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SHORT COMMUNICATION

Zoological institutions as hotspots of gastrointestinal parasites that may affect the success of ungulate reintroduction programmes

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Abstract

Background: Ungulates from zoological institutions are frequently used as founders in reintroduction programmes. These animals are subject to specific parasite management as parasitic infections have previously been associated with failed Bovidae reintroductions.

Methods: Questionnaires to obtain data on how these institutions screen for seasonal parasite presence and the clinical signs they induced in threatened ungulates were sent to 65 institutions involved in European *Ex situ* Programmes (58.5% response rate). Temperature and relative humidity data were also obtained to categorize each zoological centre.

Results: *Strongyloides* spp. (52.6%), *Trichuris* spp. (42.1%), Trichostrongylidae family (39.4%) and *Eimeria* spp. (36.8%) were the most frequently reported parasites in the received questionnaires. Climatic variables did not influence parasite presence.

Conclusion: Our results suggest that artificial microenvironments created by husbandry practices and enclosure design in zoos could create hotspots for gastrointestinal parasites. To maximise the success of reintroduction projects, we recommend that the influence of microclimates on parasite burdens be evaluated.

KEYWORDS
gazella cuvieri, microclimate, nanger dama mhorr, oryx dammah, parasite management, reintroduction programme

1 | INTRODUCTION

Zoological institutions (hereafter zoos) play an important role in biodiversity conservation, particularly by means of translocations to re-establish viable populations back in the wild.[1–3] European *Ex situ* Programmes (EEPs) intensively manage *ex situ* populations to ensure that they remain healthy and viable for the foreseeable future and provide animals for reintroductions when appropriate.[4] Many ungulate species listed as threatened in the IUCN Red List of Threatened Species are managed through EEPs including the scimitar-horned oryx *Oryx dammah* (extinct in the wild), the Mohor gazelle *Nanger dama mhorr* (critically endangered) and the Cuvier's gazelle *Gazella cuvieri* (vulnerable).[5]

Ungulate conservation translocations, including reintroductions, sometimes partially or completely fail, with parasitic diseases being one of the most commonly registered problems.[6,7] Consequently, it is widely acknowledged that prevention and control of parasites is essential to avoid health problems in *ex situ* populations and for successful reintroduction programmes.[8] However, a balance must be struck in the development of resistance to parasites in *ex situ* animals, and ensuring that reintroductions do not inadvertently introduce these pathogens to naive wild populations. This issue is particularly complex, and each individual EEP will need to evaluate it for their programme.[9] This challenge increases because of the presence of parasitic species in climatic regions that are not theoretically suitable for them. Their widespread presence, despite the prevailing climatic conditions, may be due to the husbandry regimes and enclosure design that lead to soil contamination and high animal density creating favourable conditions that the parasite would not encounter in the wild in its original climatic zone. These *ex situ* conditions may create a micro-environment that favours many gastrointestinal parasites, especially those with a direct biological cycle.[10,11] Particular attention should be paid to microclimates created in zoo facilities,[12] which may favour the presence of parasites under particular artificial microenvironments, as previously described for a population of captive gazelles.[13] This is likely to lead to different parasitofauna between the founders from *ex situ* institutions and wild ungulate populations with the potential to compromise the success of reintroduction or conservation translocation programmes.[14] Our objective is to determine the most frequent parasites found in a range of zoos and their relationships with environmental factors such as temperature and humidity, which are closely related to the biological cycle of parasites. To achieve this, a questionnaire based survey was used to obtain information about parasite species in captive ungulates housed in different latitudinal gradients, highlighting the possible implications of these parasitic infections in failed Bovidae reintroductions.

2 | MATERIALS AND METHODS

In July 2010, a modified version of the questionnaire by Isaza et al[15] was sent to 65 European Association of Zoos and Aquaria (EAZA)-member zoos holding any of the three focal ungulate species: scimitar-horned oryx, Mohor gazelle and Cuvier's gazelle. All three species have been subject to efforts to reintroduce them from *ex situ* populations to their former range. Questions focused on the search (closed question: 'yes' and 'no'), detection (open question: to enumerate the parasites found), clinical signs (closed question: 'yes' and 'no'; but with the option to add additional information on observed clinical signs) and seasonality (closed question: 'spring', 'summer', 'autumn' and 'winter') of ectoparasites, blood parasites and other endoparasites that shed eggs in faeces for these three species. Each zoo was considered as a sample unit, and parasites that were present in at least 20% of zoos were included in the analyses.

