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Serological Survey of Antibodies to *Mannheimia haemolytica* and *Pasteurella multocida* in Camelids from Argentina

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Abstract

South American camelids are a source of livestock wealth in Andean countries. In Argentina, there is little information about camelid pathogens, and most of the literature data available are seroprevalence works against virus. Besides, little is known about the immunological status against bacterial agents affecting these animals. In an effort to explore the serological status of Argentinean camelids, we evaluated the presence of serum antibodies against bacterial pathogens involved in pneumonic diseases (*Pasteurella multocida* and *Mannheimia haemolytica)* in llamas from different regions of the country. By ELISA, a high seroprevalence for both pathogens was found in the serum samples; higher optical density (OD) values were obtained when the sera were incubated with heat-killed *P. multocida* as coating antigen compared to *M. haemolytica*. In addition, a large number of sera analyzed presented high OD values for both microorganisms independently of their origin region. Serum avidity was also evaluated, by means of an assay based on antibody desorption by urea. No correlation was found between the high ODs obtained for *P. multocida* and the serum avidity. On the other hand, samples reacting with *M. haemolytica* had lower OD values but higher avidity index. The antigenic recognition pattern for both microorganisms was determined by western blot. Unlike *P. multocida*, the antigenic recognition pattern of *M. haemolytica* did not differ among serum samples obtained from animals living in different areas. In summary, we found that camelids can synthetize antibodies that recognize *M. haemolytica* with high avidity for different antigens of the bacterium, suggesting that Argentinean camelids are in contact with *M. haemolytica* which is probably a causative agent of subclinical infections. Conversely, specific antibodies for *P. multocida* were also found, but these sera presented low avidity that is probably the result of a colonization process by this bacterium, or else, to be a consequence of cross-reactivity phenomena.

Keywords: South American camelids; *Pasteurella multocida*; *Mannheimia haemolytica*; Specific antibodies; ELISA; Avidity test; Western blot

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Introduction

The term pasteurellosis refers to the infection with bacteria of the genera *Pasteurella* and *Mannheimia*, which occurs in animals and humans. Some species and strains of *Pasteurella* and *Mannheimia* can also act as primary pathogens, and can occasionally cause epidemics of pneumonia or septicemia with significant mortality in domestic and, more occasionally, wild animals [1]. Both bacteria are causative agents of numerous diseases that are of significant economic importance to the livestock activity. *Pasteurella multocida*, a normal inhabitant of the oral and pharyngeal bacterial flora, is one of the most common bacteria associated with diseases that are of economic importance [2]. This bacterium may proliferate and invade tissues leading to disease due to an impairment of host's immunity caused by stress factors. Apart from vectors such as ticks and fleas, aerosol or direct contact may also favor transmission [1]. Various strains of *P. multocida* are considered primary causative agents of fowl cholera in birds, hemorrhagic septicemia in ungulates, atrophic rhinitis in pigs, and snuffles in rabbits [3,4]. In addition, *P. multocida* can be a major contributor to not well-defined conditions of the respiratory tract such as the Bovine Respiratory Disease, enzootic pneumonia in sheep, swine, and calves. *Mannheimia haemolytica* is a normal resident of the nasopharynx and tonsils of cattle and sheep, and also an opportunistic pathogen [5,6]. In cases of immunosuppression caused by various combinations of environmental stress factors and complex interactions among several infectious agents, including virus and bacteria, the host may

fail to control the replication of *M. haemolytica* [5]. After that, *M. haemolytica* invades the lungs and infects the lower respiratory tract and alveolar epithelia, resulting in rapid development of fibrinous pleuropneumonia.

