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Research article

Haematology and serum biochemistry parameters in vaccinated versus unvaccinated captive Cuvier's gazelles: implications for zoo management practices

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Abstract
Wild ungulates kept in captivity have become increasingly important as stock for conservation and study. Routine preventive treatment and vaccination is used to reduce parasite density and/or minimise parasite transmission in multispecies captive facilities such as zoos. But vaccination also has disadvantages: animals are not allowed to develop a natural immune response, commensal parasites performing beneficial roles are also removed, handling the host species is difficult, and so forth. Even more problems arise when captive wild animals are bred for reintroduction into the wild, as the use of parasite-naive individuals may lead to failure. In this study we evaluated the need for such treatment in a captive population of Cuvier's gazelle. Our results show that there are no major differences in body weight or health status between sanitised (dewormed and vaccinated) individuals and those that are not. These results challenge the need for routine preventive vaccination in wild animals in captivity. We suggest that the advantages and disadvantages of vaccination of the study population should be weighed and balanced, and recommend that in absence of symptoms, regular coprological analysis be performed, vaccinating only when the parasite burden becomes pathological.

Introduction

As a result of declining free-ranging populations, wild ungulates kept in captivity have become increasingly important as stock for conservation and study. Therefore, knowledge of their diseases must be acquired, especially when they are bred for reintroduction into the wild (Kock et al. 2007; Sainsbury et al. 2012). Parasitic diseases play an important role in wild animals in captivity whose health status varies with factors such as inbreeding and loss of immunogenic variation, management, feeding, sanitation, and seasonal variation such as temperature and humidity (Shrikhande et al. 2008). The risk of infection to captive animals also increases with more restricted enclosures, because reinfection can occur due to environmental contamination (Ortiz et al. 2006).

In the wild, animals might be naturally resistant to parasitic infection or live in balance with their parasites. But the change in environment and living conditions from freedom to captivity influences the animals' ecology and might increase their sensitivity to parasitic infections (Goossens et al. 2005). In zoos, preventive vaccination is used to reduce parasite density and/or minimise parasite transmission in these multispecies captive facilities, but vaccination does not always induce a significant increase in antibody titres (Risi et al. 2012). On the other hand, the idea of maintaining captive populations as 'parasite-free' has come under scrutiny as, in the first place, the parasites themselves are important components of biodiversity (Pérez et al. 2006), and secondly, reintroducing parasite-naive individuals may lead to failed reintroduction if released individuals encounter a pathogen to which they have no resistance (Ewen et al. 2012a).

The Cuvier's gazelle (*Gazella cuvieri*) is an endangered Sahelo-Saharan species managed under an EEP (European Endangered Species Programme). The programme began at 'La Hoya' Experimental Field Station (EEZA-CSIC) in Almería, Spain, in 1975. In October 1980, 3:5 individuals (males:females) were sent to Münchener Tierpark Hellabrum (Germany). From 1982 to 1988 some of their descendants (6:9) were sent from

Münchener Tierpark Hellabrum to the San Diego Zoo (USA), and are the origin of the captive populations in North American zoos (Moreno and Espeso 2008). As described for other wild and semi-wild ungulates, Cuvier's gazelles at La Hoya host a wide spectrum of gastrointestinal (GI) nematodes (Ortiz et al. 2001, 2006), and are treated annually with ivermectin and Basquin®. More details of health and veterinary protocols used are given by Moreno and Espeso (2008).

This paper evaluates the probable advantages (disease prevention in hosts) of vaccination in a captive population of Cuvier's gazelle by comparing body mass and haematological and serological parameters in a vaccinated group and another unvaccinated group. We would expect vaccinated animals to be in better condition (body weight and health status) than the unvaccinated ones. The goal of our study was to evaluate the need for this kind of treatment in Cuvier's gazelles kept in captivity at La Hoya Experimental Field Station, as this is the EAZA institution housing the largest population of this species in the world, and as such, plays a very important role in its conservation at global level. Although vaccination can be an effective way to control parasites in captivity, such treatment also entails disadvantages, such as removal of other commensal parasites that may perform beneficial roles (Ewen et al. 2012b), and in zoos, difficulties in handling the host can also be a practical drawback.

