue. Pentastomid Granuloma Type 3 (figure 2) (n=3; 25%) were characterised by tissue loss and a centre surrounded by concentric bands of inflammatory infiltrate constituted in centrifugal order by macrophages and lymphocytes interspersed with fibrous connective tissue and fibroblasts. Occasional foci of mineralisation were randomly identified. Finally, Pentastomid Granuloma Type 4 (figure 2) (n=1, 8.33%) presented a mineralised necrotic centre, surrounded by an inflammatory infiltrate composed of lymphocytes, macrophages and fibroblasts in similar proportions surrounded by areas of dystrophic mineralisation.

These results are similar to those described in humans, which have been used as a reference to make presumptive diagnoses attributable to *L. serrata* (Ma *et al* 2002). However, unlike those observed in human studies, sometimes the granulomas of the present study had a thin eosinophilic, translucent, layered, and acellular structure at its centre compatible with the chitin cuticle surrounding a nymph (figure 1). This structure could appear sectioned, folded or completely covering the interior of the lesion, in intimate contact with macrophages or cellular debris. In such

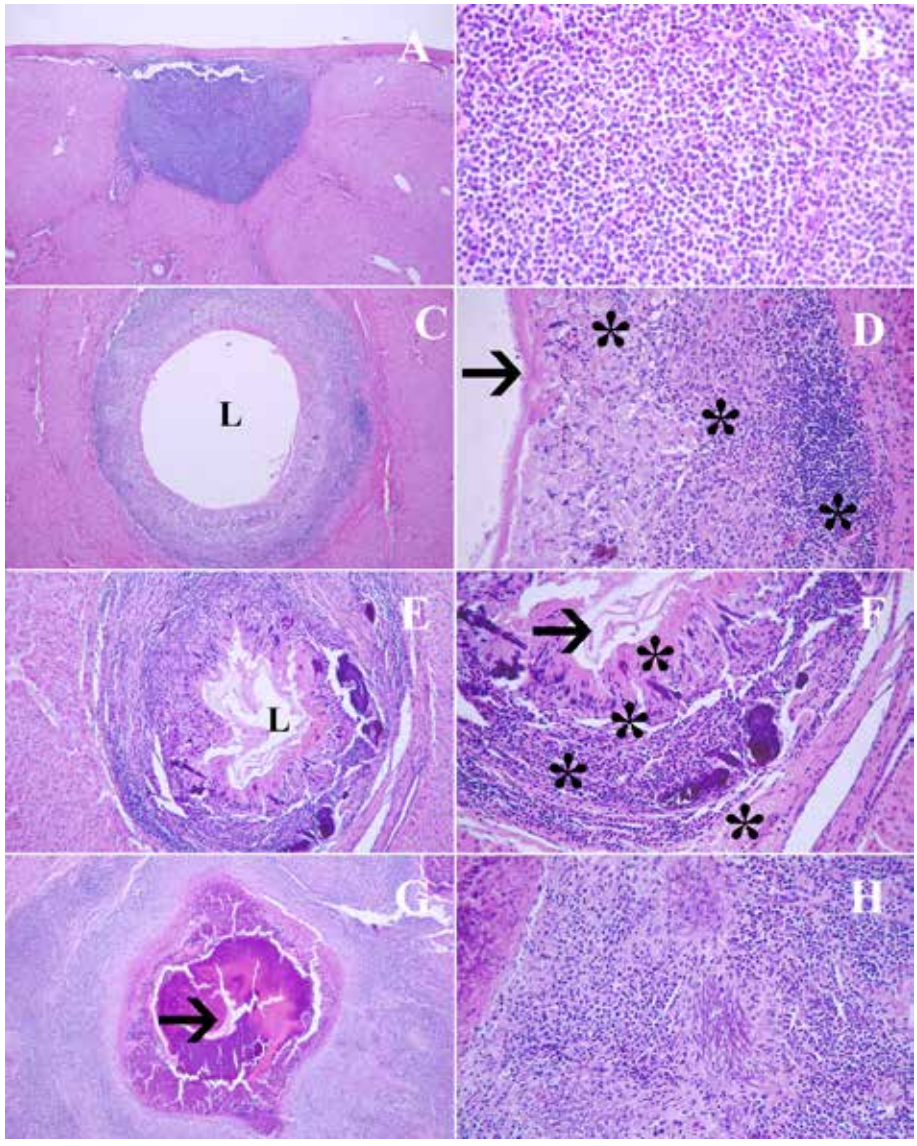

Figure 2. Pentastomid gramulomas in liver, Hematoxylin-eosin stain. A. Subcapsular granuloma type 1 (10X). B. Mixed inflammatory infiltrate with predominantly eosinophilic cells (40X). C. Granuloma type 2 with central lumen (L) (40X). D. Eosinophilic connective tissue around the lumen (arrow) and mixed inflammatory cellular infiltrate (asterisks). E. Granuloma type 3 with irregular lumen and foci of mineralization (10X). F. Eosinophilic structure intraluminal compatible with pentastomid cuticle (arrow), connective tissue (arrowhead) and different inflammatory cellular infiltrates (asterisks). G. Granuloma type 4 with a necrotic and mineralized centre and abundant peripheral inflammatory infiltrate (10X). H. Mixed inflammatory infiltrate around the necrotic tissue (40X).

cases, the pentastomid granulomas would be delivering a sub-etiopathological diagnosis (Tappe and Büttner 2009). In the case of Pentastomid Granuloma Type 1 (n = 1), this was composed of a predominantly eosinophilic inflammatory infiltrate, similar to that observed in lesions with the presence of a nymph and an integral capsule; but no nymphal structures were visualised. Rabeeh *et al* (2017) describe fibrosis of the infected lymph nodes around the parasite in different animals, in some cases indicating a granulomatous reaction formed by giant cells, macrophages, lymphocytes, and eosinophils around the degenerated nymphs.

In this study, it is proposed that all lesions correspond to the different stages and modifications suffered by the nymph in their natural course of degradation and reactions with the surrounding tissue. We propose that the lesion progresses with a nymph with a viable capsule that induces an early eosinophilic response, passing through the progressive capsular and cuticular rupture with almost complete parasitic destruction which leaves a cavity, with parallel recruitment of large numbers of macrophages and lymphocytes around the parasite jointly with the formation of a granuloma similar to the foreign body reaction type. The latter is characterised by the deposit of connective

tissue showing a contraction of the lesion with folding or fragmentation of the internal structures, leading to the filling of the cavity left by the nymph with inflammatory cellular debris and mineralisation.

Regarding the few histopathological descriptive reports existing in animals, further contributions with more detailed characterisations of the lesions induced by *L. serrata* are needed to reach a more consistent presumptive diagnosis. Moreover, recognising and describing the predominant nature of inflammatory responses in lesions could generate a basis for further discussion and a better understanding of the host-parasite interaction of this agent which has importance in both animal productivity and public health.

REFERENCES

Aguilar-Barojas S. 2005. *Fórmulas para el cálculo de la muestra en investigaciones de salud*. Vol.11. Secretaría de Salud del Estado de Tabasco, México, Pp 333-338.

Azizi H, Nourani H, Moradi A. 2015. Infestation and pathological lesions of some lymph nodes induced by *Linguatula serrata* nymphs in sheep slaughtered in Shahrekord Area (Southwest Iran). *Asian Pac J Trop Biomed* 7, 574-578.

Carrillo M, Morales P, Carvallo F, Abarca C. 2017. Prevalencia de linguatulosis hepática en bovinos beneficiados en una planta faenadora de carnes, Talca, Chile. *Rev Investig Vet Perú* 28, 169-177.

Castro J, Naupay A, Fajardo N, Trevejo G, Almeyda V, *et al.* 2015. Prevalencia de ninfas de *Linguatula serrata* en ganado bovino en camales de Lima, Perú. *Rev Investig Vet Perú* 26, 310-316.

Farjanikish G, Shokrani H. 2016. Prevalence and morphopathological characteristics of linguatulosis in one-humped camel (*Camelus dromedarius*) in Yazd, Iran. *J Parasitol Res* 115, 3163-3167.

Gibson-Corley K, Olivier A, Meyerholz D. 2013. Principles for valid histopathologic scoring in research. *Vet Pathol* 50, 1007-1015.

Gunn A, Pitt SJ. 2012. Arthropod parasites. In: *Parasitology: An Integrated Approach*. Wiley Blackwell, West Sussex, UK, Pp 151-153.

Hajipour N, Soltani M, Mirshekar F. 2019. Effect of age, sex, and season on the prevalence of *Linguatula serrata* infestation in mesenteric lymph nodes of goats slaughtered in Tabriz, Iran. *Trop Anim Health Prod* 51, 879-885.

Hajipour N, Tavassoli M. 2019. Prevalence and associate factors of *Linguatula serrata* infection in definitive and intermediate hosts in Iran and other countries: A systematic review. *Veterinary Parasitology: Regional Studies and Reports* 16,100288.

Hamid T, Hossein YD, Mehran BB, Masood F, Hamid E. 2012. A case report of *Linguatula serrata* infestation from rural area of Isfahan City, Iran. *Adv Biomed Res* 1, 42.

Ma K, Qiu M, Rong Y. 2002. Pathological differentiation of suspected cases of pentastomiasis in China. *Trop Med Int Health* 7, 166-177.

Machado MA, Makdissi FF, Canedo LF, Martino RB, Crescentini F, *et al.* 2006. Unusual case of pentastomiasis mimicking liver tumor. *J Gastroenterol Hepatol* 21, 1218-1220.

Ministerio de Salud. 2002. *Norma general técnica sobre inspección médico veterinaria de las reses de abasto y de sus carnes y criterios para la calificación de aptitud para el consumo humano*. Ministerio de Salud, Santiago, Chile.

Paredes E, Muñoz M. 2016. Descripción histopatológica de ninfas de *Linguatula serrata* en hígados de bovinos faenados en Valdivia, Chile. *Rev Med Vet* 9, 73-81.

Tabaripour R, Fakhar M, Alizadeh A, Reza Youssefi M, Tabaripour R, *et al.* 2017. Prevalence and histopathological characteristics of *Linguatula serrata* infection among slaughtered rumiants in Mazandaran Province, northern Iran. *Comp Clin Pathol* 26, 1259-1265.

Tappe D, Winzer R, Bütnner D, *et al.* 2006. Linguatulosis in Germany. *Emerg Infect Dis* 126, 1034-1036.

Tappe D, Büttner D. 2009. Diagnosis of human visceral pentastomiasis. *PLoS Negl Trop Dis* 3, 320.

Taylor M, Coop R, Wall R. 2016. *Veterinary Parasitology*. 4th ed. Wiley Blackwell, West Sussex, UK, Pp 257-258.

Valenzuela G, Bascuñan M, Bayer L, Ernst S. 1995. *L. serrata* infections in bovine livers in Valdivia, Chile. *Arch Med Vet* 27, 29-34.

Yakhchali M, Tehrani AA. 2013. Histopathological changes caused by the nymph stage of *Linguatula serrata* in the mesenteric lymph nodes of goats. *Acta Vet Hung* 61, 36-41.

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SHORT COMMUNICATION

Variability of cranial morphometrical traits in Suffolk Down Sheep

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ABSTRACT. The widespread use of measures and indices associated with the head for racial analysis suggests that such measures have a strong relationship with the underlying bone structure. Few studies analyse the variability of the bones of the head and the relationship with their external expression. The objective of this work was to identify and measure the magnitudes of the main skull parameters in Suffolk Down adult sheep. This study was carried out on sixteen adult Suffolk Down sheep skulls at INIA Butalcura. Their skeletons were obtained and digital morphometry was performed. Each skull was photographed from dorsal, ventral, lateral and nuchal views with a total of 28 parameters evaluated (10 dorsal, 5 ventral, 6 lateral and 6 nape). The results indicate that the externally observable variability in the cranial zone of a sheep cannot be extrapolated to the rest of the bony components of the cranial zone, either in length, width or height. It was observed that the variability of a cephalic dimension can be contrasted with the variability of individual bones that participate in a certain dimension as part of a plasticity adjustment mechanism independent of the genetic variability of each bone separately. The cranial dimensions are still useful in defining the productive potential of a sheep population; however, they should be taken cautiously for racial definitions, where the individual variability of the bones could be a better reflection of the genetic structure of the population and the dimensionality could be biased by adaptive plasticity.

Key words: morphology, sheep, skull, animal ethnology.