South American camelids are a source of livestock wealth in Andean countries. This group of animals includes two domestic species, alpaca (*Lama pacos*) and llama (*Lama glama*), and two wild species, vicuña (*Lama vicugna*) and guanaco (*Lama guanicoe*). Over the last decades, the studies on the production of South American came lids have been intensified in order to promote the production of alternative regional products to enhance the development of small regional economies [7]. According to this, llamas represent an important source of meat, fiber and dung, and they are essential in the culture of the native high Andean populations [8,9]. Infectious diseases constitute a limiting factor in the production of domestic camelids due to the high mortality and morbidity, in young and adults, which leads to serious economic losses. Even though there are few studies that suggest the existence of exclusive camelid pathogens [8], in general, camelid infectious diseases are the result of co-existence between species. In this regard, Rosadio et al. [10] have demonstrated the co-existence of bovine virus (parainfluenza type 3 and/or bovine respiratory syncytial) and bacteria (*P. multocida* and/or *M. haemolytica*) as responsible of the acute pneumonia in Andean alpacas [10].

The production of South American camelids is increasing in Argentina. In this country only a few epidemiological reports have been conducted to study camelid pathogens, and most of the literatures available are seroprevalence works against virus [8,11-13]. To date, literature data on bacterial agents is scanty. In an attempt to explore the situation in Argentina, we aimed our effort to evaluate the presence of serum antibodies against bacterial pathogens involved in pneumonic diseases (*P. multocida* and *M. haemolytica)* in llamas from different regions of the country. Specific antibodies were evaluated by ELISA, an avidity test and western blot. In addition, the results obtained in the avidity test and western blot were compared to those obtained with sera from mice immunized with a commercial vaccine for Bovine Respiratory Disease (BRD).

Material and Methods

South American camelids

Healthy South American camelids of different provinces of Argentina were bled by jugular vein puncture. A total of 122 llamas from different provinces of Argentina were incorporated in this study: 38 animals from Catamarca, 18 from Jujuy, 32 from Buenos Aires, 24 from Entre Ríos, and 10 animals from San Luis. In addition, 12 guanacos from Chubut were included in this study. All animal experiments were carried out in accordance with the EC Directive 86/609/European Economic Community for Animal Experiments, and the Guiding Principles of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. Individual serum samples were separated and stored to -20° until used.

Mice

Female BALB/c mice were obtained from the Animal Facility at the Facultad de Ciencias Veterinarias (Universidad de Buenos Aires, Argentina) and kept in the Animal Facility of our Institute. Animals were housed (n=5 mice/cage) under specific conditions according to the "Guide for the Care and Use of Laboratory Animals" (National Research Council of the National Academies, USA) [14], with controlled air temperature (20°C to 22°C), humidity, and 12 h light/dark cycles. Food and water were provided *ad libitum*. All mice were 6 weeks old at the beginning of the experiment.

Bacterial suspensions

P. multocida originally isolated from a clinical case of human bacteremia was purchased from the Special Bacteriology Department, INEI-ANLIS Dr. Carlos G. Malbrán. *M. haemolytica* was kindly provided by Biogenesis-Bagó laboratories. Both bacteria were subjected to phenotypic profile identification and 16S rRNA sequencing (Macrogen Inc., Korea). Further microbial identification was performed by means of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Microflex, Bruker Daltonics GmbH, Bremen, Alemania). Bacterial cultures were stored at -70°C in Brain Heart Infusion (BHI) broth (Biokar Diagnostics) containing 20% vol/vol glycerol. Initial inocula were prepared by streaking onto 5% rabbit blood agar plates and incubating overnight under aerobic conditions at 37°C.