Methods

Study species

The Cuvier's gazelle is a mountain ungulate of the Maghreb (Africa) that is in sharp decline in most of its range (Morocco, Tunisia, Algeria; Beudels et al. 2005) and classified as endangered by the IUCN (2010). It is a medium-sized sexually dimorphic gazelle with adult males 24% heavier than adult females (average body mass of adult females: 26.4 kg, range 21–32 kg; adult males: 32.6 kg, range 24.5–40 kg; Moreno and Espeso 2008). Sexual maturity is reached at 8–9 months for the female and at 12–13 months for males. Gestation is about 5.5 months (Moreno and Espeso 2008).

Management of the captive population and experimental procedure

Animal manipulations were performed in accordance with the Spanish Regulation for Animals in Research, RD53/2013, which conforms to European Union Regulation 2010/63/UE on the protection of animals used for scientific purposes. Moreover, this study was approved by the EEZA's Animal Ethics Committee.

As a general rule, breeding herds in La Hoya consist of one adult male and a group of five to eight adult females. Males used in this study were born in four different breeding groups in May 2009. Animals were kept in their breeding herds until they were six months old. At this age, juvenile males were removed from the breeding enclosure and split into two bachelor groups in separate enclosures (area 200 m^2 each), one with 10 individuals (labelled for the purpose of this study the 'vaccinated group') and another with 11 individuals ('unvaccinated group'). Unfortunately, one male from the vaccinated group was injured and had to be removed. Animals had ad libitum access to commercial pellets, fresh alfalfa, barley and water throughout the study (Moreno and Espeso 2008).

The vaccinated group was treated as follows: at the age of six months, animals were inoculated with ivermectin (a broad-spectrum antiparasitic and anthelmintic) and Basquin® (inactivated enterotoxemia and colibacillosis vaccine, also used for treatment of respiratory processes in sheep and goats) at the time of their transfer from the breeding to the bachelor herds. They were inoculated again with the same drugs in May 2011, at the age of two years (this is the normal vaccination procedure at La Hoya). Blood samples were taken from this group just before the second inoculation (sample I) and again one month later (sample II). The unvaccinated group, as their name implies, was not vaccinated during the study period (May 2009–June 2011). Blood samples in this group were taken only once, coinciding with sample II from the vaccinated group. All the gazelles were clinically normal at the time of sampling.

Individuals were captured using a hand net, and no sedatives were administered for sample collection (Moreno and Espeso 2008) in accordance with the Spanish animal protection regulation RD53/2013, and European Union Regulation 2003/65/CE. Immediately after capture, their legs were tied and their faces masked to reduce stress (Andrade et al. 2001). At capture, all animals were weighed and blood samples were collected from the jugular vein with disposable syringes and needles (0.9 mm, approximately 20 g). Immediately afterwards, blood was transferred to 5 ml tubes without additives for serum analysis and a 5 ml BD Vacutainer® EDTA-K3 tube for haematology. Blood samples for serum analysis were centrifuged at 4000 rpm for 15 minutes and the serum harvested for analysis. Haematology samples were refrigerated at 4º C and analysed within 24 h. Serum chemistries were analysed using a Menarini Falcor 350 analyser. Determinations included concentrations of total protein (TP), albumin, α-1 globulin, α-2 globulin, β-globulin, γ-globulin, and the albumin/globulin (A/G) coefficient was calculated. Haematological analyses were performed using an ABX Pentra 60 Haematology Analyser. The following haematological measures were calculated: erythrocyte count (RBC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), leucocyte, lymphocyte, neutrophil, and monocyte count.

Statistical analyses

A t-test for dependent samples was used to look for any differences in samples within the vaccinated group (sample I vs sample II), as they were taken from the same individuals at different times. For differences between vaccinated and unvaccinated groups, generalised linear mixed models (GLMM; vaccinated group sample I vs unvaccinated group; vaccinated group Sample II vs unvaccinated group) was used. As inbreeding may affect the parasitic burden of individual gazelles as well as their immunological response to parasites (Cassinello et al. 2001), the inbreeding coefficient was included in the analyses as a covariate. All analyses were performed using InfoStat (Di Rienzo et al. 2008).