INTRODUCTION

The body architecture of an animal biotype is the result of several factors that influence the predominant gene pool of a population and the expression of the characteristics that codify these genes (Thiagarajan and Jayashankar 2012). As a consequence, the animal architecture of sheep has been used for racial characterisation (Álvarez *et al* 2000, Riva *et al* 2004, Herrera and Luque 2009, Parés *et al* 2010, Bravo Sepúlveda 2010, De la Barra *et al* 2016, Baranowsky 2017). Zoometric indexes have also been used for this purpose, especially cephalic ones, because the cranial zone would be less influenced by environmental and management factors (Parés *et al* 2010, Özcan *et al* 2010, Ilayperuma 2011, Mohamed *et al* 2016), given that aspects such as the founder effect, hybridisation, inbreeding, and selection itself would alter animal body architecture by privileging certain genetic combinations in the offspring to the detriment of other gene mixtures (Sierra 2001, Latorre *et al* 2011, Chirinos 2011, De la Barra *et al* 2016). In this regard, the dimensions of the skull are those that allow a better taxonomic affiliation of an animal, and can even provide valuable information about the changes that occur over time in a population as a result of selection (Brüenner *et al* 2002, Cobb & O'Higgins 2007, Parés *et al* 2010, Ilayperuma 2011). In this way, several authors point out that the main racial differentiators are found in the head of the animal (Aparicio 1960, Sánchez Belda 1964, Sotillo and Serrano 1985, Agüera *et al* 1988, Miró *et al* 1988, Parés *et al* 2010), that is, the differences at the skull level are more defining of a breed than those found in the rest of the skeleton. Thus, craniocephalic topography allows obtaining topometric data that facilitate breed discrimination (Miró *et al* 1988, Mohamed *et al* 2016).

Despite the aforementioned, the skull in mammals is a complex of assembled bones with great adaptive capacity (Ravosa *et al* 2000, Thomason *et al* 2001), which could indicate a certain degree of variability at the level of its components. This could allow a greater specificity when using body measurements or zoomometric indexes in breed evaluation. With regard to animal selection, it is necessary to consider that, in the absence of other means of selection, morphology is an essential visual characteristic to differentiate and select biotypes, since it allows projecting the distinctive attributes of the corporality of an animal population in their offspring using such phenotypic criteria (Sierra 2001). In this sense, several authors agree that biological functionality is conditioned by the underlying form of the animal biotype (Bravo and Sepúlveda 2010, Chirinos 2011, De la Barra *et al* 2016, Macedo *et al* 2016, Popoola and Oseni 2018).

Thus, it is relevant to generate detailed information about the cranial parameters of the sheep to determine if the externally observed aspects are confirmed in the internal cranial structure, making the diagnosis and racial evaluation more accurate. The working hypothesis was that the variability of the skull components is similar to that of their total dimensions. The objective of this work was to identify and measure the magnitudes of the main skull parameters in adult Suffolk Down sheep.

MATERIAL AND METHODS

The present study was performed on sixteen skulls extracted from the skeletons of adult Suffolk Down sheep from the research flock of INIA Butalcura. Suffolk Down

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was used since it is a selected and widely distributed breed that allows its measurements to be confirmed by different researchers, and at the same time it can be used as a comparison pattern with other breeds. All the evaluated animals were females between 7 and 8 years of age. The weight of the animals varied between 80 and 90 kg. The skulls came from 16 sheep unrelated to each other. The animals corresponded to females selected according to racial origin that came from different hatcheries and were integrated as a basal breeding nucleus in a plant. Their offspring were later selected by birth weight and weaning weight. However, these selection criteria were not applied to the selection. The skeletons had no anomalies or evidence of diseases that affected their normal development. To eliminate the remains of fat, meat and connective tissue, the skeletons were macerated in water with the addition of potassium hydroxide, using the technique of Olopade and Onwuka (2004), subjecting them to heating at 100 °C for 60 minutes. Subsequently, a water change was made and they were subjected again to the same temperature and time. Once cleaned, digital morphometry was performed on the skulls. Each skull was photographed from four angles (dorsal, ventral, lateral and nape) using a digital camera installed in a lectern, and a measurement scale was included next to each skull. The set of photographs was digitally measured using tools from the Power Point program. Twenty-eight parameters were measured (11 dorsal, 5 ventral view, 6 lateral and 6 nape view) (figure 1) and analysed using the Excel program. The normality of the data was analysed through the Llilie test (Kolmogorov Smirnov). The parameters to be evaluated, which were defined by Choudhary and Singh (2016), are detailed below:

Dorsal view

1. Lsk: Distance between the highest points of the parietals to the middle of the rostral margin of the incisive bone.
2. Wcr: Maximum distance between the bases of the horn buttons.
3. Lfa: Distance from the frontonasal suture to the centre of the incisive bone.
4. Wfa: Distance between the caudal extents of the orbital rims.
5. Lna: Distance from the central point of the frontonasal suture to the rostral end of the internasal suture.
6. Wna: Maximum distance across the nasal bones or maximum distance between the nasomaxillary sutures.
7. Lfr: Maximum length between parietofrontal suture and frontonasal suture.
8. Lfrs: Length of the frontal suture.
9. Ls: Length of the parietal bone.
10. Ws: Maximum distance between the zygomatic arches (total head width)

Ventral view

11. LBsk: Distance between the midpoints of the dorsal margin of the foramen magnum to the level of the middle point of the rostral margin of the incisive bone.
12. Lpa: Distance measured from the rostral mid sutured line of incisive bone to the caudal nasal spine of the palatine bone.
13. Wpa: Maximum distance at the horizontal plate of palatine bone behind the last molar tooth.
14. Hfm: The distance between the midpoints of the dorsal-ventral rims of the foramen magnum.
15. Wfm: The maximum distance between the two occipital condyles.

Lateral view

16. Lo: The perpendicular distance between the supraorbital and infraorbital margins of the orbit.
17. Wo: The horizontal distance between the rostral and caudal margins of the orbital rim.
18. Lpm: Maximum length of premaxilla.
19. Lma: Length of maxilla.
20. Hpm: Height of premaxilla.
21. Hma: Length of lacrimal.

Nape view

22. Hoc: Distance from base of the occipital condyle to the starting point of sagittal crest.
23. Wic: Width between the lateral ends of the occipital condyles.
24. Wipc: Width between the lateral ends of the paracondylar process.
25. Hpa: Maximum height of parietal.
26. Wpa: Maximum width of parietal.
27. Woc: Maximum point of parietal to the foramen magnum.

RESULTS AND DISCUSSION

The values of the measurements made on the sheep skulls evaluated are shown in table 1. The dimensions of length, width and height of the head are determinant from the point of view of racial qualification and evaluation. In this way, the length and width of the head and skull are variables widely used in breed evaluation (Aparicio 1960, Sánchez Belda 1964, Sotillo and Serrano 1985, Agüera *et al* 1988, Miró *et al* 1988, Parés *et al* 2010, Mohamed *et al* 2016, Macedo *et al* 2016). However, this does not occur with height, since it is a dimension that is difficult to measure and, therefore, it is only qualitatively qualifiable by describing the imaginary triangle that occurs between the observable height and the length of the head.

The support behind each of these dimensions responds to different bone structures and must be analysed in detail to interpret the variations of these dimensions in different animals (Ravosa *et al* 2000, Thomason *et al* 2001) since it can be determined by the variability of a bone or the cumulative variation of all of them.

The total observable cephalic length from the dorsal view corresponded on average to 23.83 cm, ranging between 21.42 and 27.03 cm, with a coefficient of variation of

Table 1. Values of skulls parameters in adult Suffolk Down sheep.

Variable	Average (cm)	Standard deviation	Coefficient of variation (%)	Minimum (cm)	Maximum (cm)
		Dorsal view			
Lsk	23.83	2.07	8.68	21.42	27.03
Wcr	5.44	0.38	6.02	4.76	5.78
Lfa	15.33	1.85	12.07	12.24	17.17
Wfa	8.58	0.61	7.07	7.14	9.01
Lna	9.29	0.82	8.86	8.33	10.37
Wna	3.1	0.37	12.01	2.72	3.74
Lfr	8.05	0.82	10.21	7.31	9.69
Lfrs	5.19	0.22	4.27	4.93	5.61
Ls	3.56	0.54	15.02	3.06	4.42
Ws	12.87	0.59	4.56	12	14.11
		Ventral view			
LBsk	23.73	1.24	5.21	22.44	25.84
Lpa	8.42	0.33	3.87	7.99	8.84
Wpav	4.97	0.74	14.79	4.08	5.95
Hfm	1.69	0.29	16.72	1.36	2.04
Wfm	2.57	0.46	18.07	2.04	3.23
		Lateral view			
Lo	4.7	0.46	9.67	3.91	5.1
Wo	4.98	0.35	7.04	4.42	5.61
Lpm	8.75	1.14	13.06	7.48	9.86
Lma	12.32	1.74	14.11	10.71	15.13
Hpm	1.5	0.16	10.41	1.28	1.7
Hma	7.89	0.12	1.51	7.68	7.99
		Nape view			
Hoc	12.24	0.27	2.23	11.9	12.58
Wic	7.84	1.03	13.18	6.46	9.69
Wipc	11.28	0.16	1.38	11.05	11.56
Hpa	4.31	0.13	2.93	4.08	4.42
Wpan	10.56	0.11	1.03	10.54	10.71
Woc	8.59	1.22	14.2	7.14	10.74

8.68% (Lsk, table 1). The longitudinal dimension showed acceptable variability for a breed (less than 10% according to Parés *et al* 2010). Regarding the magnitude of Lsk, Agüera *et al* (1988) reported values of 28.04 and 27.28 cm for the Spanish Segureña and Merino breeds, respectively, while Parés *et al* (2010) found 26.55 cm for the Xisqueta breed, values that are much higher than those observed in this study where even the range of maximum values of the sample did not reach those previously reported by others. On the other hand, the Lsk values obtained in this study outperforms those found in Turkish breeds such as Morkmarán and Tuj (20.44 and 19.80 cm, respectively) or Iranian breeds such as Mehraban (20.06 cm), which show a fairly high coefficient of variation of 22.58% (Karimi *et al* 2011).

However, in this dimension some bones such as the occiput, parietal, frontal, nasal, and premaxilla are totally or partially involved. Therefore, several intermediate parameters are measured.

The length of the parietal bone (Ls) presented an average of 3.56 cm, in a range between 3.06 and 4.42 cm, with a coefficient of variation of 15.02%. This means that this parameter of the cranial area exhibits a variation higher than that acceptable for a racial population (Aparicio 1960), although externally this variation in total length of the head (Lsk) is not observed. Similarly, the frontal bone

in the longitudinal dimension provides two parameters, Lfrs with an average of 5.18 cm, a range of 4.93 to 5.61 cm, and a coefficient of variation of 4.27%, and Lfr with an average of 8.05 cm, a range of 7.31 to 9,69 cm, and coefficient of variation of 10.20%. Therefore, Lfrs has a low variability and Lfr is at the acceptable limit for a breed. Therefore, it can be seen that, within the appreciable longitudinal dimension from the outside of the animal, there are underlying arrangements of the set of bones that are hidden by the external variability of the head. The Lfr exceeds in magnitude the values for Turkish breeds such as Morkmarán and Tuj (7.37 and 7.00 cm, respectively), their range does not include them because they are larger (Özcan *et al* 2010), and is lower than those found in the Segureña breed (9.59 cm) by Parés *et al* (2010).

The nasal bone, represented by the facial length (Lfa), also intervenes in the longitudinal expression analysed from the dorsal view, reaching an average of 15.32 cm with a range between 12.24 to 17.17 cm and coefficient of variation of 12.07%, expressing high morphometric variability. This parameter exceeds in magnitude the values for Turkish breeds such as Morkmarán (14.03 cm) and Tuj (13.67 cm) (Özcan *et al* 2010). It is also higher than the value for the Iranian breed Mehraban (12.54 cm) which exhibits a coefficient of variation of 9.88% (Karimi *et al* 2011).

Finally, the premaxillar bone is also involved through the nasal length (Lna), with an average of 9.29 cm, varying between 8.33 and 10.37 cm and with a variation coefficient of 8.86%. This parameter was lower than that reported for Turkish breeds such as Morkmarán (14,03) and Tuj (13.67 cm), (Özcan *et al* 2010), although it is higher than the value shown by the Iranian race Mehraban (8.08 cm) which reports a slightly higher coefficient of variation, with 11.75% (Karimi *et al* 2011).

Although the total head length (Lsk) showed a normal variability, out of the 5 evaluated parameters intervening in the complex of bones that constitute the longitudinal dimension, three (Ls, Lfr and Lfa) overcome the accepted variability in a well-structured breed population. This suggests that an external expression of low or normal variability does not necessarily indicate that such variability reaches those canons in all the bones that make up that dimension.