Preparation of *P. multocida* and *M. haemolytica* antigens

A single colony from each strain was inoculated into 400 ml of BHI broth and incubated at 37°C under aerobic conditions with continuous shaking for 18 h. After incubation, cultures were harvested by centrifugation (4,000 rpm, 15 min, 4°C) and washed three times with phosphate-buffered saline (PBS) pH 7.4. Supernatants were discarded and the pellets resuspended in 10 ml PBS. Bacterial suspensions were divided to prepare the antigens to be employed in the antibody analysis; heat-killed whole-cell suspensions were used for ELISA and bacteria lysates for western blotting. To prepare the killed whole-cell suspensions, bacteria were killed by heating at 100°C for 1 h. To prepare the bacteria-lysate antigen for western blot, bacteria were subjected to 3 cycles of freeze-thawing by immersion in liquid nitrogen for 5min and then placed in the water bath at 70°C for 5 min. Bacterial cells were then sonicated (3 pulses of 15 sec) (Transsonic 540 Sonicator, Germany), centrifuged at 4,000 rpm for 10 min at 4°C, and lysates were stored at -20°C until used. Protein content in both antigen preparations was assessed by the Bicinchoninic acid method (Bio-Rad Protein Assay, USA).

Immunization schedule

BALB/c mice were divided in two groups: immunized mice (n=10), and non-immunized control mice (n=10). Mice were immunized subcutaneously on days 1 and 15 with 0.25 ml of a commercial national vaccine (containing aqueous suspension of inactivated Bovine Herpesvirus types 1 and 5, Bovine Viral Diarrhea types 1 and 2, Parainfluenza 3 virus, *Pasteurella multocida, Mannheimia haemolytica* and *Histophilus somni*). Twelve days after the last immunization, mice were bled.

Levels of specific antibodies against *P. multocida* and *M. haemolytica*

Specific camelid and murine antibody levels were measured by indirect ELISA in individual serum samples, employing heat-killed *P. multocida* or *M. haemolytica* as coating antigen in PBS. Briefly, polystyrene microplates (96 well, Nunc MaxiSorp) were coated separately with 1 µg protein/well of each antigen diluted in PBS. For mice and camelid samples, 100 µl of diluted (1/100) serum was added to each well in duplicates and incubated at 37°C for 1 h. Specific antibodies were detected using a HRP-conjugated H+L goat anti-llama (1/7,000, Bethyl) or HRP-conjugated anti-mouse IgG serum (1/6,000, Cappel) as secondary antibodies. Absorbance values

(optical density, OD) were obtained after spectrophotometric reading at 450 nm in an ELISA plate reader (Thermo, Electron Corporation, Original Multiskan Ex).

Avidity test

A similar indirect ELISA was performed in 13 serum samples of mice and llama employing 8M urea as chaotropic agent for dissociation. After the incubation of the sera, the well content was discarded and 100 µl of 8M urea was added and incubated at room temperature for 10 min. The reaction was continued as above. The OD obtained from these wells was considered as the remaining OD (OD_{UREA}) and was compared to the OD of the same serum sample but without the addition of the dissociating agent (OD_{TOTAL}). The avidity index (AI) was calculated as follows: $AI = (OD_{UREA}/OD_{TOTAL}) \times OD_{UREA} \times 100$.

Protein electrophoresis and western blot

In order to compare the antigen recognition pattern of the sera, western blots were performed. Bacteria-lysate proteins (30 µg/lane) were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using 12% running gel (Tris-HCl buffer, pH 8.8). The electrophoresis was carried out in Tris-Glycine chamber buffer at a constant current of 50 mA for 90 min. SDS-PAGE Standards (Bio-Rad) with a range of 6.5 kDa to 200 kDa were used as a molecular weight standards. After the electrophoresis, bacterial proteins were transferred onto the PVDF Plus Transfer membrane (0.22 micron, GE Water & Process Technologies) using an immunoblot system (Bio Rad) at a constant current of 300 mA for 90 min. Diluted hyper-immune anti-mouse sera and anti-camelid sera (1/100 in PBST- 3% BSA) were incubated for 1 h at room temperature. Bound antibodies were detected by addition of a goat anti-llama H+L HRP-conjugated serum (1/7,000, Bethyl) or a goat anti-mouse H+L HRP-conjugated serum (1/7,000, Jackson). Membranes were then exposed to luminol and hydrogen peroxide using an enhanced chemiluminescence kit (Pierce ECL WB Substrate, Thermo, USA) and revealed with a photographic film (Kodak, Rochester, NY, USA).

