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ORIGINAL ARTICLE

Study of the Kinetics of Antibodies Titres Against Viral Pathogens and Detection of Rotavirus and Parainfluenza 3 infections in Captive Crias of Guanacos (*Lama guanicoe*)

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Summary

A longitudinal study was conducted to investigate the presence of antibodies (Ab) to Rotavirus (RV), Parainfluenza-3 virus (PI-3), Bovine Herpesvirus-1 (BoHV-1), Bovine Viral Diarrhoea virus (BVDV-1) and Bluetongue virus (BTV) in eleven guanaco's crias (chulengos) relocated from Rio Negro to Buenos Aires Province (Argentina) and reared in captivity for a year in an experimental field. Serum samples were collected periodically to detect the evidence of viral infections. Faecal samples were collected to investigate RV shedding. We detected the evidence of Ab to RV from the beginning of the experience, suggesting the presence of maternal Ab against the virus. RV infection was detected in seven of the eleven chulengos, by seroconversion (4), virus shedding in stools (1) or both (2). In all cases, the RV strain was typed as [P1]G8, the same G/P type combination detected in captive chulengos with acute diarrhoea sampled in Rio Negro, in 2001. In contrast, we could not detect antibodies against PI-3, BoHV-1, BVDV or BT in any of initial samples. No Abs against BoHV-1, BVDV or BTV were detected in the chulengos throughout the study. However, all the chulengos became asymptomatically seropositive to PI-3 by the 7 month after arrival. This study suggest that wild-born guanacos raised in captivity can be relatively susceptible to common livestock viral infections, such as RV and PI-3, which are easily spread among chulengos.

Introduction

Three of the four species of South American camelids (SACs), llamas (*Lama glama*), guanacos (*Lama guanicoe*) and vicuñas (*Vicugna vicugna*), inhabit Argentina, being the first one domestic and the other two wild species.

The guanaco has the broadest geographical distribution, ranging from sea level in Tierra del Fuego to up to 4000 m in the Andes. It is the most abundant free-ranging ungulate of arid environments and the native species characteristic of the Patagonian region (Franklin, 1983).

The numbers of free-ranging guanacos in South America declined from 30 000 000 to 35 000 000 (Raedeke, 1979) individuals prior to the Spanish conquest to 400 000–600 000 guanacos with more than 90% of the total population inhabiting the Patagonia (Franklin, 1983; Torres, 1985; Amaya and von Thüngen, 2001). Guanacos have suffered the loss of habitat because of sheep and domestic cattle feeding competition introduced by Europeans, legal overhunting and poaching. This situation has led to the species being listed in appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and

Flora (CITES), which allows using the species under strict regulations on its trade (Montes, et al., 2006).

The current guanaco management programmes in Argentina include capture, shearing and release of wild populations of guanacos (Montes et al., 2006) and farming systems (Amaya and von Thüngen, 2001). However, the intensive breeding of wild animals involves inappropriate captive environments that have deleterious effects on the behaviour and physiology of mammals (Carlstead, 1996), including a diminished immune competence of animals, which affects the survival of the individuals when faced with pathogens (Teixeira et al., 2007). Severe acute diarrhoea, respiratory, and reproductive problems, mainly caused by viral diseases, were previously described in captive guanacos (Fowler, 1998; Mattson, 1994; Parreño, et al., 2001).

Even though SACs are susceptible to infectious agents that cause disease in domestic animals (Parreño and Marcoppido, 2006), little is known about viral infections affecting this species. In addition, this information is limited to surveys in domestic camelids. Previous studies have reported serological evidence of exposure to Rotavirus (RV), Parainfluenza-3 virus (PI-3), Bovine Herpesvirus-1 (BoHV-1), Bovine Viral Diarrhoea virus (BVDV-1), Foot-and-mouth disease virus (FMDV) and Bluetongue virus (BTV) in llamas, most of which did not cause clinical symptoms of the disease (Mattson, 1994; Fowler, 1998; Saraiva, 2004; Celedón et al., 2006; Evermann, 2006; Cabello et al., 2006; Parreño and Marcoppido, 2006). Viral antigens have been also detected from aborted foetus and nasal discharge in domestic SACs that correlated with the presence of clinical signs of disease for BVDV and Adenovirus (Mattson, 1994; Mattson et al., 2006; Parreño and Marcoppido, 2006; Foster et al., 2007). Nonetheless, current available information on wild SACs is scarce. We have recently reported the detection of wild vicuñas with high Ab prevalences to RV and PI-3 (100% and 37%, respectively) and just a few wild vicuñas seropositive for BoHV-1 and BVDV-1 (0.78%) in the Andean Puna (Marcoppido et al., 2010). Previous serological surveys conducted in free-ranging guanacos from Argentina and guanacos and vicuñas from Chile did not detect Ab against BoHV-1, BVDV, PI-3, BTV or FMDV (Karesh et al., 1998; Celedon et al., 2001). However, a study carried out in the Argentinean Patagonia region on a population of wild guanacos kept in captive conditions reported 95% antibody (Ab) prevalence to RV and the first isolation and characterization of RV in chulengos with acute diarrhoea (Parreño et al., 2001, 2004). As little disease prevalence information is available from captive guanacos, the purpose of this longitudinal study was to evaluate the antibody response against a panel of livestock-affecting viruses in chulengos relocated from their natural environment in Patagonia to Buenos Aires Province.