When analysing the longitudinal dimension in the ventral view there are three parameters involved, one in the occipital bone area (Hfm), the second between the palatine and maxilla (Lpa) and a longer third, which includes part of the sphenoid, palatal, maxillary, and premaxillary bones (Lbsk) (figure 1). The length of the foramen magnum (Hfm) had an average of 1.68 cm, varying between 1.36 and 2.04 cm and reaching a variation coefficient of 16.71%, which reveals a high variability for this component. In its

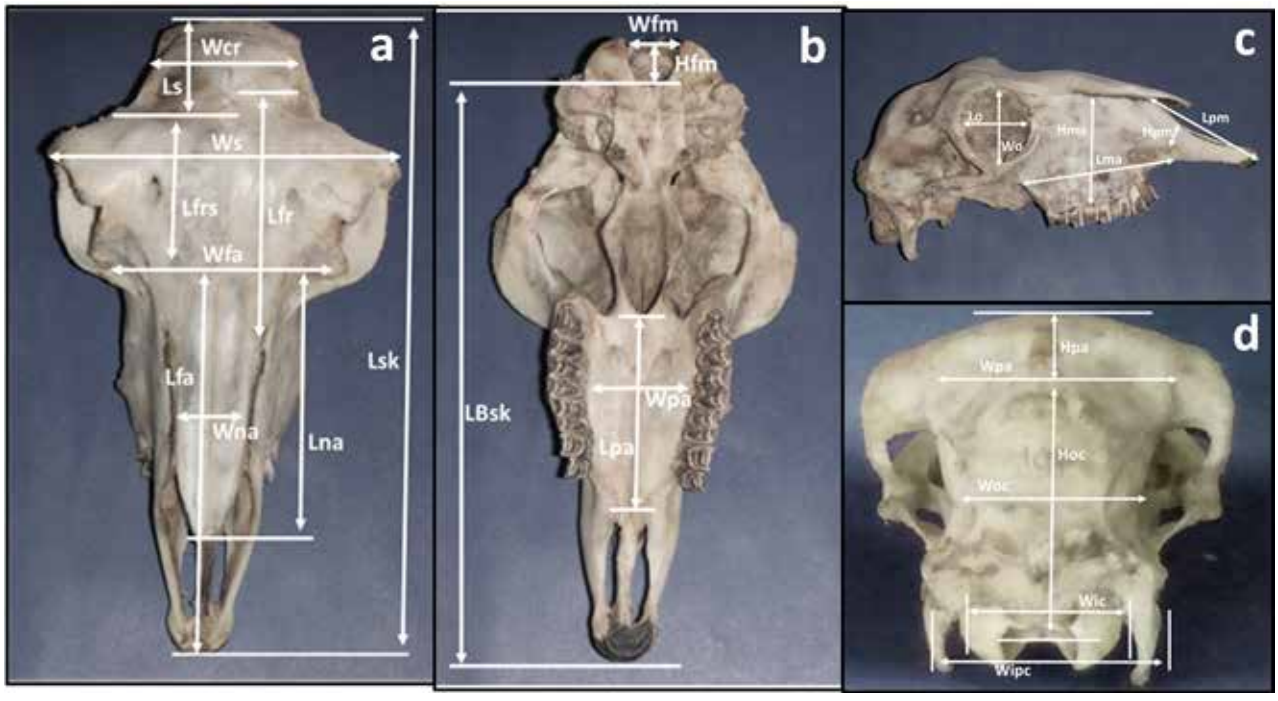

Figure 1. Skull measurements; a) Dorsal view showing skull length (Lsk), cranial width (Wcr), facial length (Lfa), facial width (Wfa), nasal length (Lna), nasal width (Wna), frontal length (Lfr), frontal short length (Lfrs), parietal bone length (Ls) and total head width (Ws); b) Ventral view showing skull length (LBsk), palatine length (Lpa), palatine width (Wpa), height (Hfm) and width (Wfm) of the foramen magnum (Hfm); c) Lateral view showing length of orbit (Lo), width of orbit (Wo), length of premaxilla (Lpm), length of maxilla (Lma), height of premaxilla (Hpm) and height of maxilla Hma; c) Nape view showing height of occipital (Hoc), intercondylar width (Wic), interparacondylar width (Wipc), height of parietal (Hpa), width of parietal (Wpa) and occipital width (Woc).

magnitude, the foramen magnum length was lower than that of Turkish breeds such as Morkmarán and Tuj (1.94 and 1.78 cm, respectively), but including them in the range (Özcan *et al* 2010). It is also lower than the value of the Iranian race Mehraban (1.92 cm), which exhibits a coefficient of variation of 6.25% (Karimi *et al* 2011). The palatine length (Lpa) reached an average of 8.41 cm (range of 7.99 to 8.84 cm), showing a low variability with a variation coefficient of 3.87%. The Lbsk parameter, with a length that goes from the ventral rims of the foramen magnum to the rostral margin of the incisive bone, exhibited an average of 23.73 cm (range of 22.44 to 25.84 cm) with a coefficient of variation of 5.20%. In Morkmarán and Tuj sheep, this parameter reaches 18.23 and 17.55 cm, respectively (Özcan *et al* 2010), and in the Iranian sheep Mehraban is also smaller (21.48 cm) with a coefficient of variation of 10.01%, which is normal for a breed (Karimi *et al* 2011), while in Xisqueta breed it is 22.31 cm (Parés *et al* 2010). These data indicate a higher value for these breeds. In the context of a cephalic total length (Lsk) it exhibits normal to low variability. In the case of Lbsk, the Xisqueta sheep shows a coefficient of variation of 3.2% (Parés *et al* 2010), suggesting that in the bone complex that intervenes in the longitudinal dimension, the low variability of some parameters may hide the high variability of others or vice versa, being part of the plasticity proposed by Thomason *et al* (2001).

Three parameters intervene in the side view, which are the length of the eye socket (Lo), the length of the premaxilla (Lpm), and the length of the maxilla (Lma) (figure 1). Lo exhibited an average of 4.70 cm (range of 3.91 to 5.10 cm) with a coefficient of variation of 9.67%. In its magnitude, Lo exceeded Turkish breeds such as Morkmarán and Tuj (3.61 and 3.66 cm, respectively) and its range was higher, and therefore it does not include them (Özcan *et al* 2010).). It is also higher than the value in the Iranian breed Mehraban (3.64 cm), which exhibits a coefficient of variation of 5.76% (Karimi *et al* 2011). The length of the premaxilla showed an average of 8.75 cm (range of 7.48 to 10.20 cm), with a coefficient of variation of 13.05%. In its magnitude, the Lpm exceeded the mentioned Turkish breeds (6.22 and 5.85 cm, respectively) and its rank does not include them because it is higher (Özcan *et al* 2010). On the other hand, the length of the maxilla (Lpm) showed an average of 12.31 cm (range of 10.88 to 15.13 cm) with a coefficient of variation of 14.01%. In this case, one of the three parameters (Lo) exhibited a normal variability, while the other two (Lpm and Lma) showed high variability. The parameters of the nape view do not influence the longitudinal dimension.

A second dimension is given by the total head width (Ws), which reached a dorsal view average (figure 1) of 12.87 cm (range 12.0 to 14.11 cm), with a coefficient of variation of 4.56% (table 1), showing low variability than desirable for a well-structured breed population. This dimension is higher than that observed in Iranian Mehraban sheep (10.44 cm), which also exhibit a reduced coefficient of variation of 7.37% (Karimi *et al* 2011). It is interesting to note that, in dorsal view, the width only involves the frontal bone. In this dimension, three parameters were evaluated: the cranial width parameter (Wcr), with a mean value of 5.44 cm (range 4.76 to 5.78 cm), with a coefficient of variation of 6.02% (figure 1; table 1); the facial width (Wfa), which partially incorporates the frontal and lacrimal bone and exhibited a mean (figure 1) of 8.58 cm (range of 7.14 to 9.01 cm) with a coefficient of variation of 7.07 (table 1). The value is lower than that of Mehraban (10.68 cm), which exhibits a similar coefficient of variation (7.20%) (Karimi *et al* 2011), and finally, the nasal width (Wna) involving only the nasal bone, that showed a mean (figure 1) of 3.10 cm (range 2.72 to 3.74 cm) with a coefficient of variation of 12.00% (table 1). The value is higher than the value thrown by the Mehraban breed (2.88 cm), which exhibits a coefficient of variation of 13.54% (Karimi *et al* 2011).

In the dorsal view of the three parameters evaluated, two showed low variability (Wcr and Wfa), while Wna had a variability of over 10%.

The dimension of the width in the ventral view was evaluated through two parameters: the width of the palatine (Wpav) and the width of the foramen magnum (Wfm). The width of the palatal (Wpav), which involves as its name indicates the palatal bone, but also partially to the maxilla, exhibited a mean (figure 1) of 4.97 cm (range of 4.08 to 5.95 cm) with a coefficient of variation of 14.79% (table 1). On the other hand, the width of the foramen magnum (Wfm), which only involves the occipital bone, showed a mean (figure 1) of 2.57 cm (range of 2.04 to 3.23 cm) with a coefficient of variation of 18.07% (table 1). The reference shown by Mehraban breed (1.97 cm), is smaller and exhibits a coefficient of variation also smaller (6.59%) (Karimi *et al* 2011). A high variability at the width of the palatal and the foramen magnum was appreciated in the ventral view.

The analysis of the lateral view only considered the analysis of the width of the ocular orbit (Wo), which involves the frontal, zygomatic, and lacrimal bone. This parameter showed a mean (figure 1) of 4.98 cm (range of 4.42 to 5.61 cm) with a variation coefficient of 7.04% (table 1). The value is slightly lower than that of the Mehraban sheep (5.11 cm), which exhibited a coefficient of variation of 9.98% (Karimi *et al* 2011). The occipital, parietal and interparietal bones participate in the nape view (figure 1). Four parameters were evaluated; the parietal width (Wpan), the occipital width (Woc), the intercondylar width (Wic) and the interparacondylar width (Wipc). The parietal width (Wpan) showed a mean (figure 1) of 10.56 cm (range 10.37 to 10.71 cm) with a coefficient of variation of 1.03% (table 1), that is, practically without variation. The occipital width (Woc) exhibited a mean (figure 1) of 8.59 cm (range 7.14 to 10.71 cm) with a coefficient of variation of 14.20% (table 1). On the other hand, the

intercondylar width (Wic), showed a mean (figure 1) of 7.84 cm (range 6.46 to 9.69 cm) with a coefficient of variation of 13.18% (table 1). This parameter broadly exceeded Turkish breeds such as Morkmarán and Tuj (4.41 and 4.45 cm, respectively), and the range also exceeds them (Özcan *et al* 2010). Finally, the interparacondylar width (Wipc) reached a mean (figure 1) of 11.28 cm (range of 11.05 to 11.56 cm) with a coefficient of variation of 1.38% (table 1). For this parameter, the measurements also exceed those of the Turkish breeds (5.78 and 5.72 cm, respectively), and even the range excludes them (Özcan *et al* 2010). In this dimension of the nape view, there was wide variability in the occipital (Woc) and intercondylar (Wic) width. In contrast, the parietal width (Wpan) and the interparacondylar width (Wipc) showed a very low variability.

In the cephalic altitudinal dimension, the dorsal dimension and ventral view do not intervene. In the side view, two parameters that can affect the height are considered: the height of the premaxilla (Hpm) and the height of the maxilla (Hma). The height of the premaxilla (Hpm) showed a mean (figure 1) of 1.49 cm (range of 1.28 to 1.70 cm) with a coefficient of variation of 10.40% (table 1). On the other hand, the height of the maxilla (Hma) had a mean (figure 1) of 7.89 cm (range of 7.68 to 7.99 cm) with a coefficient of variation of 1.51% (table 1). The first shows variability in the acceptable limit for one breed, and the second a very low variability.

The dorsal and ventral view does not intervene in the cephalic altitudinal dimension. In the side view, two parameters that can affect the height were considered, the height of the premaxilla (Hpm) and the maxilla (Hma). Hpm showed a mean (figure 1) of 1.49 cm (range 1.28 to 1.70 cm) with a coefficient of variation of 10.40% (table 1), in the acceptable limit for one breed. On the other hand, Hma showed a mean (figure 1) of 7.89 cm (range 7.68 to 7.99 cm) with a very low coefficient of variation (1.51%) (table 1).