Statistical analysis

Absorbance values obtained by ELISA were analyzed calculating the mean, median and standard deviation for each camelid and mouse serum from different areas. Comparison between groups was analyzed by means of one-way ANOVA if normality of residuals, by Shapiro-Wilk test, was reached. When hypothesis of equality was rejected, a posteriori DGC test based on clusters, was applied [15]. When normality was not reached, non-parametric one-way ANOVA, Kruskal-Wallis, followed by a multiple comparison Dunn test, was appropriate. Double sided reference intervals of 95% for each region were calculated. Softwares used were: InfoStat (2014 version, Grupo InfoStat, Universidad Nacional de Córdoba, Argentina), GraphPad Prism 5.0 (GraphPad Software, USA), and MedCalc version 15.2.2. Values were considered significantly different at $^{*}P<0.05$, $^{**}P<0.01$, or $^{***}P<0.001$.

Results

The presence of antibodies against *M. haemolytica* and *P. multocida* in sera from South American camelids of different provinces of Argentina was analyzed. PBS and sera of immunized mice were used as controls. PBS incubation did not show detectable OD values demonstrating the specificity of goat anti-llama HRP-conjugated serum. Immunized mice sera were used to know the OD values of a positive sample even if it is another species. Sera from non-immunized mice showed low OD values (0.27 ± 0.10 for *P. multocida* and 0.37 ± 0.20 for *M. haemolytica).* When mice were immunized with the commercial vaccine, the OD values measured in the sera were increased (2.26 ± 0.82 for *P. multocida* and 1.28 ± 0.51 for *M. haemolytica*). A single dilution for each of 133 camelid serum samples was studied by indirect ELISA and OD values obtained for each bacterium are shown in the (Figure 1). The majority of sera analyzed presented reactivity against both microorganisms; only a few animals showed an OD value lower than 0.5 (1/133 for *P. multocida* and 7/133 for *M. haemolytica*). For *P. multocida*, a high percentage of animals (40%) had OD values >1.5 while that for *M. haemolytica* only a 6% of samples presented these high values. As observation, higher OD measures were obtained when the sera were confronted with heat-killed *P. multocida* as coating antigen compared to *M. haemolytica* (Figure 1). Animals involved in this study inhabited in different provinces of Argentina. In order to evaluate whether the origin of animals influences the presence of specific antibodies, the distribution of OD values by provinces was analyzed. The dispersion of OD values was different for both pathogens, being homogeneous in some provinces and more dispersed in others (Figure 2A and 2B). Camelid sera from Buenos Aires and Chubut presented a more diverse response against *P. multocida* while a slightly more uniform response was found in other provinces like Catamarca, Jujuy and Entre Ríos (Figure 2A). Similarly, OD values were included in reference intervals according to a normal distribution for each microorganism and each province (Table 1). Conversely, the response against *M. haemolytica* was more compact along the different provinces (Figure 2B). These results are also reflected in the reference intervals. The OD values for both bacteria were then divided in levels (0-0.5; 0.5-1; 1-1.5; 1.5-2 and >2) (Figure 3). When *P. multocida* was employed as coating antigen, sera of camelids from Chubut, Buenos Aires and San Luis showed higher OD values (>1.5 OD) as compared to the rest of the provinces (Figure 3A). While Chubut, Buenos Aires and San Luis presented more than a 60% of animals with these high antibody levels (67%, 60%, and 90%, respectively); only 20% to 28% of camelids from Catamarca (22%), Entre Ríos (21%) and Jujuy (28%) showed these OD values. Regarding *M. haemolytica*, the OD values measured for most sera were situated within the ranges of 0.5-1 (48%) and 1-1.5 (41%) (Figure 3B). When the differences between provinces for both microorganisms were evaluated dissimilar groups were observed (Table 2). For *P. multocida*, the group constituted by Entre Ríos, Catamarca and Jujuy showed similar OD values. These provinces presented values that were different from of those of Buenos Aires