Materials and Methods

Animals

In February 2002, eleven wild-born male chulengos ranging from 3 to 4 weeks of age were relocated from a breeding farm in Río Negro Province (Patagonia region), Argentina, to the experimental field of the Centro de Investigaciones en Ciencias Veterinarias y Agronomicas (CICVyA), Instituto Nacional de Tecnología Agropecuaria (INTA), in the province of Buenos Aires. The crias were relocated for physiological studies and raised in captivity according to proper management practices for the species (Zapata et al., 2002). The captive chulengos were studied over a 7-month period. During this time, the animals were placed in two paddocks (five and six animals each) that were interconnected through a common pass with a larger paddock. The animals were bottle-fed three times a day, with 2% of their body weight of bovine pasteurized powder milk (Nestle®, Saladillo, Buenos Aires, Argentina) and supplemented with alfalfa hay. Weighing, physical examination and specimen collection took place in a handling pen. At 1 year of age, the chulengos were relocated to a breeding farm in the west of Buenos Aires province.

Sampling protocol

Blood samples were obtained under manual restraint of the animals by jugular veni-puncture (Fowler, 1998). Chulengos were sampled monthly from 1 to 7 months of age. The sampling was repeated in all the chulengos 10 months after the relocation, at 17 months of age. An additional sampling was conducted in three animals, at 20 months of age. The serum samples were obtained by centrifugation and stored at −20°C until use. Faecal samples were collected monthly and stored at −20°C until use.

Laboratory Testing

Faecal samples were screened for group A RV by ELISA, using reagents and techniques previously described by Cornaglia et al. (1989). This technique was adapted for the detection of Ab to RV in serum samples from SACs using I-801 NCDV Cody P[1]G8 BRV reference strain as positive antigen and mock-infected MA-104 cells as negative control. Serum samples were tested in serial 4-fold dilution and a commercial peroxidase-labelled polyclonal goat anti-llama IgG (H+L) (Bethyl, Lab Inc, Montgomery, CA, USA) at a 1 : 2000 dilution was used as conjugate to develop the assay (Parreño et al., 2001).

Rotavirus (RV) genomic ds-RNA was extracted from the rotavirus-positive faeces (Sambrook et al., 1989), and a RT-PCR analysis was performed as previously described for RV typing (Parreño et al., 2004).

Virus neutralization tests (VN) for BoHV-1 and BVDV-1 were carried out following standard methods for domestic cattle (OIE, 2008), using reference strains BoHV-1 Los Angeles and BVDV-1 Singer (cytopathic biotype 1a), respectively. Antibodies to PI-3 were detected by a hemagglutination-inhibition test (HI) (Collins et al., 1996) specially adapted for SAC sera, using PI-3 reference strain ST20. Briefly, potassium periodate-glycerol pretreated sera were diluted from 1/5 to 1/320 in 'U'-shape microtitre plates and mixed with an equal volume of PI-3 containing 8 hemagglutination units (HU). After 1-h incubation at room temperature, two volumes of 0.25% guinea-pig erythrocyte suspension in PBS were added. Serum Ab titre was expressed as inhibitory hemagglutination units (IHU) at the highest serum dilution showing complete inhibition of the viral hemagglutination activity, multiplied by the 8 HU of the virus used in the test. Antibodies against BTV were detected using a commercial immunodiffusion test (BTID) following the manufacturer's instructions (Veterinary Diagnostic Technology, Inc, Wheat Ridge, CO, USA). For RV and PI-3, sera from naturally infected guanacos served as positive controls (Parreño et al., 2001).