Meteorological data (monthly mean temperature, relative humidity and maximum and minimum temperature) were collected in 2010 from the closest official meteorological stations to the zoos for the time period covered for the questionnaire (Table S1). A spatial analysis was carried out using ArcGIS v10.1 to detect the spatial autocorrelation (SA) of the absence-presence of *Strongyloides* spp., *Trichuris* spp., Trichostrongylidae family and *Eimeria* spp. The applied tool was Global Moran's I, based on the locations of the zoos simultaneously, evaluating whether the pattern was clustered or random. The seasonal average values obtained from the monthly meteorological records were included as independent variables in spatial regression analysis to determine their likely correlation with the presence of the gastrointestinal parasites described above. This regression analysis allowed us to explore spatial relationships using ordinary least squares (OLSs), helping to explain the climatic factors behind the observed spatial patterns. Among the statistics provided is the Jarque-Bera (JB) statistical index, which we used to assess model bias and indicate if the residuals were normally distributed. The assessment of stationarity was carried out using the Koenker (BP) statistic (Koenker's studentized Bruesch-Pagan statistic) to determine if the explanatory variables in the model had a consistent relationship to the dependent variable both in geographic and data space. Variance inflation factor (VIF) measured redundancy among explanatory variables. Finally, to assess residual SA, Moran's I tool was applied to the regression residuals to ensure that they were spatially random. Statistically significant clustering of high and/or low residuals would indicate if a key variable was missing from the model.

3 | RESULTS

Completed questionnaires were received from 38 EAZA institutions (58.5%) from 12 European countries and Israel. The scimitar-horned oryx was the most commonly kept species in zoos (86.8%), followed by Mohor (23.7%) and Cuvier's gazelles (10.2%). For more details see Moreno Mañas et al.[9]

Most respondent institutions (97%) detected eggs and oocysts of endoparasites in faeces. The most common parasites reported by the zoos were gastrointestinal nematodes and protozoa species: *Strongyloides* spp. (52.6%), *Trichuris* spp. (42.1%) and Trichostrongylidae family (39.5%) eggs, and *Eimeria* spp. (36.8%) oocysts. These parasites were found by most zoos (range: 65.7–81.6%) but were rarely associated with clinical signs (weight loss and diarrhoea) in animals (range: 7.9%–21.1%). The season with the highest presence of parasites was autumn (range: 21.1%–34.2%), followed by spring (range: 18.4%–31.6%), winter (range: 10.5%–26.3%) and summer (range: 13.2%–18.4%) (Figure 1a).

The analysis of the SAs showed a random pattern for all the parasites analysed (Table S2). Spatial regression and statistical analyses (JB, K [BP], VIF, SA) did not reveal significant associations between the presence of parasites and climatic variables registered in the areas where the zoos were located, showing a low climate-dependent geographical distribution of parasitic specimens in zoos of our study area (Table 1), as evidenced in Figure 1b. In detail, the exploratory regression provides the values of the