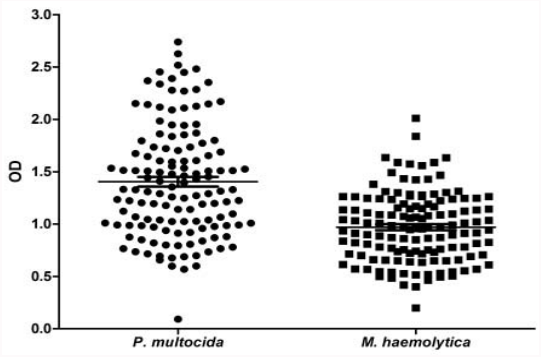

Figure 1: Antibodies against *P. multocida* and M. *haemolytica* in camelid sera. A single dilution for each serum sample was analyzed by indirect ELISA. Optical density (OD) values obtained for each bacterium are shown. $^{***}P<0.001$.

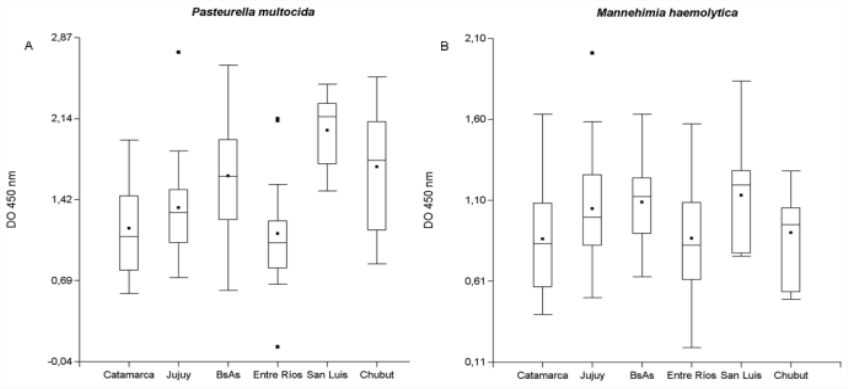

Figure 2: Box and whiskers plots showing the distribution of OD values among provinces. OD values for specific *P. multocida* A) and M. *haemolytica* B) antibodies for each province are shown. Dots inside the box represent the average.

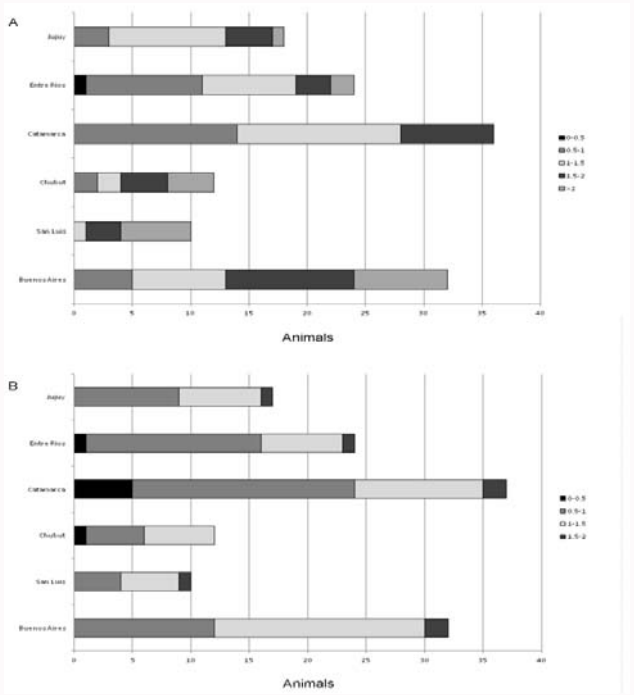

Figure 3: Distribution of OD values among provinces. OD values of camelid sera from each province were divided in levels (0-0.5; 0.5-1; 1-1.5; 1.5-2 and >2). Distributions for specific *P. multocida* A) and *M. haemolytica* B) antibodies are shown.