The positive controls for BoHV-1 and BVDV-1 were obtained from vaccinated llamas. For BTV, sera from naturally infected domestic cattle were used as positive control. All the tests included sera from llamas as negative controls. Seroconversion rates were defined as an increase of 1 $\log_{10}$ in the Ab titre between two consecutive serum samples.

Results

All sampled crias had serum Ab against group A RV upon arrival at the experimental field (1 month old). At the beginning of the experience, RV Ab titres were highly variable among individuals and ranged from 64 to 65 536. Geometric mean Ab titres of the group of chulengos remained high up to 7 months of age (Fig. 1).

One month after arrival, RV circulation was detected in seven of eleven chulengos (Fig. 2). The infection was evidenced by Ab seroconversion and virus shedding in two crias, whereas in four chulengos the infection was evidenced by Ab seroconversion only. The faeces of one chulengo (CH 17) that died during the second month of the study were also positive to group A RV. This chulengo showed the lowest Ab titre of the group (RV Ab titre = 64).

Rotavirus (RV) circulation was first detected in March (Figs 1 and 2). Virus shedding was detected in two chulengos (CH 3 and CH 7) with moderate RV Ab titres (256 and 1024, respectively). Antibody seroconversion in these crias occurred immediately after the initial detection of virus shedding, in April. Additional seroconversions were observed between February and March in two chulengos (CH 2 and CH 5) and between March and April in the other two crias (CH 6 and CH 8) (Fig. 2).

Rotavirus (RV) infection either was asymptomatic (9/11) or showed mild diarrhoea (2/11), at 2–3 months of age, approximately 1 month after arrival at the experimental field. During April, the mean RV Ab titres reached a peak value (GM = 16 384). After the peak of infection, the mean Ab titres decreased over time, even after the relocation of the animals, at 17 and 20 months of age (Fig. 1). RV ELISA-positive stools were typed as [P1]G8 by multiplex RT-PCR.

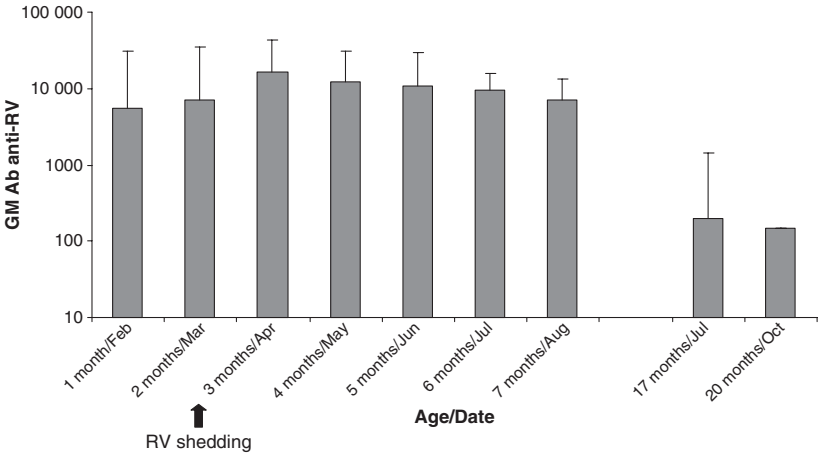

Fig. 1. Geometric mean Ab titres to RV of the group of chulengos during the sampling.

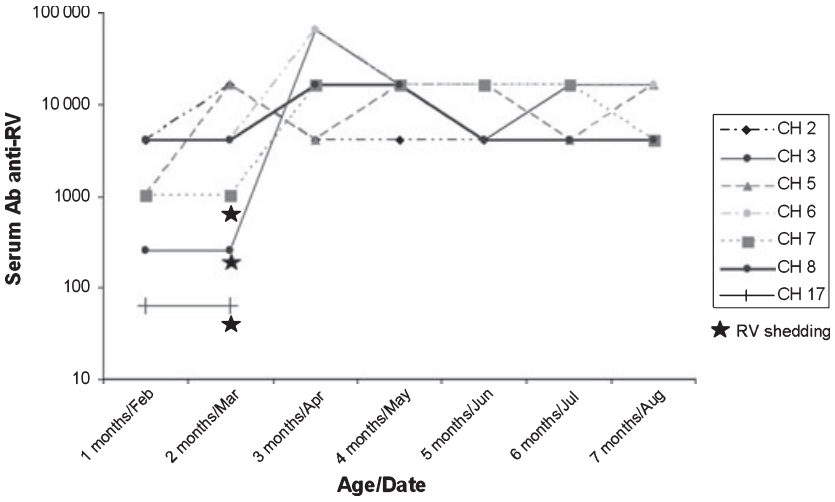

Fig. 2. Virus shedding and Ab seroconversion against RV detected in the chulengos sampled.