The cephalic height analysed from the nuchal view considers the intervention of the occipital and parietal bones and is carried out through the measurement of two parameters, the occipital (Hoc) and parietal (Hpa) height (figure 1). Hoc showed a mean (figure 1) of 12.24 cm (range 11.90 to 12.58 cm) with a coefficient of variation of 2.22% (table 1). Hpa exhibited a mean (figure 1) of 4.31 cm (range 4.08 to 4.42 cm) with a coefficient of variation of 2.93% (table 1), both parameters having low variability.

There are not many specific studies carried out on cranial parameters in sheep; however, there are relevant differences between the different breeds, suggesting the need for further investigations. Since the bibliography reports a high cranial variability intraspecies, this is a relevant aspect to consider because a significant number of studies to characterise sheep breeds use cranial measurements (Rodríguez *et al* 1990, Álvarez *et al* 2000, Riva *et al* 2004, Özcan *et al* 2010, Parés *et al* 2010, Latorre *et al* 2011, Mujica *et al* 2012). In this sense, indexes such as cranial or cephalic are used to describe the potential or productive specialisation of a sheep breed (Bravo and Sepúlveda 2010, De la Barra *et al* 2016, Baranowsky 2017).

However, it is possible to speculate that since the bone structure would be less influenced by specific environmental effects (Parés *et al* 2010, Özcan *et al* 2010, Ilayperuma, 2011, Mohamed *et al* 2016), complex bone arrangements could be made that allow the animal to acquire a biomechanical functionality through the plastic adaptation of the cranial components, generating an immediate adjustment mechanism, beyond the genetic variability of each bone separately (Thomason *et al* 2001). From this point of view, the cranial dimensions are still useful in defining the productive potential of a sheep population; however, they should be taken with caution for racial definitions, where the individual variability of the bones could be a better reflection of the genetic structure of the population.

Finally, regarding the analysed data, it is not possible to state that the externally observable variability in the cranial area of a sheep can be projected to each of the bone components of this area, whether in length, width, or height dimensions. Therefore, it is possible to observe that the external variability might contrast with the variability of the individual bones that participate in a certain dimension. A more comprehensive and multivariate analysis could be required to analyse the underlying shape to perform more precise analyses (Salako 2006, Toro *et al* 2010). However, it can be speculated that, since the bone structure would be less influenced by specific environmental effects (Parés *et al* 2010, Özcan *et al* 2010, Ilayperuma 2011, Mohamed *et al* 2016), complex bone arrangements could be made, that allow the animal to acquire a biomechanical functionality through the plasticity or a mechanism of adaptation of the cranial components, generating an immediate adjustment mechanism, beyond the genetic variability of each bone separately (Thomason *et al* 2001). From this point of view, the cranial dimensions are still useful in defining the productive potential of a sheep population; however, they should be taken with caution for breed definition purposes, where the individual variability of the bones could be a better reflection of the genetic structure of the population instead of the dimensional variability, which could have a relevant environmental bias.

It is concluded that it is not possible to affirm that the externally observable variability in the cranial area of a sheep can be projected to each one of the bony components of the cranial zone, whether in the dimension of length, width, or height. It was observed that the variability of a cephalic dimension, whether to the length, width or height, can be contrasted with the variability of the individual bones that participate in a given dimension as part of a mechanism of wide plasticity or adjustment, independent of the genetic variability of each bone separately. The cranial dimensions are still useful in defining the productive potential of a sheep population; however, they should be taken with caution for breed definition purposes, where

the individual variability of the bones could be a better reflection of the genetic structure of the population, and the dimensionality could be biased by adaptive plasticity.

REFERENCES

Agüera S, Castejón F, Diaz A, Miró F, López Rivero J. 1988. Diferenciación radiológica en ovejas manchegas y merinas. *Archivos de Zootecnia* 37, 205.

Alvarez S, Fresno M, Capote J, Delgado J, Barba C. 2000. Estudio para la caracterización de la Raza ovina Canaria. *Archivos de Zootecnia* 49, 209-215.

Aparicio G. 1960. *Zootecnia especial. Etnología compendiada*. Imprenta Moderna, Córdoba, Argentina.

Baranowsky P. 2017. Craniometric characteristics and cranial indices of Polish Heath sheep rams - extended data. *Int J Morphol* 35, 133-140.

Bravo S, Sepúlveda N. 2010. Indices zoométricos en ovinos criollos Araucanos. *Int J Morphol* 28, 489-495.

Brüenner H, Lugon-Moulin N, Balloux F, Fumagalli L, Hausser J. 2002. Taxonomical re-evaluation of the Valais chromosome race of the common shrew. *Sorex araneus* (Insectivora: Soricidae). *Acta Theriol* 47, 245-275.

Carneiro H, Louvandini H, Paiva S, Macedo F, Mernies B, *et al*. 2010. Morphological characterization of sheep breeds in Brazil, Uruguay and Colombia. *Small Rum Res* 94, 58-65.

Chirinos Z. 2011. La funcionalidad animal, herramienta esencial para el mejoramiento del rebaño bovino. In: *Innovación y tecnología de la ganadería doble propósito*. Universidad del Zulia, Zulia, Venezuela, Pp 217-223.

Choudhary P, Singh I. 2016. Morphological and radiographic studies on the skull of Indian blackbuck (*Antilope cervicapra*). *Int J Morphol* 34, 775-783.

Cobb S, O´Higgins P. 2007. The ontogeny of sexual dimorphism in the facial skeleton of the African apes. *J Hum Evol* 53, 176-190.

De la Barra R, Martínez ME, Carvajal AM. 2016. Morphostructural relationships and productive functionality of sheep breeds used for terminal crossbreeding in Chile. *Int J Morphol* 34, 958-962.

Herrera M, Luque M. 2009. Morfoestructura y sistemas para el futuro en la valoración morfológica. In: Sañudo AC (ed). *Valoración morfológica de los animales domésticos.* Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid, España, Pp 79-109.

Ilayperuma I. 2011. Evaluation of cephalic indices: A clue for racial and sex diversity. *Int J Morphol* 29, 112-117.

Karimi I, Onar V, Pazvant G, Hadipour M, Mazaheri Y. 2011. The mranial morphometric and morphologic characteristics of Mehraban sheep in Western Iran. *Global Veterinaria* 6, 111-117.

Latorre E, Uribe H, Martínez ME, Calderón C, De la Barra R. 2011. Morphology differentiation and structural functionality of ewes due to uncomplete crossbreeding. *Int J Morphol* 29, 954-959.

Macedo R, Arredondo V, Cervantes A. 2016. Head and tail morphology of Pelibuey, Katahdin and Blackbelly rams in Colima, México. *Vet México* 3, 1-9.

Miró F, Diaz A, López-Rivero J, Regodon S. 1988. Determinación de algunos parámetros cefálicos del vacuno de raza Retinta. *Arch Zootec* 37, 75.

Mohamed R, Driscoll M, Mootoo N. 2016. Clinical anatomy of the skull of the Barbados Black Belly sheep in Trinidad. *Int J Curr Res Med Sci* 2, 8-19.

Mujica F, Mella J, De la Barra R, Blanco A. 2012. Phenotipic characterization of the sheep breed Creole Chilota and two sheep breeds that predominate in southern Chile. *Actas Iberoamericanas de Conservación Animal* 2, 67-70.

Olopade J, Onwuka S. 2004. Morphometric studies of the cranio-facial region of the West African Dwart goat in Nigeria. *Int J Morphol* 22, 145-148.

Özcan S, Aksoy G, Kürtul I, Aslan K, Özüdogru, Z. 2010. A comparative morphometric study on the skull of the Tuj and Morkaraman sheep. *Kafkas Univ Vet Fak Derg* 16, 111-114.

Parés I, Kamal S, Jordana J. 2010. On biometrical aspects of the cephalic anatomy of Xisqueta sheep (Catalunya, Spain). *Int J Morphol* 28, 347-351.

Popoola MA, Oseni SO. 2018. Multifactorial discriminant analysis of cephalic morphology of indigenous breeds of sheep in Nigeria. *Slovak J Anim Sci* 51, 45-51.

Ravosa M, Noble V, Hylander W, Johnson K, Kowalski E. 2000. Masticatory stress, orbital orientation and the evolution of the primate postorbital bar. *J Human Evol* 38, 667-693.

Riva J, Rizzi R, Marelli S, Cavalchini L. 2004. Body measurements in Bergamasca sheep. *Small Rum Res* 55, 221-227.

Rodríguez P, Tovar J, Rota A, Rojas A, Martín L. 1990. El exterior de la cabra Verata. *Archivos de Zootecnia* 43, 57.

Salako A. 2006. Application of morphological indexes in the assessment of type and function in sheep. *Int J Morphol* 24, 13-18.

Sánchez-Belda A. 1964. *Merinos entrefinos. Fomento y mejora del ganado lanar.* Ministerio de Agricultura, Madrid, España.

Sierra I. 2001. El concepto de raza: evolución y realidad. *Archivos de Zootecnia* 50, 547-564.

Sotillo J, Serrano V. 1985. *Producción animal. Etnología zootécnica*. Tomo I. Imprenta Flores, Albacete, España, Pp 111-116.

Thiagarajan R, Jayashankar M. 2012. Effect of genetic and no genetic factors on staple length in indigenous and crossbreed sheep. *Research Journal of Animal Sciences* 6, 1-3.

Thomason J, Grovum L, Deswysen A, Bignell W. 2001. *In vivo* surface strain and stereology of the frontal and maxillary bones of sheep: Implications for the structural design of the mammalian skull. *The Anatomical Record* 264, 325-338.

Toro I, Manríquez S, Suazo G. 2010. Morfometría geométrica y el estudio de las formas biológicas: De la morfología descriptiva a la morfología cuantitativa. *Int J Morphol* 28, 977-990.

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SHORT COMMUNICATION

Mycobacterium avium subsp. *paratuberculosis* (MAP) infection in the endangered huemul deer (*Hippocamelus bisulcus*) in Patagonia

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ABSTRACT. In a huemul (*Hippocamelus bisulcus*) population sympatric with cattle, we found evidence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection. Three huemul faecal pellet samples and two cows pats were collected and cultured for MAP presence. DNA was then extracted for PCR analysis of all signal-positive cultures. To assess whether MAP isolates obtained from huemul faeces were associated with typical MAP isolated from livestock, positive confirmed culture samples were sub-typed using a combination of five Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat Analysis and one Short Sequence Repeat analysis markers. All faecal samples from both species were MAP positive. One huemul presented a different bacteria profile genotype not described before, suggesting that huemul and cattle in Patagonia could carry a unique MAP strain.

Key words: Patagonia, huemul, cattle, spillover, paratuberculosis, typing.

INTRODUCTION

The International Union for the Conservation of Nature estimates that over 24% of the world's extant mammals are currently threatened with extinction, yet infectious diseases have only been listed as a major threat for a small fraction (1.1%)[1]. It is likely that diseases are underrepresented as a contributing threat to wildlife extinction, especially given that less than half (39%) of critically endangered and endangered artiodactyls, carnivores, and primates from the 2006 IUCN Red List have any published records of pathogens from their wild populations (Pedersen *et al* 2007). Recently, evidence linking huemul (*Hippocamelus bisulcus*), an endemic and endangered deer species from southern Chile and Argentina (Corti *et al* 2010), with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection has been reported (Salgado *et al* 2017). MAP is the causative agent of Johne's disease, infection mostly affecting domestic ruminants, but it has been shown to pose a threat also to wildlife (Manning and Collins 2001, Salgado *et al* 2015). However, the pathogen has also been isolated from non-ruminants species and even from humans, associating it with Crohn's disease (Chiodini *et al* 2012).

Although huemul inhabits remote areas with limited contact with domestic animals/livestock and exists at low population density, the presence of pathogens like MAP may be interpreted as an indicator of spill-over infections from domestic animals. Then, to improve our knowledge about this current issue in huemul, a cross-sectional study was conducted on few huemul and cattle co-inhabiting the same area to inquire possible epidemiological interactions at Torres del Paine National Park in Chilean Patagonia (figure 1).