Results and Discussion

Table 1 shows mean, standard deviation and range for the inbreeding coefficient, weight, serum and haematological parameters in vaccinated and unvaccinated groups. Differences are shown in Table 2 and Figures 1 and 2.

Individual inbreeding had no effect on any of the variables studied ($p>0.05$ in all cases). Cassinello et al. (2001) showed a positive relationship between nematode egg counts in faeces and individual coefficient of inbreeding in Cuvier's gazelles, and suggested a decrease in heterozygosity as the reason for the increased susceptibility to parasitism. The discrepancy between these findings and ours may have arisen from the coefficient of inbreeding used by Cassinello et al. (2001) having been calculated assuming a founder population formed by unrelated individuals, which does not seem to be the case (Ruíz-López et al. 2009).

The lower MCH and MCHC found in sample I than in sample II from vaccinated animals (Table 2; Figure 1 A and B) and MCHC in sample I from the vaccinated group compared to the unvaccinated group (Table 2; Figure 1 C), with no significant differences in RBC counts or haematocrit, would indicate a lower haemoglobin

Table 1. Mean, standard deviation (SD) and range for the inbreeding coefficient, weight, haematological and serum parameters in vaccinated and unvaccinated gazelles.

	Vaccinated group (Sample I)			Vaccinated group (Sample II)			Unvaccinated group		
Parameter	n	Mean ± SD	Range	n	Mean ± SD	Range	n	Mean ± SD	Range
RBC (mill/mm3)	9	11.29 ± 0.82	10.44 - 12.44	9	11.55 ± 0.68	10.6 - 12.32	11	11.67 ± 0.73	10.51 - 13
Hb (g/dl)	9	14.75 ± 1.06	13.5 - 16.3	9	15.25 ± 0.86	13.9 - 16.4	11	15.57 ± 0.97	14.4 - 17.5
HCT (%)	9	48.2 ± 3.74	44.3 - 53.3	9	49.2 ± 2.96	43.9 - 53.9	11	49.92 ± 3.07	46.3 - 55.6
MCV (fL)	9	42.73 ± 1.22	41.3 - 44.8	9	42.6 ± 1.32	40.8 - 44.6	11	42.77 ± 1.05	41.1 - 44.6
MCH (pgr)	9	13.05 ± 0.29	12.6 - 13.5	9	13.22 ± 0.31	12.6 - 13.6	11	13.34 ± 0.4	12.8 - 14
MCHC (%)	9	30.61 ± 0.47	29.9 - 31.5	9	31.01 ± 0.49	30.4 - 31.7	11	31.2 ± 0.25	31 - 31.8
RDW (%)	9	14.24 ± 0.3	13.8 - 14.8	9	14.3 ± 0.4	13.9 - 15.1	11	14.1 ± 0.52	13 - 14.7
Leucocytes (%)	9	4.88 ± 0.7	3.9 - 5.8	9	4.99 ± 0.73	3.5 - 5.7	11	4.71 ± 1.08	3.7 - 7.5
Neutrophils (%)	9	50.75 ± 8.51	35 - 58	9	44.5 ± 10.84	32 - 67	11	61.82 ± 9.37	51 - 76
Lymphocytes (%)	9	42.75 ± 8.51	31 - 58	9	47.25 ± 11.17	25 - 63	11	32 ± 9.52	17 - 43
Monocytes (%)	9	6.5 ± 3.16	2 - 11	9	5.25 ± 2.05	3 - 8	11	5.91 ± 1.45	3 - 8
Total protein (g/dl)	9	5.79 ± 0.3	5.34 - 6.22	9	5.61 ± 0.28	5.15 - 6	11	6.15 ± 0.47	5.33 - 7
Albumin (%)	9	55.85 ± 4.33	49.74 - 61.28	9	61.93 ± 3.39	56.1 - 66.2	11	53.2 ± 7.78	39.77 - 64.95
α 1 - Globulin	9	6.14 ± 0.76	5.08 - 7.57	9	5.56 ± 0.59	4.72 - 6.52	11	7.13 ± 0.83	5.94 - 8.47
α 2 - Globulin	9	14.44 ± 2.59	11.64 - 18.89	9	10.2 ± 1.8	7.81 - 13.96	11	12.02 ± 4.34	8.21 - 20.58
β Globulin	9	11.94 ± 1.31	10.3 - 14.83	9	10.39 ± 1.54	8.46 - 12.74	11	12.99 ± 2.28	8.46 - 16.29
γ Globulin	9	11.76 ± 2.73	7.5 - 16	9	11.9 ± 2.09	9 - 14.1	11	14.66 ± 2.8	10 - 18.4
A/G	9	1.28 ± 0.23	0.98 - 1.58	9	1.64 ± 0.23	1.27 - 1.96	11	1.19 ± 0.37	0.66 - 1.85
Weight (kg)	9	30.54 ± 3.75	25.2 - 36.3	–	–	–	11	33.8 ± 4.02	26.3 - 39.5
Inbreeding coefficient	9	0.25 ± 0.03	0.22 - 0.31	–	–	–	11	0.23 ± 0.02	0.2 - 0.27

