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CROSS-SECTIONAL STUDY OF *EIMERIA* SPP. INFECTION IN THREE ANTELOPE SPECIES (*ADDAX NASOMACULATUS*, *GAZELLA DORCAS* AND *ORYX DAMMAH*) MAINTAINED IN THE SOUSS-MASSA NATIONAL PARK (MOROCCO)

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Eimeria spp. are prevalent specific intestinal protozoa in many host species with a very variable degree of pathogenicity, found worldwide. Wild ruminants are susceptible hosts to such infections; these infections become important especially when held under stressing captivity conditions. We present herein a cross-sectional study to estimate the *Eimeria* spp. prevalence and abundance in three threatened antelope species, namely *Gazella dorcas*, *Oryx dammah*, and *Addax nasomaculatus* reintroduced to Souss-Massa National Park (Morocco) after they disappeared from their natural North African biotope. A total number of 254 faecal samples (80 from *A. nasomaculatus*, 81 from *O. dammah* and 93 from *G. dorcas*) were collected and analysed by the qualitative flotation and the quantitative McMaster methods. The infection prevalence was 36.25%, 22.58%, and 29.63% for *A. nasomaculatus*, *G. dorcas*, and *O. dammah*, respectively. The average infection abundance values were 21.25 ± 4.7, 136.56 ± 52.4, and 20.37 ± 5.8 for *A. nasomaculatus*, *G. dorcas*, and *O. dammah*, respectively. Among the three studied antelopes, *G. dorcas* was the species that was shedding the highest amount of oocysts. This study should be completed by morphological and molecular characterisation of different *Eimeria* parasites in each antelope host species.

Key words: abundance, coccidian infection, prevalence, Sahelo-Saharan antelopes, threatened species

Introduction

In addition to macroparasites (e.g., nematodes, trematodes, cestodes) that may be a threat for wildlife health, microparasites like protozoans could also be responsible for parasitic disorders in several animal species. Coccidian parasites consists of several groups of protozoans (Apicomplexa), among them the genus *Eimeria* spp*.,* which infect several species of vertebrates (Bowman, 2014). *Eimeria* infections are responsible for severe disease in birds and livestock, inducing large economic losses. The life cycle of Eimeriidae is often homoxenous or facultatively heteroxenous. They are extremely host-specific and the whole development occurs mainly in intestines and in a few cases in other organs (e.g., kidneys, gallbladder) of the host with a very variable pathogenic effect (Upton, 2000). Unlike domestic mammals in which coccidian have been studied widely, for economic reasons, few studies were published about wild mammals' infection by *Eimeria*. However, *Eimeria* spp. were isolated from a large number of wild mammals species, including ungulates, insectivores, marsupials, rodents and others, regardless of their health impact (e.g., Samuel et al., 2001).

For wildlife conservation purposes, animals are held under particular conditions of sequestration and space limitations, in captivity (zoos) or in semi-captivity (e.g., national parks and nature reserves), making them vulnerable to many health disorders including parasitic infections, which are relatively exacerbated by both a high animal population density and captivity stress (Thomas et al., 2005; Wobeser, 2007).

Therefore, adopting a combined approach of animal welfare and biodiversity conservation, when dealing with threatened species, forces us to elaborate adequate health management strategies. These strategies should be compatible with the field circumstances by establishing guidelines that indicate when wildlife managers (e.g., conservationists, veterinarians, epidemiologists) should act to resolve health-related problems at different scales, viz. individuals, population and ecosystem levels (Aguirre et al., 2002; Delahay et al., 2009).

This is the case in the Souss-Massa National Park (Agadir, Morocco), where three endangered species of Sub-Saharan antelopes are hosted. They are included on the Red List of threatened species of the International Union for Conservation of Nature (IUCN), namely *Gazella dorcas* Linnaeus, 1788 classified as «Vulnerable» (IUCN SSC Antelope Specialist Group, 2017), *Addax nasomacculatus* De Blainville, 1816 classified as «Critically endangered» (IUCN SSC Antelope Specialist Group, 2016a), and *Oryx dammah* Cretzschmar, 1826 classified as «Extinct in the Wild» (IUCN SSC Antelope Specialist Group, 2016b).