MATERIAL AND METHODS

The sampling was carried out in November 2016, at the end of the southern hemisphere spring. Three huemul faecal pellet samples, each containing from six to 10 droppings, and two cows pats were collected (Figure 1). The sampling selection criteria was that faecal material from both species was no more than one-day old, estimated according to the appearance of deer pellets and the characteristics of cow pats (Lehmkuhl *et al* 1994). To collect the faeces, we used sterile latex gloves avoiding soil contamination, and the samples were individually kept in sealed plastic bags previously labelled. Samples were stored one week under a refrigeration temperature of 5 ºC until laboratory analyses. Faecal samples were processed in a liquid culture system (BACTEC-MGIT 960, Sparks, Maryland 21152, USA) for MAP presence according to the manufacturer's protocol, and then DNA was extracted from all signal-positive cultures. Real-time IS*900* PCR was performed for MAP detection verification to confirm positive samples (Salgado *et al* 2014). The MAP confirmation consisted of a PCR system targeting the insertion element IS*900*. The PCR total reaction was 20 µl, from which 5 µl was DNA template, 10 µl were 23 TaqMan Universal MasterMix (Roche Applied Science,

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[1] IUCN, International Union for the Conservation of Nature. 2018. IUCN red list of threatened species. Available at http://www.redlist.org

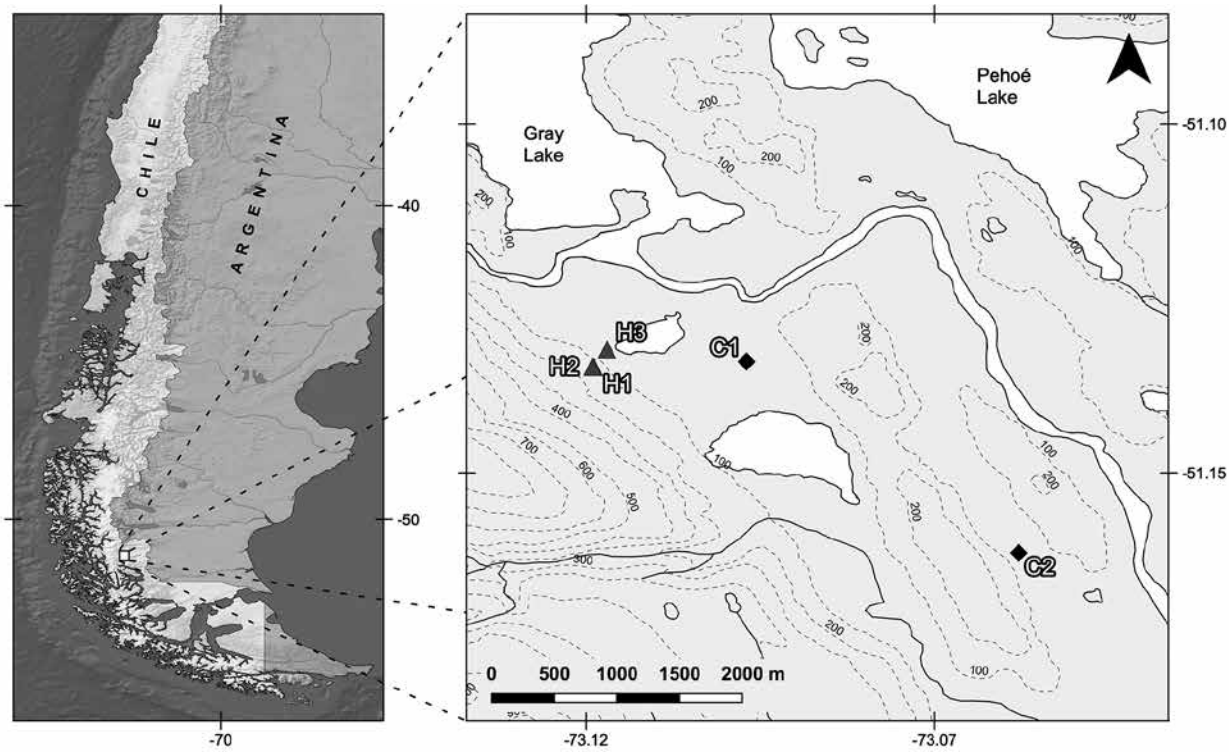

Figure 1. Geographic location of the study area and collection sites at Torres del Paine National Park in Chilean Patagonia. Black dots indicate environmental huemul (H) and cow (C) faecal samples.

Indianapolis, Indiana 46250, USA), 2 µl of each primer (0.2 µM), and 1 µl probe (0.1 µM). The sequence for IS*900* primers, which amplified a 63 nucleotide fragment of the IS*900* gene target, was 5' GACGCGATGATCGAGGAG-39 (L) and 59-GGGCATGCTCAGGATGAT-3' (R). The reactions were run in a Roche LightCycler 2.0 system (Roche Applied Science) under the following standard conditions: one cycle to 95°C for 10 min, 45 cycles with three steps of 95°C for 10 sec, 60°C for 30 sec, 72°C for 1 sec, and a final cooling step at 40°C for 30 sec. Negative and positive (MAP ATCC 19698) PCR controls were included as well as a DNA extraction negative and positive control.

To assess whether MAP isolates obtained from huemul faeces were associated with typical MAP isolated from livestock, positive confirmed culture samples were sub-typed using a combination of five Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat Analysis (MIRU-VNTR 292, 25, X3, 7, and 3; Thibault *et al* 2007), and one Short Sequence Repeat analysis markers (SSR 2; Amonsin *et al* 2004). The combination of MIRU-VNTR and SSR allele repeats formed a unique profile that allowed the allele profile to be compared to MAP sub-types obtained from a survey on huemul and other domestic animals in Chile (Salgado *et al* 2017).

RESULTS AND DISCUSSION

All three huemul faecal samples were culture positive for MAP, and the two cow faeces samples were also positive for MAP, both confirmed through PCR (table 1). All DNA

Table 1. Huemul MIRU-VNTR and SSR pattern result and its comparison with local cow strains from Torres del Paine National Park.

	Sample ID	292	x3	25	47	3	MIRU-VNTR*	INMV**	SSR-L2***
HUEMUL	134	4	2	3	3	2	42332	INMV 1	10
HUEMUL	135	4	2	3	3	2	42332	INMV 1	10
HUEMUL	137	3	2	3	3	2	32332	INMV 2	14
COW	138	3	2	3	3	2	32332	INMV 2	14

*MIRU-VNTR: Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat Analysis.
**INMV: The INRA Nouzilly MIRU-VNTR profile nomenclature, as defined by Thibault *et al* (2007).
***SSR L2: Short Sequence Repeat analysis markers-Locus 2.

of the isolated strains showed a sufficient quality to consider the results of the molecular characterisation conclusive, except for the strain obtained from one of the cows. The lack of variation between one cow and one huemul MAP strains suggests that cattle and huemul populations are sympatric, as previously stated (Salgado *et al* 2017). This result suggests that both species share the same bacteria, evidencing interspecies transmission as shown elsewhere between wildlife and livestock based on the same typing protocol (Fritsch *et al* 2012). Also, Thibault *et al* (2008) highlighted that MIRU-VNTR typing followed by SSR was the most cost-effective and efficient strategy for strain discrimination.

It has been also hypothesised (Salgado *et al* 2017) that the infection to huemul primarily may be through drinking faecal-contaminated water and secondarily through faecal-oral route since huemul species are browsers, mostly feeding on shrubs, trees, and forbs but rarely on graminoids near the ground (Vila *et al* 2009). However, one specific MIRU-VNTR and SSR genotype found in Torres del Paine National Park, from huemul faecal material, was different from those detected in cattle herds and from genotypes previously informed in Chile (table 1)(Salgado *et al* 2017).

It is highly interesting, within this restricted area, that epidemiologically associated free-ranging cattle and huemul could carry specific MAP genotypes. Therefore large-scale studies are urgently needed to gain deeper knowledge on this important sanitary issue in an endangered species like the huemul.

REFERENCES

Amonsin A, Li LL, Zhang Q, Bannantine JP, Motiwala AS, *et al.* 2004. Multilocus short sequence repeat sequencing approach for differentiating among *Mycobacterium avium* subsp. *paratuberculosis* strains. *J Clin Microbiol* 42, 1694-1702.

Chiodini RJ, Chamberlin WM, Sarosiek J, McCallum RW. 2012. Crohn's disease and the mycobacterioses: a quarter century later: causation or simple association? *Crit Rev Microbiol* 38, 52-93.

Corti P, Wittmer HU, Festa-Bianchet M. 2010. Dynamics of a small population of endangered huemul deer (*Hippocamelus bisulcus*) in Chilean Patagonia. *J Mammal* 91, 690-697.

Fritsch I, Luyven G, Köhler H, Lutz W, Möbius P. 2012. Suspicion of *Mycobacterium avium* subsp. *paratuberculosis* transmission between cattle and wild-living red deer (*Cervus elaphus*) by multitarget genotyping. *Appl Environ Microbiol* 78, 1132-1139.

Lehmkuhl JF, Hansen CA, Sloan K. 1994. Elk pellet-group decomposition and detectability in coastal forests of Washington. *J Wildl Manage* 58, 664-669.

Manning EJ, Collins MT. 2001. *Mycobacterium avium* subsp. *paratuberculosis*: pathogen, pathogenesis and diagnosis. *Rev Sci Tech Int Off Epi* 20, 33-150.

Pedersen AB, Jones KE, Nunn CL, Altizer SA. 2007. Infectious disease and mammalian extinction risk. *Conserv Biol* 21, 1269-1279.

Salgado M, Verdugo C, Heuer C, Castillo P, Zamorano P. 2014. A novel low-cost method for *Mycobacterium avium* subsp. paratuberculosis DNA extraction from an automated broth culture system for real-time PCR analysis. *J Vet Sci* 15, 233-239.

Salgado M, Aleuy OA, Sevilla IA, Troncoso E. 2015. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in a cattle/pudu interface. *Arq Bras Med Vet Zootec* 67, 1205-1209.

Salgado M, Corti P, Verdugo C, Tomckowiack C, Moreira R, *et al.* 2017. Evidence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection in huemul deer (*Hippocamelus bisulcus*) in Patagonian fjords. *Austral J Vet Sci* 49, 135-137.

Thibault VC, Grayon M, Boschiroli ML, Hubbans C, Overduin P, *et al* 2007. New variable-number tandem-repeat markers for typing *Mycobacterium avium* subsp. *paratuberculosis* and *M. avium* strains: comparison with IS900 and IS1245 restriction fragment length polymorphism typing. *J Clin Microbiol* 45, 2404-2410.

Thibault VC, Grayon M, Boschiroli ML, Willery E, Allix-Béguec C, *et al*. 2008. Combined multilocus short-sequence-repeat and mycobacterial interspersed repetitive unit-variable-number tándem repeat typing of Mycobacterium avium subsp. paratuberculosis isolates. *J Clin Microbiol* 46, 4091-4094.

Vila AR, Galende GI, Pastore H. 2009. Feeding ecology of the endangered huemul (*Hippocamelus bisulcus*) in Los Alerces National Park, Argentina. *Mastozool Neotrop* 16, 423-431.

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SHORT COMMUNICATION

Serological survey of bovine viral diarrhea (BVDV-1), brucellosis, and leptospirosis in captive white-lipped peccaries (*Tayassu pecari*) from the Midwest region in Brazil

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ABSTRACT. The present study was conducted to assess the occurrence of anti-*Brucella* sp., anti-BVDV-1, and anti-*Leptospira* spp. antibodies from captive white-lipped peccary (*Tayassu pecari*). A cross-sectional survey was performed testing 100 serum samples collected in a commercial breeding herd. All samples were submitted to the acidified antigen test (AAT), virus neutralization test (VNT) and microscopic agglutination test (MAT) with live antigens. None of the samples tested agglutinated in the AAT screening test. In the VNT, 28 samples presented a cytotoxic effect and were excluded from the evaluation. For BVDV-1, only one sample (1/72; 1.38%) was positive, with antibody titers of 40. For leptospirosis, 9% (9/100) of the samples reacted to at least one of the 24 serovars tested, with 8% (8/100) positive for serovar Patoc and 1% (1/100) for serovar Grippotyphosa. The maximum titer observed was 100. The identification of antibodies against the serovars Patoc and Grippotyphosa suggests that the sampled individuals have been exposed to the pathogen at some point during their lifetime. Regarding BVDV-1, this may be the first serological survey to describe seropositive samples in tayassuids.

Key words: infectious disease, tayassuidae, zoonosis.

INTRODUCTION

Swine and peccaries belong to the *Artiodactyla* order and to the *Suidae* and *Tayassuidae* families, respectively. Two species of peccaries are found in Brazil, the White-lipped peccary (*Tayassu pecari*) and the Collared peccary (*Tayassu tajacu*) (Keuroghlian and Eaton 2008). These two species of tayassuids are widely hunted in tropical forests to avoid their entry into certain regions and for the consumption by local communities (Bodmer *et al* 1996, Cullen, Bodmer and Valladares-Padua 2000, Altrichter and Boaglio 2004). Predatory hunting of peccaries has been motivated by their gregarious and occasional aggressive behaviour, besides food habits that promote the damage of grain and horticultural plantations (Oliver 1993). The reduction of ecological impact occasioned by predatory tayassuid hunting may be possible by implementing commercial, sustainable, conservationist alternatives for income generation for communities living in areas inhabited by those species (MMA 2001).