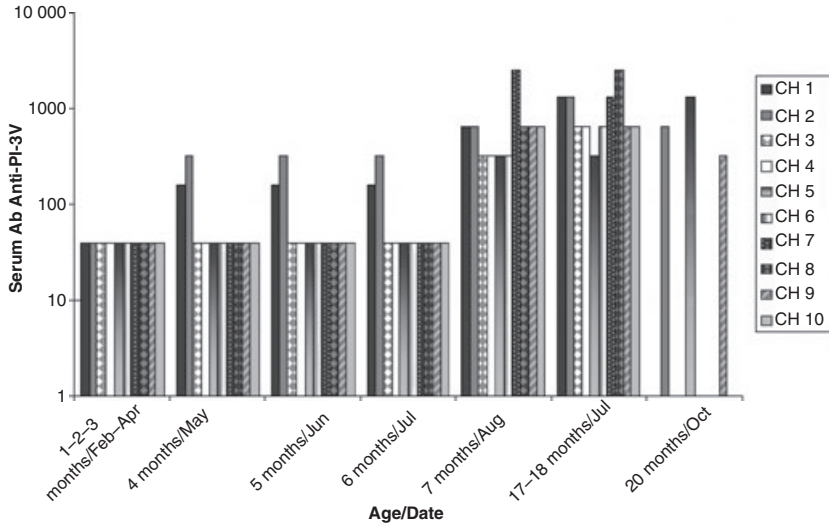

Fig. 3. Individual Ab titres to PI-3 of the chulengos during the experience.

All chulengos started the experience without detectable antibodies against PI-3. The animals remained seronegative throughout the first three samplings. At 4 months of age (May), two animals (2/10) became asymptomatically seropositive to PI-3 (IHA Ab titre range: 160–320, which remained at similar levels until the end of the study; Fig. 3).

One hundred per cent of chulengos had evidence of an immune response to PI-3 by 7 months of age, but no clinical symptoms were observed. During the last sampling carried out over three 20-month-old animals (13 months after the relocation), we detected Ab seroconversion to RV in two animals (CH 2 and 9) and Ab to PI-3 in one (CH 5). No Ab against BoHV-1, BVDV-1 or BT was detected in the chulengos during this longitudinal study.

Discussion

The captive breeding programmes of wild species such as SACs is subject to improper management conditions, affecting the well-being of the individuals. The most significant disease risks for intensive management are likely to occur when the hosts are chronically stressed, when they are exposed to novel pathogens, and/or when herd immunity is lost (Lyles and Dobson, 1993). Captivity

involves numerous stressors such as transportation and relocation, inadequate housing conditions, incorrect feeding practices, reduced space and human handling which are believed to influence the immunocompetence of the captive animals and lead to short- or chronic-term behavioural and physiological effects in the animals (Goddard et al., 1998; Moberg, 2000; Tarlow and Blumstein, 2007; Teixeira et al., 2007).

During this study, we attempted to diminish the stressful situations and events by applying strict welfare practices (Zapata et al., 2002). The chulengos gained weight monthly and remained in good physical condition during the entire study.

The high RV Ab prevalence detected in the chulengos upon arrival at the study site suggested the presence of passive maternal antibodies to this pathogen commonly found in guanacos from the Patagonia region (Parreño, 2001). RV was also described as the main cause of neonatal calf diarrhoea in Argentina (Garaicoechea et al., 2006). In addition, RV strains causing severe diarrhoea in chulengos from Patagonia have been previously characterized (Parreño et al., 2004). Our results agree with the high RV Ab prevalences found in adult camelids in South America (Rivera et al., 1987; Cebra et al., 2003) and more specifically in Argentina (Puntel et al., 1999; Parreño and Marcoppido, 2006). The circulating RV strain was typed as [P1]G8, identical to the strain previously detected in Rio Negro, which suggests that the virus shed by these animals was carried by the group of guanacos from Patagonia to Buenos Aires. Moreover, pre-exposure to the virus and/or presence of maternal Ab seems to confer immunity to infection because only chulengos with RV Ab titres below 1000 became infected. The low levels of passive Ab could be associated with an early separation from their mothers immediately after birth during the capture season, to start captive programmes. These early separations do not allow the crias to have a proper colostrum intake containing adequate titres of protective maternal Ab against the main pathogens that affect them in the wild. These management practices lead to failure in the passive transfer of Ab through colostrum and the consequent low protection against enteric and respiratory viral infections, such as RV and PI-3 viruses.