Eimeria spp. infections were mentioned in many endangered antelopes species from different Protected Areas around the world, as in some of the latest studies conducted in *Oryx dammah* from USA (Pauling et al., 2016), *Antidorcas marsupialis* Sundevall, 1847 from Namibia (Turner et al., 2016), *Pantholops hodgsonii* Abel, 1826 from China (Cao et al., 2019), *Philantomba walteri* Colyn et al., 2010 from Nigeria (Omonona et al., 2019).

The current cross-sectional study aimed to estimate *Eimeria* infection prevalence and abundance in these three antelope species in the Souss-Massa National Park by analysing faecal samples using non-invasive coprological techniques, namely flotation and McMaster egg counting technique.

Material and Methods

Study area and animals

The Souss-Massa National Park (SMNP) is a Protected Area intended for ecological rehabilitation and natural resources conservation. It is located south of the city of Agadir, Morocco (9.666666° W, 30.083333° N) (Fig. 1). It covers a total area of 338 km^2 along the Atlantic coast that also includes the estuaries of the River Souss and the River Massa. In addition to the three antelopes (*Addax nasomaculatus*, *Gazella dorcas*, *Oryx dammah*), the SMNP hosts two threatened bird species, (*Geronticus eremita* Linnaeus, 1758 and *Struthio camelus camelus* Linnaeus, 1758), and a diversified endemic flora (e.g., *Sideroxylon spinosum* L.) (El-Bekkay et al., 2013). The population sizes of antelopes in the SMNP were estimated at 230 individuals of *O. dammah*, 440 individuals of *A. nasomaculatus*, and 850 individuals of *G. dorcas*. To avoid hybridisation-related problems between *A. nasomaculatus* and *O. dammah*, *A. nasomaculatus* and a part of *G. dorcas* individuals are sharing a geographic spot, while *O. dammah* and another part of *G. dorcas* are sharing another geographic spot within the Protected Area.

Sampling methodology

A total of 254 fresh individual faecal samples (80 from *A. nasomaculatus*, 81 from *O. dammah*, and 93 from *G. dorcas*) were picked up randomly from the ground between January and July 2015. All samples were labelled, transported immediately to the laboratory, and kept at +4°C until analysed.

Laboratory analyses

Faecal samples were examined by classical coprological methods, simple tube flotation for qualitative microscopic observation and McMaster method to oocysts per gram (OPG) estimation, using the RVC/FAO guide to veterinary parasitology diagnostics (Gibbons et al., 2014), as described by Hansen & Perry (1994). Consequently, 4 g of crumbled faecal samples were added to 56 ml of flotation solution, mixed and filtered before filling tubes and McMaster cells. In this technique, we used 1.27-specific gravity Sheather's sugar flotation solution (Dryden et al., 2010). For McMaster, two chambers (2 × 0.15 ml) were used for counting under the 10× objective. The tube flotation observations were done under a microscope, 100× to 1000× magnifications, driven by Motic® Images Plus 2.0 software for image capture, processing and morphometric analysis.

