The Veterinary Journal 199 (2014) 308–309

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Short Communication

Differences in natural antibody titres comparing free-ranging guanacos (*Lama guanicoe*) and capybaras (*Hydrochoerus hydrochaeris*)

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ARTICLE INFO

Article history:
Accepted 31 October 2013

Keywords:
Eco-immunology
Natural antibodies
Immunological response
Wild mammals

ABSTRACT

Natural antibodies are an important component of innate humoral immunity but have not been investigated to any great extent in wild mammals. In the current study, serum natural antibody titres were measured by hemagglutination assay for two South American herbivores, the guanaco (*Lama guanicoe*) and the capybara (*Hydrochoerus hydrochaeris*). Results indicated that capybaras had antibody titres on average more than four times higher than guanacos (median titres 1:256 and 1:4, respectively), suggesting differences in investment in constitutive humoral immunity between the two species.

It has been proposed that immunocompetence is a key factor in regulating wildlife populations (Lochmiller, 1996). Components of the innate immune system might be involved in an organism's survival as they actively participate in resistance and rapid response to infection. Natural antibodies (NAb) are one component of innate humoral immunity, and are unusual compared to other immunoglobulins, in that their production is constitutive and does not require previous exposure to a particular antigen (Ochsenbein and Zinkernagel, 2000). Given that NAb confer humoral immunity independent of antigenic stimulation and are stable over time, they have the potential to be used as indicators of immune competence in wild animals. Recently, NAbs have been used as indicators of innate humoral immunity in wild birds (Mendes et al., 2006; Whiteman et al., 2006) and reptiles (Sandmeier et al., 2012), but rarely in wild mammals (Gilot-Fromont et al., 2012). For the majority of wild vertebrate species, production of NAb, as part of innate immune defence, remains poorly understood.

In South America, guanacos (*Lama guanicoe*) and capybaras (*Hydrochoerus hydrochaeris*) occupy similar trophic niches, but live in contrasting habitats. While guanacos inhabit predominantly dry environments (Monte desert and Patagonian steppe), capybaras are found in aquatic ecosystems. Studying the dynamics of health and immunity in these species would allow a better understanding of their natural history. In the current study, we report NAb levels in free-ranging populations of these South American herbivores.

The work was conducted in full compliance with the Bioethical Committee of Universidad Nacional del Litoral (Permit 100 number: 36/09). Serum and plasma samples from wild adult capybaras ($n = 64$) were obtained from a population under management of Esteros del Iberá, Corrientes Province, where sampling was conducted monthly over a 2-year period (August 2010–September 2012). Guanaco serum and plasma samples ($n = 112$; 77 adults, 18 yearlings and 17 juveniles) were obtained from wild animals captured for a sustainable shearing program in La Payunia reserve, Mendoza Province, during the Spring of 2009 and 2010. This capture procedure was conducted under a protocol that meets the animal welfare criteria established by the UICN South American Camelid Specialist Group.[1] Each capybara and guanaco was sampled only once. The samples obtained were stored at −20 °C until use.

Determination of agglutinating NAb titres was carried out in duplicate, using a hemagglutination assay described by Matson et al. (2005), with some modifications. Briefly, 25 μL of 0.01 M phosphate-buffered saline (PBS) (Sigma Aldrich) was added to columns 2–11 of 96-well round bottom assay plates (DeltaLab) and 25 μL of each sample was added to columns one and two. Doubling dilutions were made in PBS by transferring 25 μL from one well into the consecutive one, from well 2 to 11, leaving well 12 as a negative control (PBS only). A rabbit red blood cell (RBC) suspension was prepared from whole rabbit blood, diluted 50:50 with Alseveŕs solution (Sigma–Aldrich). The hematocrit was checked using capillary tubes and the RBCs were washed three times with PBS and adjusted to 1% final cell concentration. Twenty-five microlitres of the 1% stabilized rabbit RBC suspension was added to each well and plates mechanically shaken, followed by incubation for 2 h at room temperature. Titres were recorded as the last dilution

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[1] See: http://www.camelidosgecs.com.ar/documentos_ingles.html.

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http://dx.doi.org/10.1016/j.tvjl.2013.10.036

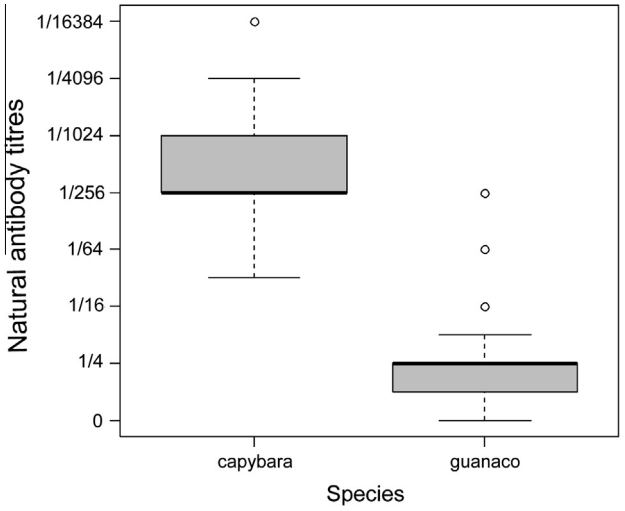

Fig. 1. Box and whisker plots showing natural antibodies titres, expressed as the last serum or plasma dilution showing clear evidence of agglutination, in wild capybaras ($n = 64$) and wild guanacos ($n = 112$). Box plots depict the median (bold bar), 25–75% quartiles (box), 10–90% quartiles (whiskers) and outliers (circles).

showing clear evidence of agglutination. Comparison between NAb titres of capybaras and guanacos was performed using a Mann–Whitney *U* test and comparison between seasons and age classes in capybaras and guanacos, respectively, using the Kruskal–Wallis test.