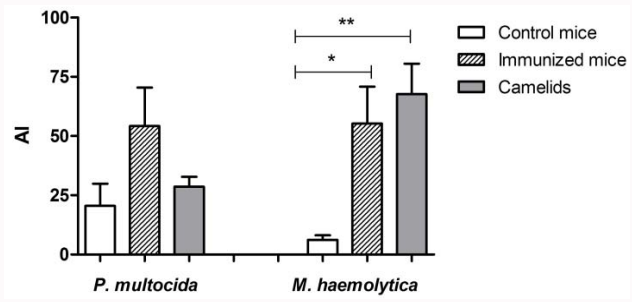

Figure 4: Avidity test. To analyze the sera avidity, ELISAs for both bacteria using 8M urea as dissociating agent were performed. The avidity index (AI) for each serum studied was calculated according to description in materials and methods. Results were expressed as mean AI ± SEM for each group. *P<0.05; **P<0.01.

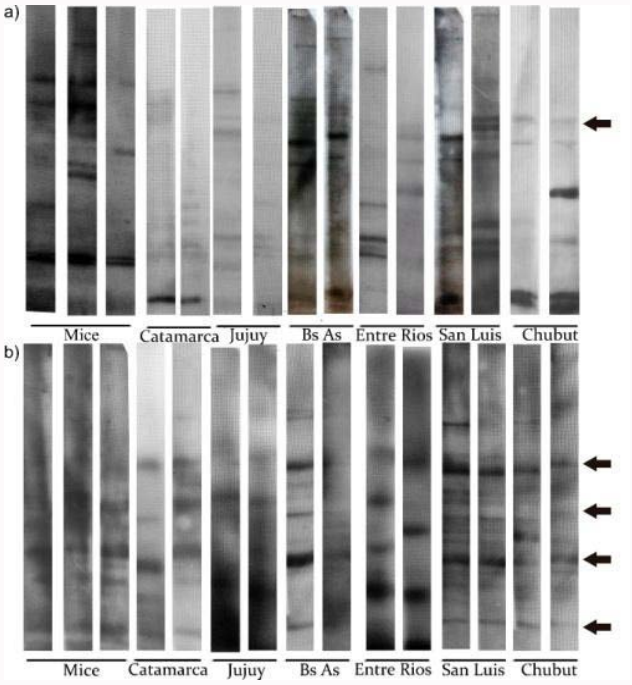

Figure 5: Western blot assays. Serum reactivity against *P. multocida* A) and *M. haemolytica* B) of sera from camelids and immunized mice were analysed by western blot. Shared bands are indicated by arrows.

and Chubut, and the OD values of sera from camelids of San Luis were different from all the other provinces. On the other hand, for *M. haemolytica* the sera exhibited a different behavior. The OD values obtained from each province after analysis were grouped in two: Catamarca, Entre Ríos and Chubut; and Jujuy, Buenos Aires and San Luis (Table 2). To analyze the sera avidity, ELISAs for both bacteria using 8M urea as dissociating agent were performed. The avidity index (AI) for each serum studied was calculated as described in Materials and Methods section. Similar AIs were obtained for sera specific to *M. haemolytica* between camelids and immunized mice and they were found to be significantly higher than those obtained in control mice (Figure 4). However, when *P. multocida* was employed as antigen, no significant differences were observed between groups. Finally, western blot assays were carried out to discriminate the antigen recognition patterns of camelid and mouse serum samples. For *P. multocida*, sera showed a diverse behavior: similar band patterns were observed between llamas of the same province; however it was not possible to establish a common antigen recognition pattern between

Table 1: Reference intervals for a normal distribution of OD values for specific *P. multocida* and *M. haemolytica* antibodies obtained in animals from different provinces.