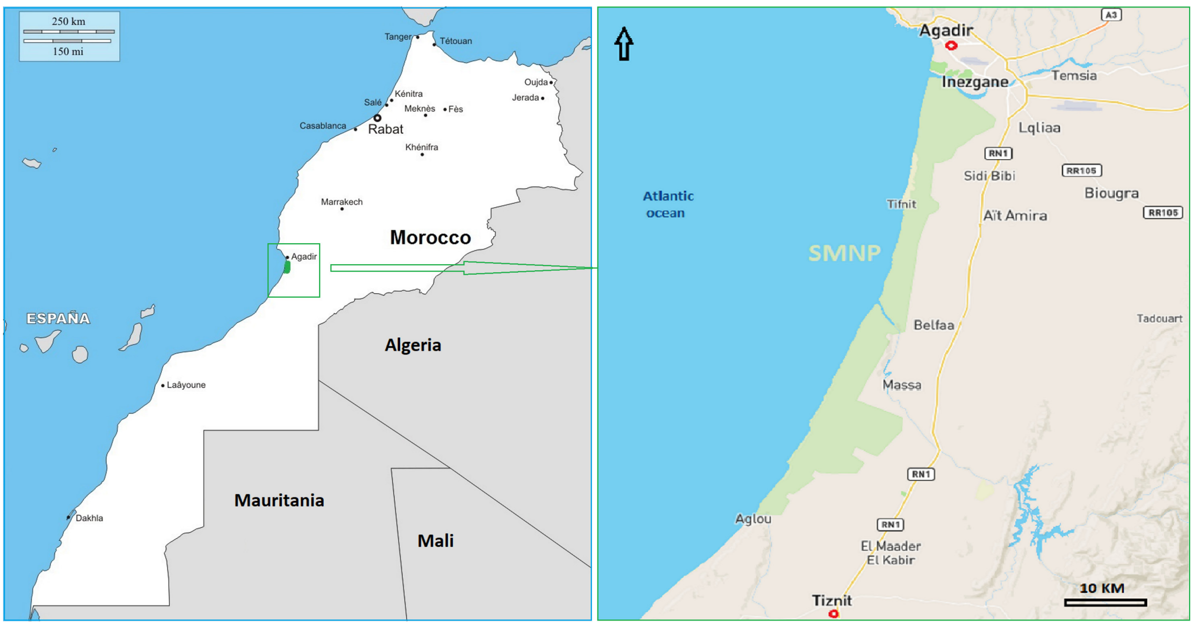

Fig. 1. The localisation and geographical delimitations of the Souss-Massa National Park (indicated in green) in Morocco.

Statistical analysis

Two parasitological indicators were estimated. We used the universal parasitological terminology (Bush et al., 1997) and the following formulas:

$$\text{Infection prevalence (\%)} = 100 \times \frac{\text{Number of infected animals}}{\text{Number of examined animals}},$$

$$\text{Mean abundance} = \frac{\text{Total number of Eimeria spp. oocysts}}{\text{Number of examined animals}}.$$

The differences in prevalence were analysed using the χ^2 test, parasite infestation abundance among the three antelope populations were compared with one-way analysis of variance (ANOVA) and the Newman-Keuls multiple comparison test at 0.05 threshold value (GraphPad PRISM® v5.00 software, USA). Data are presented as percentages and standard deviation for prevalence, and means ± mean standard deviation for parasite abundance.

Results and Discussion

Infection prevalence

The infection prevalence of *Eimeria* infection was estimated to 36.25 ± 5.40%, 22.58 ± 5.07%, and 29.63 ± 4.30% for *A. nasomaculatus*, *G. dorcas*, and *O. dammah*, respectively (Fig. 3). However, there were no statistically significant differences in *Eimeria* prevalence between the antelope species ($p > 0.05$).

Three different patterns of *Eimeria* spp. oocysts were observed: *Eimeria* sp.1 measuring 27 × 16 µm from *Gazella dorcas* (Fig. 2A,B,C), *Eimeria* sp.2 measuring 28 × 21 µm from *Oryx dammah* (Fig. 2D,E), and *Eimeria* sp.3 measuring 25 × 20 µm from *Addax nasomaculatus* (Fig. 2F).

Infection abundance

The infection abundance was statistically higher for *Gazella dorcas* (136.56 ± 52.40) compared to *Addax nasomaculatus* (21.25 ± 4.70) and *Oryx dammah* (20.37 ± 5.80) ($p < 0.05$) (Fig. 3).

The three studied antelopes showed a low mean infection abundance (less than 300 OPG), except for four *G. dorcas* individuals that shed a high number of oocysts (higher than 1000), even though the faecal pellets seem to be normal without any diarrheic aspect.