Studies regarding health aspects of tayassuids are important because they provide essential information considering the development and widespread captive breeding of peccary species in several Latin American countries (Nogueira and Nogueira-Filho 2011), taking into account the risks attributed and the environmental impact. Serological surveys conducted on *T. pecari* and *T. tajacu* populations revealed antibodies against *Leptospira* spp. (Ito *et al* 1998, Mayor *et al* 2006, Freitas *et al* 2010, Navas-Suárez *et al* 2017), vesicular stomatitis virus and pseudorabies virus (Corn 1987), *Orbivirus* spp. (Gerber *et al* 2012) and *Brucella* spp. (Corn 1987, Gruver and Guthrie 1996, Ito *et al* 1998, Mayor *et al* 2006) which are important agents causing infections in domestic pigs. Some molecular studies in *T. pecari* and *T. tajacu* have also detected antigens of *Erysipelothrix rhusiopathiae* (Coutinho *et al* 2012), *Trypanosoma evansi* e *T. cruzi* (Herrera *et al* 2008), porcine circovirus type 2, herpesvirus suis type 1, *Mycoplasma hyopneumoniae* and *Pasteurella multocida* (Castro *et al* 2014, Navas-Suárez *et al* 2017). To date, antibodies and molecular detection of bovine viral diarrhoea virus (BVDV) have been described exclusively in wild boars (Sedlak, Bartova and Machova 2008, Weber *et al* 2016).

Evidence has pointed to the risks of transmission of pathogenic agents among domestic swine, tayassuids and wild boars. Based on this assumption, the tayassuids may serve as susceptible hosts and potential reservoirs of agents that can infect domestic and wild pigs in addition to other species (Nava and Cullen 2003, Herrera *et al* 2008, Freitas *et al* 2010, Coutinho *et al* 2012). Freitas *et al* (2010) demonstrated the presence of anti-*Leptospira interrogans* antibody titers in *T. pecari* that maintained close interaction with local communities and other domestic animals, indicating that the close relationship among humans, domestic animals, and tayassuids resulted in the contact with the pathogen. In this scenario, the present study assessed the occurrence of antibodies against *Brucella* sp., BVDV-1 and

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Leptospira spp. in white-lipped peccary (*Tayassu pecari*) from a commercial breeder located in the Cerrado area, Central-Western region of Brazil.

MATERIAL AND METHODS

STUDY SITE

The study was conducted in a commercial farm (process Ibama nº 02001.004190/1999-54) composed of a set of properties located in the Cerrado area which has about 330 km^2 bordering the state of Minas Gerais, state of Goiás and state of Bahia. The farm is dedicated to the commercial breeding of *T. pecari*, *T. tajacu*, Ema (*Rhea Americana*) and red-footed tortoise (*Chelonoidis carbonaria*). The tayassuids breeding on the farm began in 2001. In 2008, the facilities were restructured and animal nutrition was reformulated by increasing the capacity and number of animals within the picket in an intensive breeding system. Each animal species was bred at a different site and each site consisted of 180 m^2 pickets, which housed groups of 6 to 10 animals. The pickets of the tayassuids consisted of an earthen floor without vegetation, surrounded by walls and fences over the walls. The pickets were located next to each other on both sides of a long corridor, and within them, there were feeders and water fountains (figure 1). This study was approved by the Ministry of the Environment (SISBIO nº 56725-1).

ANIMALS

In total, the property had 365 *T. pecari*. The family groups and groups with defined hierarchy were considered the selection criteria for sampling and a total of 100 animals were sampled, 72 females and 28 males.

Animals from 14 pickets were collected and, of those, four pickets with ten animals per group (two pickets with animals between three and six months of age and two pickets with animals between seven and 10 months of age) and ten pickets of families with six animals per group (all ages). The number of animals sampled per group according to sex and age is presented in table 2.

SAMPLES

In July 2017, the samples were collected in a single time point. For the blood sample collections, each group of animals was transferred to the management area. All

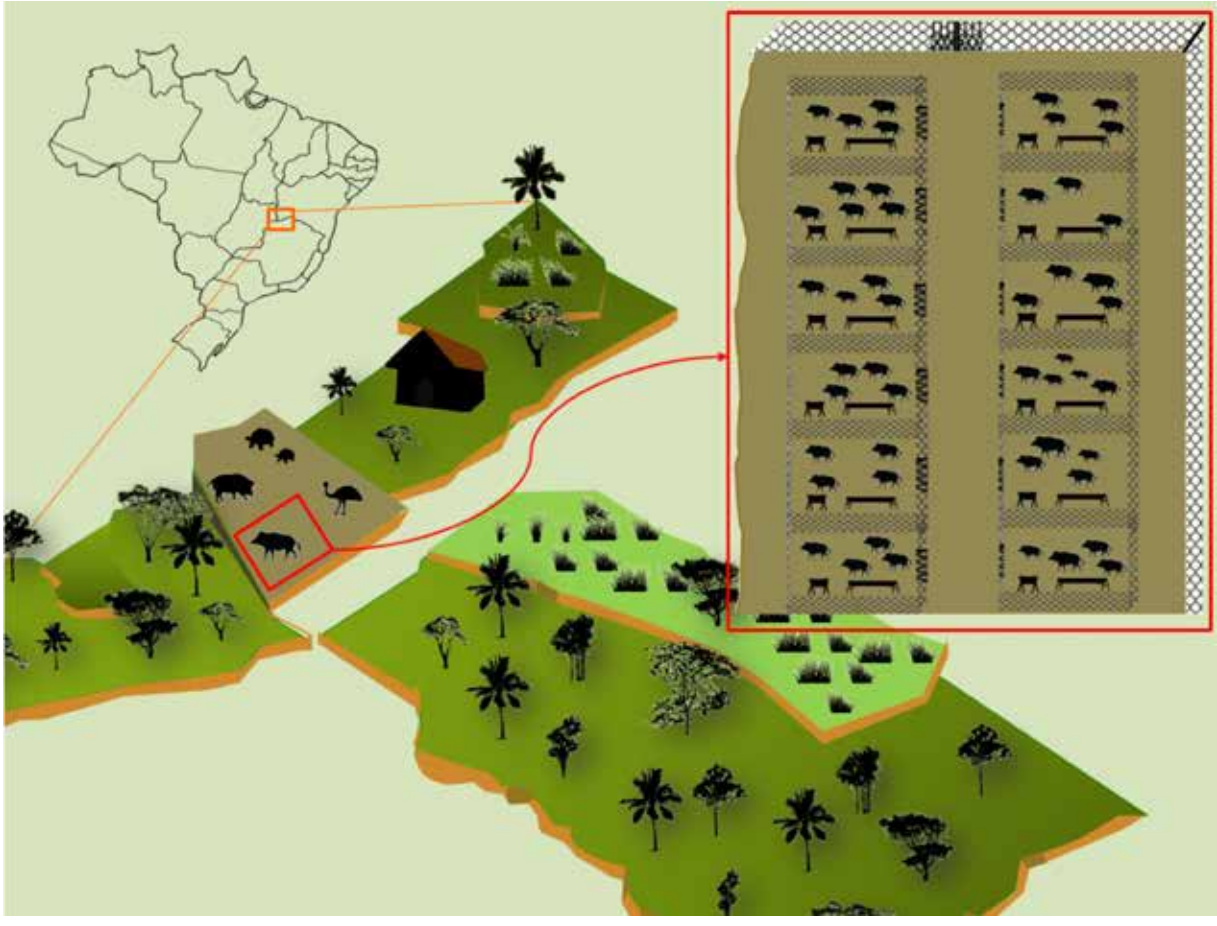

Figure 1. Schematic representation of the study site, surrounded by Cerrado vegetation and bordering the state of Minas Gerais, state of Goiás, and state of Bahia. In detail, the spatial organisation of *Tayassu pecari* pickets arranged side by side.

Table 1. Frequency of anti-*Leptospira* agglutinins by microscopic agglutination test (MAT) in 100 *Tayassu pecari* and risk factor analysis for positive reactions by sex by Fisher's exact test (P>0.05).

Serogroup		Total positive reactions (%)	
		CI** 95(%)	Maximum titer
Patoc	8 (8%)	7.94 to 8.05	100
Grippotyphosa	1 (1%)	0.17 to 5.44	100
Icterohaemorrhagiae	4 (4%)	1.56 to 9.83	50*
Shermani	1 (1%)	0.17 to 5.44	50*
Butembo	1 (1%)	0.17 to 5.44	50*
Pyrogenes	1 (1%)	0.17 to 5.44	50*
Bratislava	2 (2%)	0.55 to 7	50*
Patoc	16 (16%)	15.92 to 16.07	50*
Overall	100 (100%)		
Sex as a risk factor	Odds ratio	CI 95(%)	*P*-value
Male (3|28)*** Female (6|72)	1.28	0.19 to 6.51	0.71

*Reagents in the screening test (titer <100).
**Confidence interval
***Positive for any serovar| total individuals by sex

Table 2. Distribution of males and females of *Tayassu pecari* collected in 14 pickets with three groups, submitted to serodiagnosis of brucellosis, bovine viral diarrhea and leptospirosis.

Picket No.	Individuals by gender		Group
	Male	Female	
1	4	6	3 to 6 months
2	3	7	
3	4	6	7 to 10 months
4	4	6	
5	2	4	Families
6	1	5	
7	1	5	
8	2	4	
9	1	5	
10	2	4	
11	1	5	
12	1	5	
13	1	5	
14	1	5	

animals had a microchip and earrings with a registration number for tracking information. In the management area, the animal was placed inside of a structure similar to a cattle chute, developed in an appropriate size for the mechanical restraint of *T. pecari*. The cage had side openings for accessing to the punctured region. A total of 10 ml of blood of which animal was collected from the jugular, or cephalic or saphenous veins, prioritizing the easy access for each situation, in an attempt to avoid the animal excessive or prolonged stress. The blood sampling lasted on average two minutes per animal. The serum samples were identified and maintained at –20 °C, for further serological tests.

LABORATORY PROCEDURES

Acidified antigen test (AAT). The AAT was performed as recommended in the Manual of the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis (Brazil 2006). The method consists of

placing 0.03 mL of the serum in contact with 0.03 mL of the antigen in a checkered glass plate followed by slight homogenisation, then keeping the plate in rotational and under constant movements until the moment of the reading, which was done four minutes after the reaction using a box with light (or agglutinoscope) to observe the formation of agglutination lumps. The antigen used in this technique was prepared with a *Brucella abortus* sample 1119/3 at 8.0% cell volume, stained with Bengal rose, pH 3.65. The AAT tests, as well as the following tests, were applied following the recommendations for domestic pigs (*Sus scrofa domesticus*) and they were not standardised for *T. pecari*.

Viral neutralization test (VNT). The samples were submitted to the VNT for the detection of antibodies against BVDV-1a (Singer strain), as recommended by the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (OIE 2015) with modifications. All seropositive samples at screening were duplicated for repeatability of the result. On each plate, negative and positive controls were added. A sample was considered positive when the total neutralisation of 100 $TCID_{50}$ occurred in the serum and no cytopathic effect (CPE) was observed in the cell layer in serum dilutions higher than 1:10 (OIE 2015) with modifications. Antibody titers were expressed as the reciprocal of the highest dilution at which viral neutralisation was verified, and the final titer was the geometric mean of the titers found in the duplicates.

Microscopic agglutination test (MAT). The MAT was performed for the diagnosis of leptospirosis using a collection of live antigens that included 24 serological variants of pathogenic leptospires of the following serogroups (serotype representatives): Australis (Australis, Bratislava), Autumnalis (Autumnalis, Butembo), Ballum (Castellonis), Batavie (Batavie), Canicola (Canicola), Celledoni (Whitcombi),Cinoptery (Cinoptery), Grippotyphosa (Grippotyphosa), Hebdomadis (Hebdomadis), Icterohaemorrhagie (Copenhageni, Icterohaemorrhagiae), Javanica (Javanica), Panama , Pomona (Pomona), Pyrogenes (Pyrogenes), Sejroe (Hardjo, Wolffi), Shermani (Shermani), Tarassovi (Tarassovi), Djasiman (Sentot) and two saprophytic leptospires: Andamana (Andamana) and Seramanga (Patoc) (Lee *et al* 2017).