No information is currently available on PI-3 infections in camelids, but the seroprevalence rates found in previous surveys conducted in adult camelids, and the present study indicated that PI-3 was a common pathogen affecting SACs (Cabello et al., 2006; Parreño and Marcoppido, 2006). However, the chulengos were negative for Ab to PI-3 at arrival, suggesting the absence of maternal antibodies to this virus at the place of origin (Patagonia). The source of the PI-3 virus that infected two chulengos at 4 months of age and subsequently all animals by August is unknown, although this is a commonly found infection in cattle in Argentina. Because of a long rainy season, chulengos were placed in paddocks adjacent to other paddocks with calves that manifested respiratory symptoms; an inter-species transmission might have occurred. Bovines usually become infected with PI-3 as young calves after maternally derived antibodies decline, and during the period of acute infection, they shed large amounts of virus which can result in the infection of other susceptible species like camelids (Mattson, 1994). In addition, weather inclemency has been cited as a risk factor contributing to a higher circulation of PI-3 in alpacas, which could have contributed to this respiratory infection during a wet and cold winter season (Victorio et al., 2004). Lastly, the PI-3 virus might have been carried from Patagonia by the two crias detected as asymptomatically seropositive at an earlier stage.

Although this study did not detect evidence of BoHV-1 infection in chulengos, this agent was previously isolated from three different cases of bronchopneumonia and neurological syndromes in a genetically related species, llamas (Mattson et al., 1994). No serological evidence of BVDV-1 infection was detected in this study either, but other reports described it as a cause of abortion, weight loss, excessive nasal discharge or diarrhoea, ill thrift and ultimately death in young camelids in the USA (Parreño and Marcoppido, 2006). Moreover, we have recently reported the detection of high Ab prevalences to RV and PI-3 and a few wild vicuñas seropositive for BVDV-1 in the Andean Puna. In all the cases, the animals were in good conditions and presented no clinical signs of disease (Marcoppido et al., 2010).Although persistent BVDV infection in alpacas has also been described (Carman et al., 2005; Mattson et al., 2006), in this particular study we analysed serial samples of the chulengos during a year, and no seroconversion against BVDV was observed in the population indicating that no PI animals were present in the herd. The results of this study agree with those of others conducted in wild SACs where no antibodies against BoHV-1 and BVDV-1 were detected (Rosadio et al., 1993; Karesh et al.,1998; Celedón et al., 2001). As BTV is transmitted by vectors, the lack of detection of Ab in the chulengos sampled, which agrees with previous reports on domestic and wild SACs and bovines from the north of the country (Puntel et al., 1999), could be because of the lack of detection of *Culicoides spp* (Lager, 2004), the principal vector of the virus, both in Buenos Aires and in Patagonia.

This study suggests that wild-born guanacos raised in captivity might be susceptible to common livestock disease, such as RV and PI-3, and extreme sanitary and preventive schedule must be enforced during husbandry. Therefore, this study highlights the risk regarding the

relocation of wild animals, because they can either become infected with agents already present in the area or introduce new pathogenic agents into naïve regions (Woodford and Rossiter, 1994). The latter could be the case of the RV strain [P1]G8, which is shed by the chulengos and has not been reported to be circulating in bovines in Buenos Aires so far (Garaicoechea et al., 2006). These results also highlight the importance of avoiding captive breeding programmes in guanacos because of the possibility of an outbreak of RV and/or of PI-3 infection, two indigenous diseases present in SACs, during captive conditions. Overall, this longitudinal study highlights the need for future research to understand the potential role of wild SACs in the epidemiology of these important cattle diseases. A better understanding of the viral pathogens that affect SACs is crucial to achieve appropriate systems for sustainable management of wild populations.

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