Infected domestic animals may shed a high amount of oocysts without developing any clinical disorders (Jacobs et al., 2015), and the variation of excreted oocysts could be influenced by several factors. In addition to the parasite species, they can be intrinsic, such as the age of animals (young animals are more vulnerable), hormonal status (corticosteroids), variation in females (increase of oocysts shedding during calving and suckling periods), stress or extrinsic due to environmental conditions related to the animal host population density and breeding conditions (Apio & Wronski, 2004; Koutny et al., 2012; Bowman, 2014).

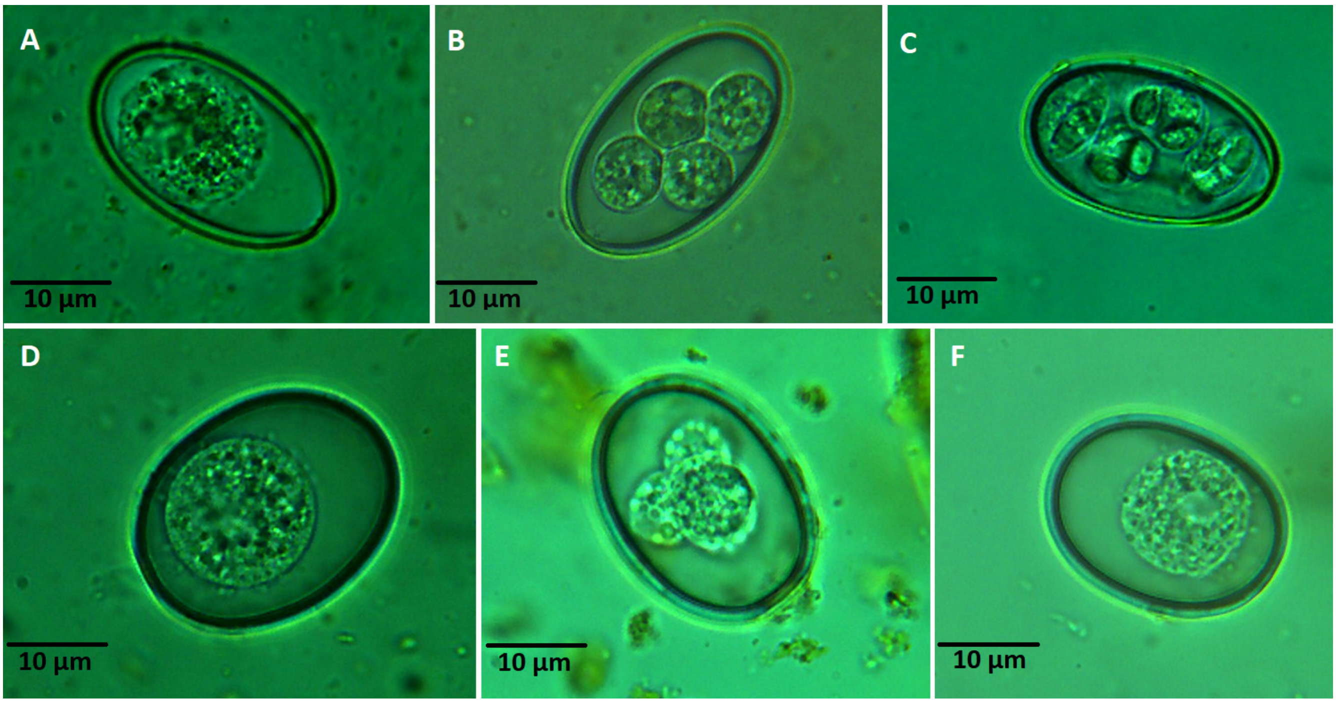

Fig. 2. Different *Eimeria* spp. oocysts recovered from fecal samples of antelopes in the Souss-Massa National Park (Morocco). Designations: A, D, F – non-sporulated oocysts; B, C, E – sporulated oocysts.