Table 2. Summary of the comparison of the measured blood parameters between the three study groups. Letters in parentheses refers to graphs in Figure 2. Italics indicate significant differences.

	t-test for dependent samples	ANOVA	ANOVA
Parameter	Vaccinated group - Sample I vs Sample II	Vaccinated group - Sample I vs Unvaccinated group	Vaccinated group - Sample II vs Unvaccinated group
RBC (mill/mm3)	t: -1.09; p= 0.31	F: 1.16; p= 0.29	F: 0.1; p= 0.7581
Hb (g/dl)	t: -1.69; p= 0.13	F: 3.11; p= 0.096	F: 0.49; p= 0.4929
HCT (%)	t: -0.97; p= 0.36	F: 1.21; p= 0.287	F: 0.25; p= 0.6222
MCV (fL)	t: 1.42; p= 0.19	F: 0.01; p= 0.9282	F: 0.16; p= 0.6924
MCH (pgr)	*t: -3.33; p= 0.01 (A)*	F: 3.01; p= 0.1009	F: 0.42; p= 0.5245
MCHC (%)	*t: -3.68; p= 0.007 (B)*	*F: 12.42; p= 0.0026 (C)*	F: 0.76; p= 0.3944
RDW (%)	t: -0.41; p= 0.69	F: 0.45; p= 0.5108	F: 0.94; p= 0.3444
Leucocytes (%)	t: -0.42; p= 0.68	F: 0.14; p= 0.7093	F: 0.2; p= 0.6564
Neutrophils (%)	t: 1.62; p= 0.14	*F: 6.96; p= 0.0172 (D)*	*F: 12.62; p= 0.0023 (F)*
Lymphocytes (%)	t: -1.08; p= 0.31	*F: 6.44; p= 0.0213 (E)*	*F: 8.99; p= 0.0077 (G)*
Monocytes (%)	t: 1.21; p= 0.26	F: 0.3; p= 0.5895	F: 0.83; p= 0.3735
Total protein (g/dl)	t: 1.85; p= 0.1	F: 3.88; p= 0.0644	*F: 9.04; p= 0.0076 (H)*
Albumin (%)	*t: -7.96; p= 0.0001 (A)*	F: 0.83; p= 0.374	*F: 9.76; p= 0.0059 (I)*
α 1 - Globulin	*t: 3.37; p= 0.009 (B)*	*F: 7.46; p= 0.0137 (F)*	*F: 22.55; p= 0.0002 (J)*
α 2 - Globulin	*t: 10.82; p= 0.0001 (C)*	F: 2.15; p= 0.1602	F: 1.38; p= 0.2553
β Globulin	*t: 5.58; p= 0.0005 (D)*	F: 2.43; p= 0.1368	*F: 8.48; p= 0.0093 (K)*
γ Globulin	t: -0.17; p= 0.86	*F: 5.47; p= 0.031 (G)*	*F: 6.01; p= 0.0247 (L)*
A/G	*t: -8.57; p= 0.0001 (E)*	F: 0.41; p= 0.5288	*F: 10.07; p= 0.0053 (M)*
Weight (kg)	–	–	F: 3.44; p= 0.0801