95% Reference Intervals	*Pasteurella multocida*	*Mannheimia haemolytica*
Catamarca	0.39-1.92	0.25-1.48
Jujuy	0.46-2.22	0.33-1.77
Buenos Aires	0.62-2.60	0.56-1.63
San Luis	1.35-2.73	0.48-1.79
Entre Ríos	0.23-1.99	0.20-1.54
Chubut	0.55-2.87	0.34-1.47

Table 2: Statistical analysis of OD values. Mean differences of OD values for specific *P. multocida* and *M. haemolytica* antibodies between provinces were analysed. *P<0.05.

P. multocida				*M. haemolytica*			
Province	**Mean**	**n**		**Province**	**Mean**	**n**	
Entre Ríos	1.11	24	A*	Catamarca	0.87	37	A*
Catamarca	1.16	36	A	Entre ríos	0.87	24	A
Jujuy	1.34	18	A	Chubut	0.90	12	A
Buenos Aires	1.63	32	B	Jujuy	1.05	18	B
Chubut	1.71	12	B	Buenos Aires	1.09	32	B
San Luis	2.04	10	C	San Luis	1.13	10	B

Test: DGC post ANOVA α=0.05
*Different letters indicate significant differences.

camelid sera from different regions. Furthermore, different antigen specificity profiles were found between immunized mice and camelid sera (Figure 5A). In contrast, for *M. haemolytic*, a similar recognition pattern was observed between llamas of the same province (Figure 5B). In addition, shared bands were found between camelids and mice. Likewise, the sera of immunized mice showed a homogeneous response for *M. haemolytica* (Figure 5B).

Discussion

South American camelids constitute a strategic resource since they provide products such as meat, hides, and wool. Although zoonotic diseases affecting these animals have been scarcely reported, the occurrence of infectious diseases in camelids is important for both, domestic and wild species. In the llama, these infectious diseases influence the production and have an impact in socio-economic aspects at various levels. As for the respiratory infectious diseases, there are reports demonstrating the interconnection between viral etiologic agents responsible of the respiratory diseases in bovines with those affecting camelids [10,16]. Moreover, Rosadio et al. [10] have reported an outbreak of *P. multocida* and *M. haemolytica* in Peru, which resulted in acute pneumonia, in association with parainfluenza type 3 and herpes viruses [16]. Since in Argentina the serological status of South American camelids for these bacterial pathogens is not known, in this study the presence of serum antibodies against *P. multocida* and *M. haemolytica*, both bacteria involved in pneumonic diseases, was evaluated. Several serum samples of *Lama glama* and *Lama guanicoe* of the different regions of the Argentina were assayed by an in-house indirect ELISA. The camelids included in the assay presented a good health status without clinical signs of disease. Additionally, none of them had been immunized with any vaccine against *P. multocida* or *M. haemolytica*. Despite the absence of signs of disease, a high seroprevalence was found in the collected serum samples indicating that the animals had been exposed to these microorganisms. It is known that the serological occurrence suggests the presence, circulation or colonization of microorganisms. *P. multocida* and *M. haemolytica* are usually found in the upper airways of cattle but, to our knowledge, no studies about the presence of these bacteria in the airways of camelids have been performed. However, due to the sympatry between camelids and other livestock species, circulation of viral and bacterial agents might occur. In this line, in camelids, other authors have reported serological evidence of exposure to viral agents typically found in bovines. Antibodies against Rotavirus, Parainfluenza-3 virus (PI3), Bovine Herpesvirus-1, Bovine Viral Diarrhea virus, Foot-and-mouth disease virus and Bluetongue virus were detected in llamas, most of which did not cause clinical symptoms of the disease [17-20]. Regarding bacterial agents responsible for respiratory diseases, a study performed in llamas from Jujuy revealed a negative serological result for *Brucella* sp. and *Mycobacterium paratuberculosis*, suggesting the absence of these microorganisms [21]. However, in these studies, an anti-bovine IgG serum was employed as secondary antibody which could have led to negative false results. Contrarily, in our study, specific llama antibodies were employed, thus conferring a great robustness to our results. Animals involved in this study were from different provinces of Argentina, a large country with a varied geography and climate, which are conditions that influence the type of food, water availability, etc. that the animals have access to. Catamarca and Jujuy are located in the north of the country where the average annual temperature is high. Buenos Aires, Entre Ríos and San Luis are in the center while Chubut is in the south of Argentine and has colder temperatures compared to other provinces. On the other hand, the co-existence of species, like cattle or domestic animals, is less frequent in warmer areas. In our study, most of sera analyzed presented high OD values for both microorganisms independently of their region of origin, indicating that the geography was not a relevant factor in this survey. Nevertheless, some other observations could be made: for *P. multocida*, sera from camelids from Chubut, Buenos Aires and San Luis showed the highest OD values (OD>1.5). Besides, these provinces presented more of 60% of animals with these high antibody levels while only 20% to 28% of camelids from Catamarca, Entre Ríos and Jujuy reached these OD values. Statistical analysis showed that the group constituted by Entre Ríos, Catamarca y Jujuy showed similar OD values, while the values corresponding to Buenos Aires and Chubut were similar. These results demonstrate that the camelids' humoral response against *P. multocida* heterogeneous. On the contrary, the antibody response against *M. haemolytica* was more homogeneous, being similar among the provinces. By western blot assay, a similar antigen recognition pattern was observed for specific *M. haemolytica* sera between llamas of the same and the different provinces. In addition, shared bands were also found between camelids and immunized mice. Sera specific to *P. multocida* showed a different behavior, not being possible to establish a common recognition pattern for bacterial antigens with the studied sera. The avidity of a serum is defined as the overall binding strength of antibodies to an antigen. Avidity maturation (from low to high) is associated with the passing of time and the increased and prolonged antigenic stimulation; the time necessary to obtain high avidity antibodies varies with different antigens [22]. In this study we also evaluated the serum avidity. The avidity test performed here showed that while higher OD values were found in sera against *P. multocida*, these values do not correlate with sera avidity. On the contrary, sera specific for *M. haemolytica* displayed a high avidity index. Moreover, the avidity of sera obtained from camelids was comparable to that obtained from immunized mice. These results suggest a different