The screening was performed at 1:50 and 1:100 dilutions. In the presence of agglutination, the sera was titrated in two-fold serial dilutions. The titer was given as the reciprocal of the highest dilution, titers of 1:100 to 1:200 are considered low positive, interpreted as exposure to *Leptospira* spp., Titer ≥1:400 are considered high positive, indicating active or recent infection (Faine *et al* 1999, Boqvist *et al* 2002). Only cultures from 4 to 14 days, which did not present contaminants or autoagglutination, were used as antigens.

STATISTICAL ANALYSIS

A descriptive analysis of the frequency results was carried out, with the calculation of the respective confidence intervals. The association between sex and the number of seropositive for *Leptospira* spp. was analysed using the Fisher exact test ($P<0.05$).

RESULTS AND DISCUSSION

In this study, none of the 100 serum samples of *T. peccary* tested had antibodies detectable by AAT for *Brucella* sp., thus, it was not necessary to perform confirmatory tests. Similar results were obtained in the northern and northeastern regions of Brazil, where researchers reported the absence of positive serological results for *Brucella abortus* in a group of 11 different species, including *T. peccary* (Minervino *et al* 2018). Previous data in the north of Brazil described 4.9% (2/41) of serum samples of *T. tajacu* reactive for *Brucella* sp., in a nursery with a history of infertility and mortality of the offspring, with the seropositive animals being euthanised due to sanitary issues (Mayor *et al* 2006, 2007). Therefore, epidemiological surveys studies regarding disease in tayassuids breeding are not clear regarding the possibility of such species serving as reservoirs or carriers of *Brucella* sp.

BVDV is a pestivirus that shows genetic and antigenic similarities to classical swine fever and other pestiviruses and has an important impact on cattle and pigs (Brodersen 2014). Out of the 100 samples submitted to VNT, 28 showed a cytotoxic effect and were excluded from the evaluation. For BVDV-1a, one animal was reactive 1/72 (1.38%, CI 95% 0.24 to 4.45), with a titer of 40. This animal was male, 48 kg, belonging to the G8 group. In the literature, there is no evidence of the detection of antibodies against BVDV in *T. pecari* or *T. tajacu*. In the present study, only one sampled animal showed anti-BVDV antibodies.

In Brazil, Gatto *et al* (2016) and Gatto *et al* (2018) identified seropositivity for BVDV-1 and BVDV-2 in pig serum samples from intensive breeding from several Brazilian states. Almeida *et al* (2017) also detected BVDV-1 and BVDV-2 seropositive samples in non-technified rearing farms at the northeast of the state of São Paulo. Wild animals infected with BVDV are described as indicative of the presence of the virus in nearby herds located within a given region, although the role of tayassuids in the epidemiology of BVDV is unknown (Milićević 2018).

Serological diagnosis of leptospirosis showed that 9% of the *T. pecari* samples were reactive to several serovars at low titers (> 1/50): Patoc, Grippotyphosa, Itereohaemorrhagiae, Shermani, Butembo, Pyrogenes, and Bratislava. However, titers higher that 1/100 were only found for serovar Patoc 8% (8/100) and 1% (1/100) for serovar Grippotyphosa, and no association was observed between sex and the occurrence of *Leptospira* spp. antibodies ($P>0.05$). The

occurrence of antibodies against *Leptospira* spp. in *T. pecari* was described by Ito et al (1998), in animals from Pantanal, the southern region of the state of Mato Grosso, which were reagents for the serovars Copenhageni, Icteratohaemorrhagiae IV, Panama, Patoc, and Autumnalis. In fragments of the Atlantic Forest located in the state of São Paulo, serum samples of *T. pecari* were seropositive to serovars Pomona and Icterohaemorrhagiae IV (Nava 2008). Freitas *et al* (2010) described the occurrence of the serovars Autumnalis, Pomona, Copenhageni, Canicola, Hardjo, Grippotyphosa, Icterohaemorrhagiae, Bataviae, Tarassovi, and Hebdomadis in *T. pecari* from an ecosystem interacting with cattle.

In Pantanal (southern region of the Mato Grosso state), Ito *et al* (1998) reported the occurrence of serovar Patoc. Seroreactivity for Butembo and Automnallis serovar was detected in pigs samples from Amazonas state (Brazil) (Mayor 2006, Mendoza *et al* 2007). In the same region, Amazonas state, a serological survey identified positive samples for Australis, Hebdomadis, Autumnalis, Bataviae, Djasiman, Grippothyphosa, Balum, Canicola, Mini, Tarassovi, and Icterohaemorragiae (Nava 2008). Pyrogenes and Patoc were described as the most prevalent in *T. tajacu*. In the Peruvian Amazon, seropositivity was reported for Iquitos, Australis, Hebdomadis, Icterohaemorrhagiae, Autumnalis, Tarassovi, Cynopteri, and Ballum (Jori *et al* 2009).

In the present study, serovar Patoc was the predominant serovar detected. The occurrence of antibodies against serovar Patoc was also described in Pantanal, southern region of the state of Mato Grosso in *T. pecari* and *T. tajacu* (Ito *et al* 1998) and only in *T. tajacu* by Nava (2008). Also, this serovar was described in wild boars in the state of São Paulo and Paraná (Marchiori Filho *et al* 2002). Only 1% of the animals were seropositive for Grippothyphosa, a result similar to that found by Freitas *et al* (2010) in *T. pecari* and by Mendoza *et al* (2007) in *T. tajacu.*

The emergence of human leptospirosis cases has been reported in several countries. The increase of human infections has been associated with increased exposure of the host to the pathogen, particularly during outdoor activities, sports practices or contact with wild animals. Thus, the sylvatic cycle of the disease is a growing concern for public health, justifying epidemiological surveillance studies regarding leptospirosis in different species of wild animals including tayassuids (Morgan *et al* 2002).

Reagent serum samples were identified for BVDV-1a and *Leptospira* spp. in *T. pecari* from a commercial breeder in the midwest of Brazil. As far as we know, this is the first serological survey to describe describe the presence of BVDV-1 (strain Singer) antibodies in *T. pecari*. Yet, we must also take into account that the diagnostic methods carried out are not standardised for *Tayassu* sp. species, and there may be false negatives and even false positives. To date, there is no consistent evidence supporting that BVDV can infect those wild species. Monitoring captive-reared wildlife is important to detect pathogen excretion which could affect human health and or other animals (wild or domestic) under production.

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REFERENCES

Almeida HMS, Gatto IRH, dos Santos ACR, Ferraudo AS, Samara SI, *et al*. 2017. A cross-sectional and exploratory geospatial study of bovine viral diarrhea virus (BVDV) infections in swines in the São Paulo State, Brazil. *Pak Vet J* 37, 470-474

Altrichter M, Boaglio GI. 2004. Distribution and relative abundance of peccaries in the Argentine Chaco: associations with human factors. *Biol Conserv* 116, 217-225.

Bodmer RE, Aquino R, Puertas P, Reyes C, Fang T, *et al*. 1996. *Manejo y Uso Sustentable de Pecaríes en la Amazonia Peruana. No. 18*. Comisión de Supervivencia de Especies, Lima, Perú.

Boqvist S, Thu HT, Vågsholm I, Magnusson U. 2002. The impact of *Leptospira* seropositivity on reproductive performance in sows in southern Viet Nam. *Theriogenology* 58, 1327-1335.

Brasil 2006. *Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose (PNCEBT). Manual Técnico*. Ministério da Agricultura, Pecuária e Abastecimento (MAPA/SDA/DAS), Brasília, Brazil.

Brodersen BW. 2014. Bovine viral diarrhea virus infections: manifestations of infection and recent advances in understanding pathogenesis and control. *Vet Pathol* 51, 453-464.

Castro AMMG, Brombila T, Bersano JG, Soares HS, Silva SOS, *et al*. 2014. Swine infectious agents in *Tayassu pecari* and *Pecari tajacu* tissue samples from Brazil. *J Wildl Dis* 50, 205-209.

Corn JL, Lee RM, Erickson GA, Murphy CD. 1987. Serologic survey for evidence of exposure to vesicular stomatitis virus, pseudorabies virus, brucellosis and leptospirosis in collared peccaries from Arizona. *J Wildl Dis* 23, 551-557.

Coutinho TA, Moreno AM, Imada Y, Lopez RPG, Neto JSF. 2012. Characterization of *Erysipelothrix rhusiopathiae* isolated from Brazilian *Tayassu pecari*. *Trop Anim Health Prod* 44, 689-692.

Cullen JRL, Bodmer RE, Valladares-Padua C. 2000. Effects of hunting in habitat fragments of the Atlantic forests, Brazil.*Biol Conserv* 95, 49-56.

Faine S, Aoler B, Bolin C, Perolat P. 1999. *Leptospira and Leptospirosis*. 2nd ed. Medi Sci, Melbourne, Australia.

Freitas TPT, Keuroghlian A, Eaton DP, Freitas EB, Figueiredo A, *et al*. 2010. Prevalence of *Leptospira interrogans* antibodies in free-ranging *Tayassu pecari* of the Southern Pantanal, Brazil, an ecosystem where wildlife and cattle interact. *Trop Anim Health Prod* 42, 1695-1703.

Gatto IRH, Arruda AG, Almeida HMS, Silva GCP, Leite AI, *et al*. 2016. A cross-sectional study to estimate the frequency of anti-bovine viral diarrhea virus-1antibodies in domestic pigs of Mossoró region in the state of Rio Grande do Norte, Brazil. *Cienc Rural* 46, 1607-1612.

Gatto IRH, Linhares DCL, de Souza Almeida HM, Mathias LA, de Medeiros ASR, *et al*. 2018. Description of risk factors associated with the detection of BVDV antibodies in Brazilian pig herds. *Trop Anim Health Prod* 50, 773-778.

Gerber PF, Galinari GCF, Cortez A, Paula CD, Lobato ZIP, *et al*. 2012. Orbivirus infections in collared peccaries (*Tayassu tajacu*) in south-eastern Brazil. *J Wildl Dis* 48, 230-232.

Gruver KS, Guthrie JW. 1996. Parasites and selected diseases of collared peccaries (*Tayassu tajacu*) in the trans-pecos region of Texas. *J Wildl Dis* 32, 560-562.

Herrera HM, Abreu UGP, Keuroghlian A, Freitas TP, Jansen AM. 2008. The role played by sympatric collared peccary (*Tayassu tajacu*),

white-lipped peccary (*Tayassu pecari*), and feral pig (*Sus scrofa*) as maintenance hosts for *Trypanosoma evansi* and *Trypanosoma cruzi* in a sylvatic area of Brazil. *J Parasitol Res* 103, 619-624.

Ito FH, Vasconcellos SA, Bernardi F, Nascimento AA, Labruna MA, *et al*. 1998. Evidência sorológica de brucelose e leptospirose e parasitismo por ixodídeos em animais silvestres do pantanal sul-mato-grossense. *Ars Veterinaria* 14, 302-310.

Jori F, Galvez H, Mendonza P, Cespedes M, Mayor P. 2009. Monitoring of leptospirosis seroprevalence in a colony of captive collared peccaries (*Tayassu tajacu*) from the Peruvian Amazon. *Res Vet Sci* 86, 383-387.

Keuroghlian A, Eaton DP. 2008. Fruit availability and peccary frugivory in an isolated Atlantic forest fragment: Effects on peccary ranging behavior and habitat use. *Biotropica* 40, 62-70.

Lee HS, Khong NV, Xuan HN, Nghia VB, Nguyen-Viet H, Grace D. 2017. Sero-prevalence of specific *Leptospira* serovars in fattening pigs from 5 provinces in Vietnam. *BMC Vet Res* 13, 125.

Marchiori Filho M, Girio RJS, Lui JF, Mathias LA, Brasil ATR. 2002. Estudo sorológico para leptospirose em populações de diferentes grupos genéticos de javalis (*Sus scrofascrofa*, Linnaeus, 1758) dos estados de São Paulo e Paraná. *Arq Inst Biol* 69, 9-15.

Mayor P, LePendu Y, Guimarães DA, Silva JV, Tavares HL, *et al*. 2006. A health evaluation in a colony of captive collared peccaries (*Tayassu tajacu*) in the Eastern Amazon. *Res Vet Sci* 81, 246-253.

Mayor P, Guimarães DA, LePendu Y, Da Silva JV, Jori F, *et al*. 2007. Reproductive performance of captive collared peccaries (*Tayassu tajacu*) in the eastern Amazon. *Anim Reprod Sci* 102, 88-97.

Mendoza P, Mayor P, Galvez HÁ, Cespedes MJ, Jori F. 2007.Antibodies against *Leptospira* spp. in captive collared peccaries, Peru. *Emerg Infect Dis*13, 793-794.