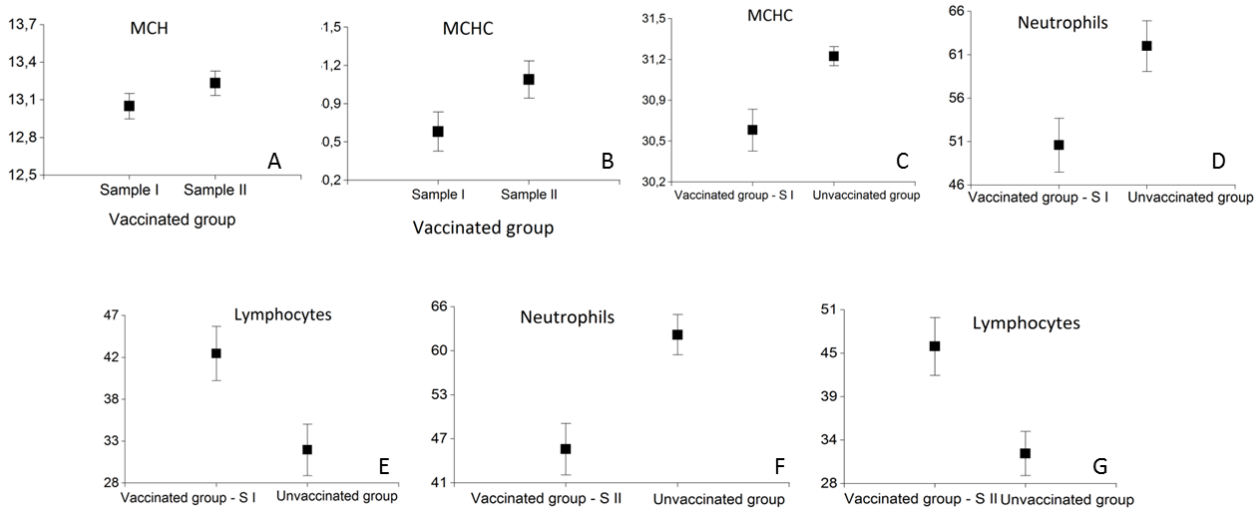

Figure 1. Significant haematological differences between vaccinated (sample I and II) and unvaccinated groups.

concentration (Hb) in sample I from the vaccinated animals, suggesting the presence of mild ferropenic anaemia in these animals, which were otherwise healthy. However, we did not find any significant differences in this parameter between groups. Note that the MCH in our results was outside the range proposed by Abaigar (1993), although it is similar to reference values for other gazelle species (Sleeman and Widdowson 1993). This discrepancy could be attributed to the use of different cell count methods by the former author.

The lower neutrophil count in vaccinated group samples I and II compared to the unvaccinated group (Table 2; Figure 1 D and F) and the significantly higher γ-globulin in unvaccinated animals than in samples I and II from the vaccinated animals (Table 2; Figure 2 G and L) are compatible with response to infection in individuals in the unvaccinated group with sustained antibody production (γ-globulin) against those putative infectious agents.

Apart from this, the higher lymphocyte count in vaccinated group samples I and II than in the unvaccinated group (Table 2; Figure 1 E and G) is compatible with lymphocyte stimulation by the vaccine.