behavior of camelids against both microorganisms and it reveals that the South American camelids from Argentina develop a maturation of the immune response against *M. haemolytica* similar to that elicited by a commercial vaccine in mice. It is feasible to postulate that Argentinean camelids be in contact with *M. haemolytica* and suffering subclinical infections. This observation is also supported by previous results of Marcoppido et al. [11] which demonstrated that guanacos bred in captivity and free of anti-PI3 antibodies, undergo seroconversion upon contact with calves in the same geographic area [11]. On the other hand, antibodies against *P. multocida* were also found but these antibodies presented low avidity and a dissimilar behavior to immunized mice. Probably, this reactivity can be a consequence of cross-reactivity or be caused by a normal colonization of this bacterium. Further studies will allow us to confirm these assumptions. Lack of a group of animals with pneumonic disease signs or with positive isolates for bacteria involved in this study inhibits us to determine seropositivity. However, preliminary studies realized in our laboratory indicate that neither *P. multocida* nor *M. haemolytica* are present in nasopharyngeal samples (data not shown). From our results we emphasize that our finding is the presence of these antibodies in camelid sera.

Conclusions

In this work we report for first time the presence of antibodies to *Mannheimia haemolytica* and *Pasteurella multocida* in Argentinean llamas. Taking into account the results obtained in the work presented herein, it can be concluded that the Argentinean camelids synthesize antibodies that recognize different antigens of *M. haemolytica* with high avidity. As mentioned above, our results would suggest that these animals are in contact with *M. haemolytica* causing them only a subclinical infection. However, these infected camelids would act as a pathogen reservoir, thus transmitting the disease to other animals. In summary, this work gives information about the serological status of South American camelids in Argentina and discloses the importance of considering these microorganisms as possible infectious agents in the camelids of the region.

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