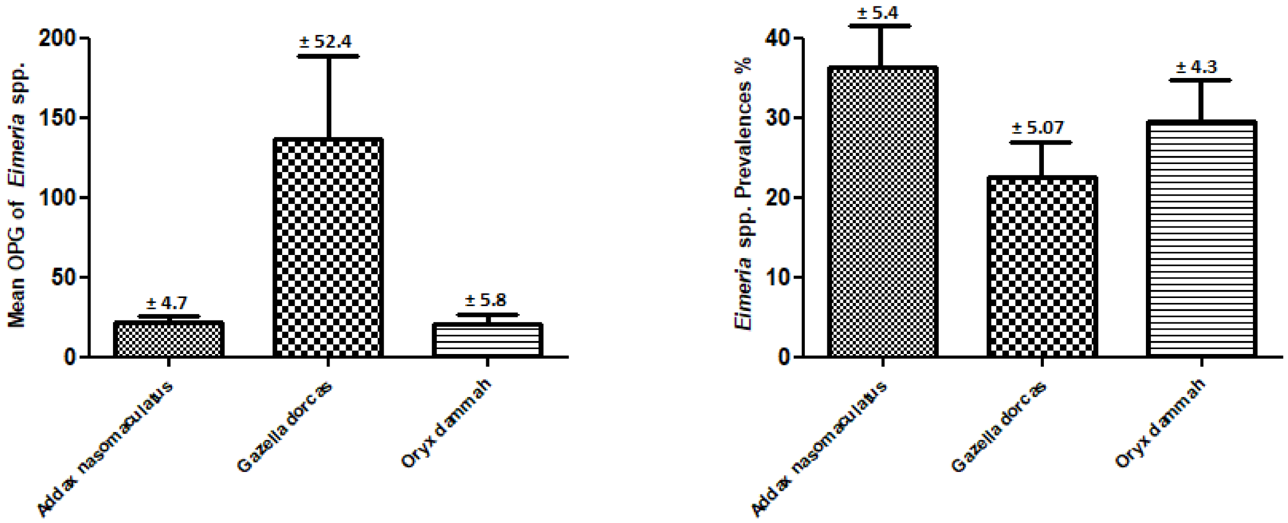

Fig. 3. *Eimeria* infection abundance (OPG: oocyst per gram in average) (left) and prevalence (right) in the three studied antelopes in the Souss-Massa National Park (Morocco).

Eimeria spp. are worldwide prevalent under diversified conditions of captivity, semi-captivity or free-ranging antelopes. For example, *E. dorcadis* Mantovani, 1966 infection was reported from *Gazella dorcas* (Mohammed et al., 2012), *E. farasanii* Omer, Apio, Wronski & Mohammed, 2011 from *Gazella gazella farasani* Thouless & Al-Bassri, 1991 (Omer et al., 2011), *E. idmii* Mohammed & Hussein, 1992 from *Gazella gazella* Pallas, 1766 (Mohammed & Hussein, 1992), *E. rheemi* Hussein & Mohammed, 1992 from *Gazella subgutturosa marica* Thomas, 1897 (Hussein & Mohammed, 1992), *E. saudiensis* Kasim & Al Shawa, 1988 from *Oryx leucoryx* Pallas, 1777 (Kasim & Al Shawa, 1988), *E. zuernii* (Rivolta, 1878) Martin, 1909 from *Oryx dammah* (Pauling et al., 2016), infecting also bovines. *Eimeria* specimens were not determined at species level by some authors, *Eimeria* sp. from *Boselaphus tragocamelus* Blainville, 1816 (Singh et al., 2009) and *Eimeria* sp. from *Antilope cervicapra* Linnaeus, 1758 (Mir et al., 2016).