Higher α1-globulin in sample I than in sample II from the vaccinated animals (Table 2; Figure 2 B) and in samples I and II

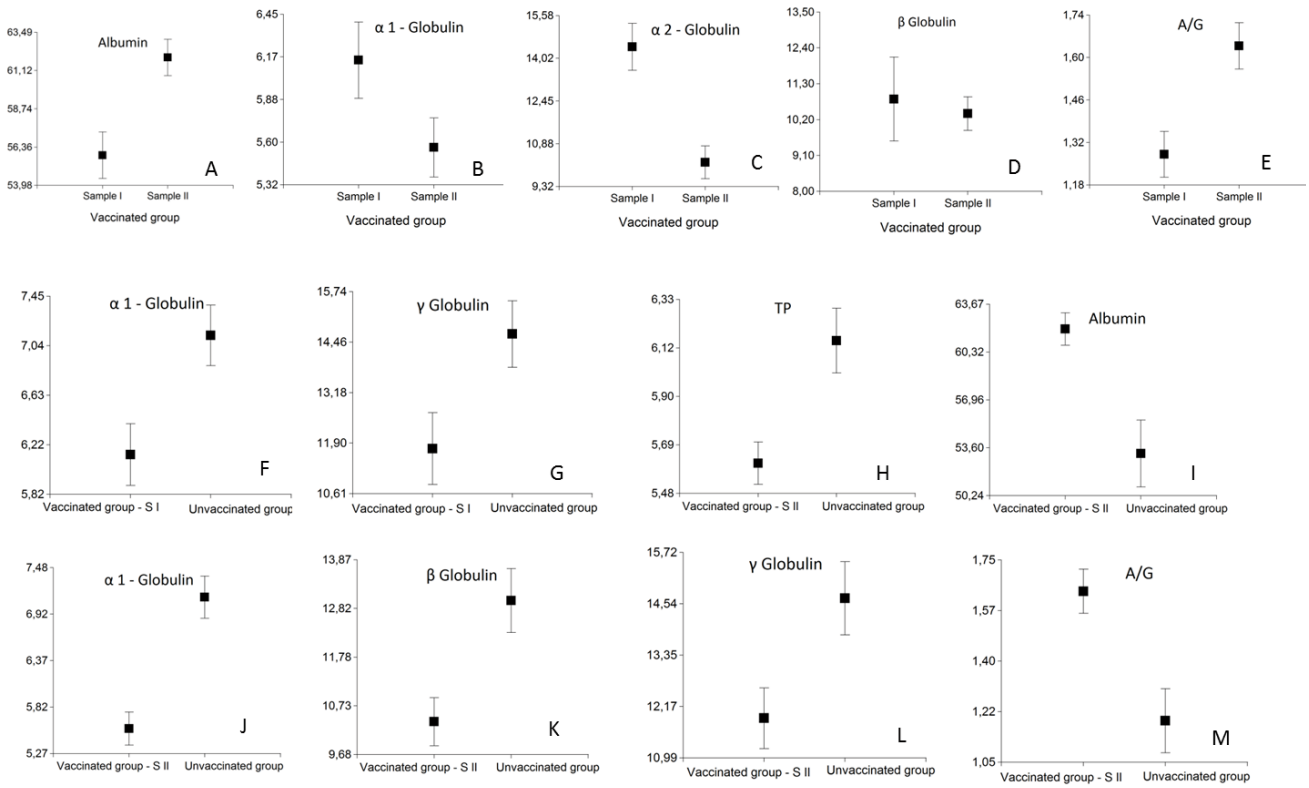

Figure 2. Significant serum differences between vaccinated (samples I and II) and unvaccinated groups.

from vaccinated animals compared to unvaccinated animals (Table 2; Figure 2 F and J) is compatible with a greater need for protection against proteases released by neutrophils (antitrypsin), which are significantly more abundant in unvaccinated animals (Table 2). However, we did not find any significant difference in this parameter between samples I and II from vaccinated animals.