In capybaras, NAb titres ranged from 1:32 to 1:16,384 (median = 1:256). There was no evidence of seasonal variation (Kruskal–Wallis chi-square = 5.97, $P = 0.113$). In guanacos, NAb titres ranged from undetectable to 1:256 (median of 1:4). The titres did not differ among age classes (Kruskal–Wallis chi-square = 1.99, $P = 0.370$). Capybaras demonstrated NAb titres greater than guanacos ($P < 0.001$). Moreover, there was almost no overlap in the titres comparing the two species (Fig. 1).

These results highlight a very different innate immunological investment comparing the two species studied and one that is worthy of more detailed investigation. A wide variety of factors may be involved in this differential immunological behaviour. The difference might simply be related to the taxonomic distance between both species (Rodentia and Cetartiodactyla), but it could also be attributable to adaptation to the different environments inhabited by each species. Micro- and macro-parasites are richer and more abundant in humid and warm environments than in dry and cold ones (Altizer et al., 2006). The capybara inhabits tropical and subtropical wetlands, where temperature and humidity are high. Guanacos, in contrast, inhabit arid regions with cool temperatures. Our hypothesis is that NAb levels, and perhaps other elements of humoral and cellular innate immunity, might differ in capybaras compared to guanacos as a result of evolutionary adaptation to their exposure to different pathogens. Similarly, caimans (*Caiman latirostris* and *Caiman yacare*), which share the same habitat with capybaras and are therefore exposed to a similar environment, rich in potentially pathogenic organisms, also show high levels of humoral innate immune activity (complement system) (Sirosky et al., 2010). It is, however, also feasible that the difference does not originate from an evolutionary divergence, but could instead result from inter-population variation caused by differential exposure to warmth and humidity. Future work should focus on whether this natural variation is a species characteristic or not.

Over interpretation of the results generated by the hemagglutination test used here should be avoided, as there could potentially be a degree of cross-reactivity with antibodies produced by the adaptive immune response. In fact, cross-reactivity can be a confounding factor for all assays of specific or natural antibodies. However, using a more sensitive and specific test, such as ELISA, would require access to secondary antibodies specific for each species studied and the lack of available immunological reagents for wild animal species is a major limitation when performing disease ecology and eco-immunology studies.

Our results suggest a need for further studies of NAb and other components of the immune system in these and other wild animal species, comparing immunological investment and pathogen exposure within and between species.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

This study was supported by Agencia Nacional de Promoción Científica y Tecnológica (PICT2010-2202), Secretaría de Estado de Ciencia, Tecnología e Innovación de la Provincia de Santa Fe (SECT-EI-21-08-12), and Universidad Nacional del Litoral (CAI+D 002-009).

We thank Pablo Carmanchahi, Carolina Marull, Virginia Rago, Sebastian Cirignoli, the Payun Matru Cooperative and The Conservation Land Trust staff for their essential support during fieldwork.

References

Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M., Rohani, P., 2006. Seasonality and the dynamics of infectious diseases. Ecology Letters 9, 467–484.

Gilot-Fromont, E., Jégo, M., Bonenfant, C., Gibert, P., Rannou, B., Klein, F., Gaillard, J.M., 2012. Immune phenotype and body condition in Roe deer: Individuals with high body condition have different, not stronger immunity. PLoS ONE 7, e45576.

Lochmiller, R.L., 1996. Immunocompetence and animal population regulation. Oikos 76, 594–602.

Matson, K., Ricklefs, R., Klasing, K., 2005. A hemolysis–hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. Developmental and Comparative Immunology 29, 275–286.

Mendes, L., Piersma, T., Hasselquist, D., Matson, K.D., Ricklefs, R.E., 2006. Variation in the innate and acquired arms of the immune system among five shorebird species. Journal Experimental Biology 209, 284–291.

Ochsenbein, A.F., Zinkernagel, R.M., 2000. Natural antibodies and complement link innate and acquired immunity. Immunology Today 21, 624–630.

Sandmeier, F.C., Tracy, C.R., Dupré, S., Hunter, K., 2012. A trade-off between natural and acquired antibody production in a reptile: Implications for long-term resistance to disease. Biology Open 1, 1078–1082.

Sirosky, P.A., Merchant, M.E., Parachú Marcó, M.V., Piña, C.I., Ortega, H.H., 2010. Characterization of serum complement activity of broad-snouted caiman (*Caiman latirostris*, Crocodilia: Alligatoridae). Zoological Studies 49, 64–70.

Whiteman, N.K., Matson, K.D., Boomer, J.L., Parker, P.G., 2006. Disease ecology in the Galápagos Hawk (*Buteo galapagoensis*): Host genetic diversity, parasite load and natural antibodies. Proceeding of the Royal Society B 273, 797–804.