The infection abundance varies among previous studies conducted in wild bovids under different circumstances of breeding. For example, *Bison bison* Linnaeus, 1758 from an Italian zoo was found to shed 500 OPG (Fagiolini et al., 2010); *Gazella benetti* Sykes, 1831 from an Indian Park shed 2200 OPG (Singh et al., 2006); *Beatragus hunteri* Sclater, 1889 from a Kenyan wildlife shed 200 OPG (Njeru et al., 2014); *Nanger granti* Brooke, 1872, *Aepyceros melampus* Lichtenstein, 1812, *Eudorcas thomsonii* Günther, 1884 from Kenyan wildlife shed 290, 1160 and 254 OPG, respectively (Ezenwa, 2003).

We emphasise that *G. dorcas* sheds more *Eimeria* oocysts than the other two studied antelopes. This could be explained by many factors, such as the animal density, which is a possible extrinsic factor that may correlate positively with the parasite abundance as reported in previous studies (e.g., Tomczuk et al., 2015; Carrau et al., 2018). Among the three animal species, the population size of *G. dorcas* (850 individuals) was much larger than those of *A. nasomaculatus* (440 individuals) and *O. dammah* (230 individuals) with which they share the pasture. which may explain such *Eimeria* abundance in *G. dorcas* compared to the other two antelope species.

Moreover, the ungulate behaviour influences *Eimeria* amounts, especially in antelopes as *Gazella* spp., because they have a more gregarious behaviour than other antelope species (Ezenwa, 2004). In addition, territorial males of gazelles are more likely to have high parasitic infection levels (Ezenwa & Snider, 2016). Both mentioned behavioural social factors may explain such a high infection levels in *G. dorcas*.

Conclusions

An efficient parasitic health management policy in national parks and nature reserves should indeed take into account the health impact of multiple infections. Hence, the excreted amount of oocysts, as a health indicator, should be mentioned together with other faecal excreted forms (helminth eggs and larvae) for gastrointestinal parasitic disease control. Finally, in the light of the foregoing, and due to the seldom-available data on wildlife, especially in those threatened antelopes, we consider to complete the current study by a combined morphometric and molecular characterisation of *Eimeria* spp. for each antelope species, particularly in *Oryx dammah* and *Addax nasomaculatus* populations.

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ИССЛЕДОВАНИЕ ИНФИЦИРОВАНИЯ ВИДАМИ РОДА *EIMERIA* ТРЕХ ВИДОВ АНТИЛОП (*ADDAX NASOMACULATUS*, *GAZELLA DORCAS* И *ORYX DAMMAH)* В НАЦИОНАЛЬНОМ ПАРКЕ СУС-МАССА (МАРОККО)

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Eimeria spp. являются всемирно распространенными специфическими кишечными простейшими у многих видов с очень разной степенью патогенности. Дикие жвачные животные являются восприимчивыми хозяевами к таким инфекциям. Эти инфекции становятся особенно важными, когда животные находятся в стрессовых условиях содержания в неволе. В настоящей работе мы представляем оценку распространенности и численности *Eimeria* spp. в трех видах антилоп, находящихся под угрозой исчезновения, а именно *Gazella dorcas*, *Oryx dammah* и *Addax nasomaculatus*, реинтродуцированных в национальном парке Сус Масса (Марокко) после их исчезновения в своем естественном биотопе в Северной Африке. В общей сложности 254 пробы фекалий (80 от *A. nasomaculatus*, 81 от *O. dammah* и 93 от *G. dorcas*) было собрано и проанализировано с помощью методов качественной флотации и количественного метода МакМастера. Степень инфицирования составила 36.25%, 22.58% и 29.63% для *A. nasomaculatus*, *G. dorcas* и *O. dammah*, соответственно. Средние значения обилия инфекции составили 21.25 ± 4.7, 136.56 ± 52.4 и 20.37 ± 5.8 для *A. nasomaculatus*, *G. dorcas* и *O. dammah*, соответственно. Среди трех видов антилоп *G. dorcas* отличался наибольшим количеством ооцист. Это исследование должно быть дополнено морфологической и молекулярной характеристикой видов Eimeria у каждого вида-хозяина антилоп.

Ключевые слова: кокцидиоз, обилие, сахельско-сахарские антилопы, степень инфицирования, угрожаемый вид