Sample II from the vaccinated animals shows significantly higher albumin than sample I from vaccinated animals or samples from unvaccinated individuals (Table 2; Figure 2 A and I), which is compatible with the fact that sample II was taken from individuals that had recently been treated with ivermectin, and the albumin molecule is the primary carrier of a variety of exogenous and endogenous proteins (e.g. bilirubin, enzymes, hormones, lipids, ions and drugs) into the bloodstream. All macrocyclic lactones (ivermectin) are systemic, i.e. after injection, ingestion or topical administration, they enter the blood stream of the host, and from there are transported 'everywhere' in the organism, killing the parasites. Moreover, the higher albumin found in sample II from the vaccinated animals is in agreement with the higher A/G ratio in sample II from vaccinated animals, than in either sample I from vaccinated individuals or the sample from unvaccinated animals (Table 2; Figure 2E and M).

α2-globulin increases due to the need for more oxygen (from promoting the synthesis of hemoglobin) and during the acute-phase reaction. In our study this parameter is significantly higher in vaccinated group sample I than in sample II (Table 2, Figure 2 C), which is consistent with a possible shortage of haemoglobin in sample I.

The amount of β-globulin was significantly higher both in sample I from vaccinated animals and in unvaccinated animals compared to sample II from vaccinated animals (Table 2; Figure 2 D and K), which is indicative of the presence of infection in both sample I from the vaccinated group and the unvaccinated group.

The higher total protein found in unvaccinated animals than in sample II from vaccinated animals (Table 2; Figure H) could be due to the higher serum globulins (α1, β and γ) in unvaccinated individuals.

We found significant differences between groups in some of the haematological and serum biochemistry parameters measured in this experiment, which would be indicative of differences in the parasitic/infectious status of the animals in the two groups. Weight loss has been associated with parasitic infestations in mammals (Chroust et al. 2012; Terio et al. 2011). But contrary to expectations, we found no significant differences in body weight between the vaccinated and unvaccinated groups (Table 1 and 2). As body weight is generally considered a good proxy of body condition in both wild (Hayward et al. 2011) and captive animals (Peixoto et al. 2007), our results suggest that neither of our groups (vaccinated or unvaccinated) had experienced such infestation to the point of negatively affecting body condition.

It is well known that the nutritional status of the host can influence the rate of acquisition of immunity to parasitic and other infections in man as well as in many animal species, including ruminants (Coop and Kyriazakis 1999, 2001). Animals often face a trade-off between investment in anti-parasite defences and other activities related to self-maintenance, survival and reproduction (Zuk and Stoehr 2002; Møller and Saino 2004). Individuals in prime body condition would invest more (or more efficiently) in antiparasitic defences and parasite infection levels would be determined by the immune system (Møller et al. 1998; Lochmiller and Deeremberg 2000). Animals at La Hoya, regardless of the antiparasitic/vaccination treatment they were subjected to, seemed to be able to cope with some amount of parasite load or infection without jeopardising their health status. The favourable environmental conditions at La Hoya, with food ad libitum, no predators, regularly cleaned enclosures, no contact with other animals (domestic or wild), etc., might partly explain this. Furthermore, even unvaccinated animals have their own immune response against pathogens, and are thus able to cope with a non-pathological parasitic load. In most environments, including multispecies facilities such as zoos, most animals are exposed to parasites and infection given the huge diversity of parasitic organisms, and the diversity of hosts. Parasites have the potential of imposing severe selective pressures on their hosts, which must have an immune system enabling them to fend off pathogens.

Conclusions

In Almería's Cuvier's gazelle population, it seems that there are no major differences between individuals that are sanitised (dewormed and vaccinated) and those that are not, in parameters normally used as body condition descriptors. Vaccination is, indeed, an essential component of zoo management, although this practice, if performed routinely, could potentially impede natural selection for pathogen resistance. Although our results may not be generalisable to all types of situations regarding wild animals in captivity, they at least suggest the need to reassess vaccination and worming practices, especially in those institutions and situations where wildlife are maintained in optimum conditions. Thus, based on our results, our husbandry recommendation for captive populations of this species is that in absence of symptoms, regular coprological analyses should be performed to assess the presence/absence of parasites and vaccination should be used only when the parasite burden becomes pathological. By following this procedure, unnecessary handling of the animals is avoided, and they are allowed to develop their own immune response against pathogens, which would be especially useful if they are included in reintroduction programmes.

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