Milićević V, Maksimović-Zorić J, Veljović L, Kureljušić B, Savić B, *et al*. 2018. Bovine viral diarrhea virus infection in wild boar. *Res Vet Sci* 119, 76-78.

Minervino AHH, Soares HS, Barrêto-Júnior RA, Neves KAL, Morini AC, *et al*. 2018. Antibodies against brucella abortus and *Leptospira* spp. in captive mammals in the states of pará and rio grande do norte, brazil. *J Zoo Wildl Med* 49, 355-360.

MMA, Ministério do Meio Ambiente. 2001. *Avaliação e identificação de ações prioritárias para a conservação, utilização sustentável e repartição dos benefícios da biodiversidade da Amazônia brasileira*. MMA, Brasília, Brazil.

Morgan J, Katz S, Manea H, Sasaki F. 2002.Outbreak of leptospirosis among triathlon participants and community residents in Springfield, Illinois, 1998.*Clin Infect Dis* 34, 1593-1599.

Nava A, Cullen L. 2003. Peccaries as sentinel species: conservation, health and training in Atlantic Forest Fragments, Brazil. *Sui form Soundings PPHSG Newsletter* 3, 15-16.

Nava AFD. 2008. Espécies sentinelas para a Mata Atlântica: as conseqüências epidemiológicas da fragmentação florestal no Pontal do Paranapanema. *Tese doutorado*, Universidade de São Paulo, São Paulo, Brazil.

Navas-Suárez PE, Soler-Tovar D, Montenegro O. 2017. Los macro y microparásitos reportados para pecaríes y su importancia en la interfaz con especies de producción. In: Diego Soler-Tovar (ed). *Conexiones de la salud global: ecosistemas, animales y humanos*. Ediciones Unisalle, Bogotá D.C., Colombia, Pp 247-272.

Nogueira SS, Nogueira-Filho SL. 2011. Wildlife farming: an alternative to unsustainable hunting and deforestation in Neotropical forests? *Biodiversity Conserv* 20, 1385-1397.

Oliver WLR. 1993. *Pigs, peccaries and hippos*. IUCN, Gland, Switzerland.

Sedlak K, Bartova E, Machova J. 2008. Antibodies to selected viral disease agents in wild boars from the Czech Republic. *J Wildl Dis* 44, 777-780.

Weber MN, Pino EHM, Souza CK, Mósena ACS, Sato JPH, *et al*. 2016. Primeira evidência da infecção pelo vírus da diarreia viral bovina em javalis. *Acta Sci Vet* 44, 1398.

OIE, World Organization for Animal Health 2015. Bovine viral diarrhoea. In: Drew T (ed). *Manual of diagnostics tests and vaccines for terrestrial animals*. OIE, Paris, France, Pp 698-710.

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CASE REPORT

Seropositivity to *Leptospira interrogans* in a herd of vicuñas (*Vicugna vicugna*) under captivity in northern Chile

Cecilia Norambuena[a,b*], Mariana Roldán[a], Christian Tuemmers[a], Gerardo Quezada[a], Oriana Betancourt[a]

ABSTRACT. Twenty-one vicuñas (*Vicugna vicugna*) from a Chilean breeding unit were tested to serovars Pomona, Canicola, Copenhageni, Ballum and Grippotyphosa of *Leptospira interrogans* using the microscopic microagglutination technique. The results showed that 23.8% of the samples reacted to the serovar Grippotyphosa which has been related to abortions in South American camelids.

Key words: leptospirosis, camelidae, *Vicugna vicugna*.

The vicuña (*Vicugna vicugna*) is a wild herbivorous mammal of the Camelidae family that inhabits the Andean peatlands called "bofedales" of the biogeographic provinces of the Puna and Altoandina of Chile, Bolivia, Peru, and Argentina. In Chile, ninety-five percent of the vicuña population is located in the Arica and Parinacota Region, above 3,000 m.a.s.l. This vicuña population has shown a progressive decrease from 25,000 individuals in 1990 to 12,061 in 2018[1].

Domestic South American camelids show low fertility in altiplanic conditions, close to 50% of pregnancy rate (Fernandez-Baca 1991). Vicuñas have shown pregnancy rates of 59.7% in the Lauca National Park (Urquieta and Rojas 1990) and 64% in a captive breeding unit (Raggi and Parraguez 2005). There have been reports of embryonic and fetal mortality in domestic camelids (Fernandez-Baca *et al* 1970) and vicuñas (Ellmen 2004) in the High Andes, as well as in well-nourished alpacas in New Zealand which had been imported from Chile (Knight *et al* 1995). The low nutritional level, high consanguinity of herds, traumatic events, congenital and acquired disorders of the reproductive tract, and infectious diseases could be the reason for the low reproductive performance in camelids (Rodríguez *et al* 2014, Pearson *et al* 2014). Among the infectious etiologies, Leptospirosis caused by spirochetes of the *Leptospira* genus, is one of the most important causes for low reproductive performance in North American camelids (Tibary *et al* 2006). The objective of the study was to determine the seropositivity of *Leptospira* spp. in a herd of vicuñas under captivity in the Chilean High Andes.

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[1] Shaw *et al* 2012, CONAF, pers. commun.

The study was carried out in the Vicuña Breeding Unit of Limani (18° 23'S, 69° 34' W; 4,385 m.a.s.l.) located in the Arica and Parinacota Region, Chile. The sampling was authorised by the Agriculture and Livestock Service of Chile (SAG). The unit has 49 vicuñas (13 males, 21 females, and 15 young vicuñas under one year of age) in a 40 Ha of bofedal. A temporary capture of 21 pregnant and non-pregnant adults from 2 to 6 years old, was carried out. Each female was tied up in sternal decubitus position without sedation, and 4 ml of blood was obtained by jugular puncture using a syringe and then collected in heparine tubes. The samples were centrifuged at 1,500 rpm for 15 min, and plasma was transported to Universidad Católica de Temuco by air, and stored at –80 °C until analysis.

The antibody reaction against the serovars Pomona, Canicola, Copenhageni, Ballum, and Grippotyphosa of *Leptospira interrogans* was determined using the microscopic microagglutination technique, in the laboratory of Veterinary Microbiology, Universidad de Concepción. The antigenic strains and positive controls were provided by SAG. Plasma samples were examined at dilutions of 1:100, 1:200, 1:400, 1:600, 1:1,800 and incubated with the antigen at 37 °C during 2 hours. Sera with ≥ 50% agglutination or more was considered positive, with titers equal to or greater than the 1:100 dilution.

Five (23.8%) out of 21 vicuñas analysed were reacted positively to the serovar Grippotyphosa of *L. interrogans* with a titer (1:100) that ruled out active infection. The results of previous studies of seropositivity to *L. interrogans* in vicuñas are diverse. In Argentina, between 7% and 62% out of 73 vicuñas from wild and captive herds, reacted to the serovars Copenhageni and Castelloni, and the study did not include the serovar Grippotypposa (Llorente *et al 2002*). In Peru, 77.4% out of 195 vicuñas from wild and captive herds were positive to the serovars Icterohaemorrhagiae and Pomona in the Huancavelica and Ayacucho Region (Rosadio *et al* 2015). On the other hand, in south Puno, only 1.9% (4/207) of the vicuñas were positive to the serovars Pomona, Autumnalis, Bratislava, and Copenhageni, including Grippotyphosa (Risco-Castillo *et al* 2014) screened against 17 serovars.

The seropositivity of the serovars Pomona (33%), Hardjo (14%), Copenhageni (11%), and Grippotyphosa (8%) has been registered in clinically healthy vicuñas of the captivity Breeding Unit of Cculicculine, Chile. The Units of Ankara and Limani were negative, although the sample size of the latter Unit was small (8 animals) (SAG 2002, Perez *et al* 2007). Based on the results of the present study, it is possible that leptospirosis could potentially affect the fertility of vicuñas in the Chilean highlands considering that serovars Pomona and Grippotyphosa were associated with episodes of abortions in North American camelids (Lohr *et al* 2007, Fowler 2010). However, the finding of serological reactivity is not enough to state that infertility of the herd can be attributed to pathogenic *Leptospira sp*. infection. It is recommended to study the prevalence of this disease in wild herds and epidemiological surveillance of the captive systems to prevent the spread of this disease. The identification of the wild species that could act as a reservoir of the causative serovars for humans and domestic species would also be helpful to have an action plan for the control of the disease. In conclusion, 23.8% (5/21) of the vicuñas under captivity in Limani were positive to the serovar Grippotyphosa of *Leptospira interrogans*.

REFERENCES

Ellmen E. 2004. Efecto de la suplementación alimentaria estratégica sobre la eficiencia reproductiva en vicuñas mantenidas en semicautiverio. *Memoria de Título*, Universidad de Chile, Santiago, Chile.

Fernández-Baca S. 1991. *Avances y perspectivas del conocimiento de los camélidos sudamericanos*. FAO, Santiago, Chile.

Fernandez-Baca S, W Hansel, Novoa C. 1979. Embryonic mortalitity in the alpaca. *Biol Reprod* 3, 243-251.

Fowler M, Bravo PW. 2010. Infectious diseases. In: Fowler M (ed). *Medicine and Surgery of Camelids.* 3rd ed. Willey-Blackwell Inc, Iowa, USA, Pp 173-230.

Knight TW, Ridland M, Scott I, Death AF, Wyeth TK. 1995. Foetal mortality at different stages of gestation in alpacas (*Lama pacos*) and the associated changes in progesterone concentrations. *Anim Reprod Sci* 40, 89-97.

Lohr CV, Bildfell RJ, Heidel JR, Valentine BA, Schaefer DL. 2007. Abortion in llamas and alpacas: a 5 year retrospective study. *Vet Pathol* 44, 5.

Llorente P, Leoni L, Martínez Vivot M. 2002. Leptospirosis en camélidos sudamericanos. Estudio de prevalencia serológica en distintas regiones de la Argentina. *Arch Med Vet* 34, 59-68.

Pearson L, Rodriguez J, Tibary A. 2014. Disorders and diseases of pregnancy. In: Cebra C, Anderson DE, Tibary A, Van Saun RJ, Johnson LW (eds). *Llama and alpaca care: medicine, surgery, reproduction, nutrition, and herd health.* Elsevier Inc., Philadelphia, USA, Pp 256-273.

Pérez C, Arredondo F, Turra L. 2007. Manejo sanitario de la vicuña. *Boletín Veterinario Oficial Nº 9*, Servicio Agrícola y Ganadero, Chile.

Raggi LA, Parraguez VH. 2005. Manejo reproductivo de la vicuña. In: Galaz J, González G (eds). *Técnicas de Manejo Productivo de la Vicuña* (*Vicugna vicugna*, Molina, 1782) *en Chile*. Corporación Nacional Forestal y Fundación para la Innovación, Santiago, Chile, Pp 209-221.

Risco-Castillo V, Wheeler J, Rosadio R, García-Pena F, Arnaiz-Seco I, *et al*. 2014. Health impact evaluation of alternative management systems in vicuña (*Vicugna vicugna mensalis*) populations in Perú. *Trop Anim Health Prod* 46, 641-646.

Rodríguez J, Pearson L, Tibary A. 2014. Infertility and subfertility in the female camelid. In: Cebra C, Anderson DE, Tibary A, Van Saun RJ, Johnson LW (eds). *Llama and alpaca care: medicine, surgery, reproduction, nutrition, and herd health.* Elsevier Inc., Philadelphia, USA, Pp 216-242.

Rosadio R, Véliz A, Castillo H, Yaya K, Rodríguez A, Rivera H, Wheeler J. 2015. Seroprevalence to pathogenic leptospira in Peruvian alpacas and vicuñas. *Small Rumin Res* 130, 256-259.

SAG. 2002. Oficio Ordinario Nº 1251 del 14 de Agosto del 2002. Resultados exámenes serológicos y parasitarios en vicuñas silvestres y en cautiverio.

Shaw AK, Galaz JL, Marquet P. 2012. Population dynamics of the vicuña (*Vicugna vicugna*): density dependence, rainfall, and spatial distribution. *J Mammal* 93, 658-666.

Tibary A, Fite C, Anouassi A, Sghiri A. 2006. Infectious causes of reproductive loss in camelids. *Theriogenology* 66, 633-647.

Urquieta B, Rojas R. 1990. Studies on the reproductive physiology of the vicuña *(Vicugna vicugna).* In: Russell B, Jane M, John I (eds). *Livestock reproduction in Latin America.* International Atomic Energy Agency, Viena, Austria, Pp 407-428.

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