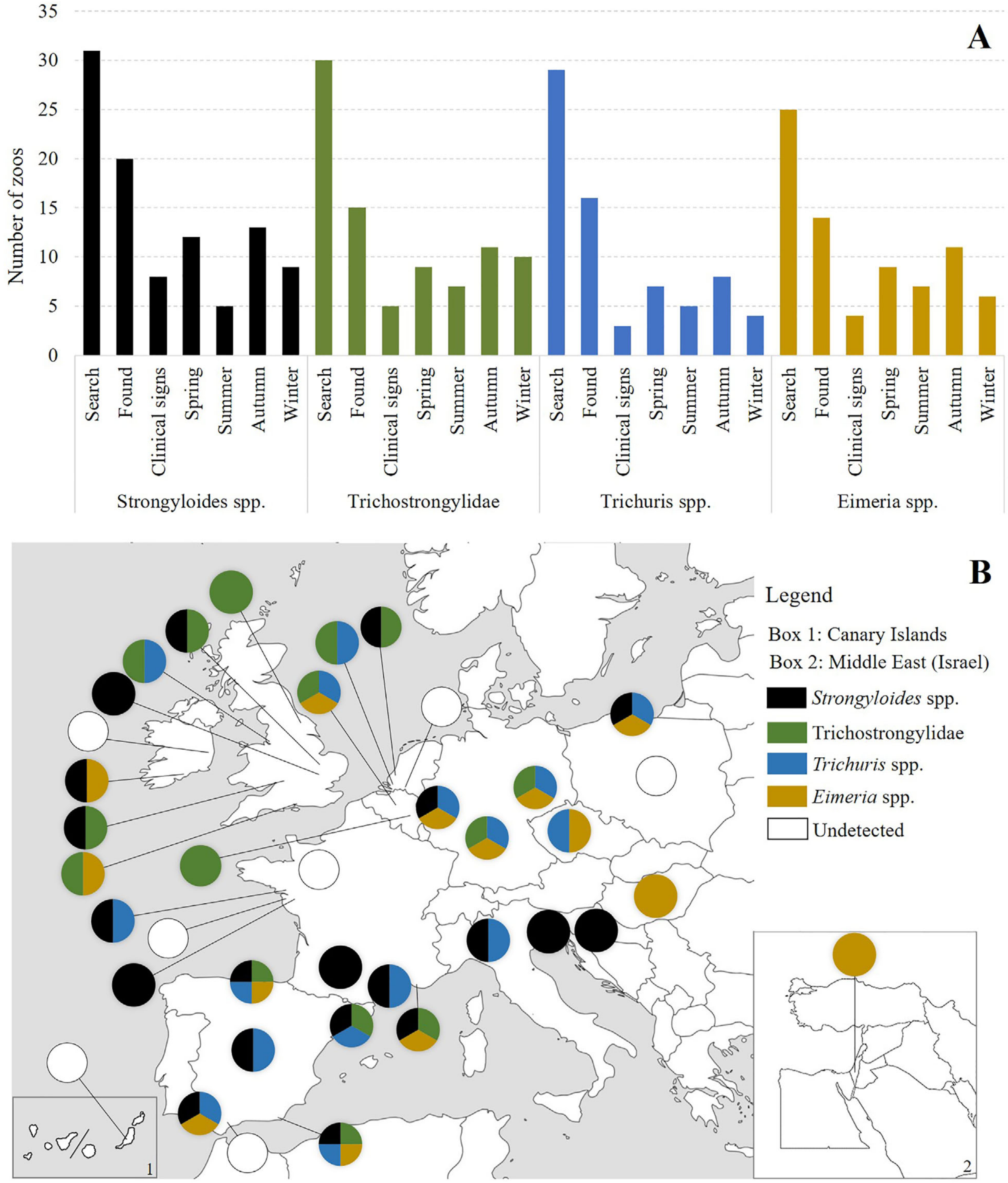

FIGURE 1 (a) Bar plot representing the number of zoos that searched and found the most reported parasitic groups (*Strongyloides* spp., Trichostrongylidae family, *Trichuris* spp. and *Eimeria* spp.), as well as their seasonal detection and the occurrence of eventual associated clinical signs. (b) Geographical distribution of zoological institutions included in the study and parasitic groups detected in each of them

TABLE 1 Exploratory regression global summary

Parasite	AdjR^2	JB	K (BP)	VIF	SA	Model	
Strongyloides spp.	0.14	0.22	0.91	4.65	0.82	-TmSpr**	+TmSum***
Trichostrongylidae family	0.22	0.41	0.37	4.22	0.45	-TmSum***	-HmWin**
Trichuris spp.	0.08	0.18	0.98	3.88	0.61	-TmAut*	-HmWin**
Eimeria spp.	0.02	0.07	0.65	1	0.98	-HmSpr	

Model variable sign (+/-); model variable significance.
Abbreviations: AdjR2, Adjusted R-Squared; JB, Jarque-Bera p-value; K(BP), Koenker (BP) Statistic p-value; SA, Global Moran's I p-value; VIF, max variance inflation factor. Variable: Aut, autumn; Hm, mean humidity; Spr, spring; Sum, summer; Tm, mean temperature; Win, winter.
*0.10.
**0.05.
***0.01.

summer and spring seasons in mean temperature as dependent variables for *Strongyloides* spp. OLS models (AdjR^2: 0.14). In the case of predictive models for Trichostrongylidae family, the candidate explanatory variables were summer temperature and winter humidity (AdjR^2: 0.22). The *Trichuris* spp. and *Eimeria* spp. models provided even lower predictive results, with adjusted R^2 values of 0.08 and 0.02, respectively.

4 | DISCUSSION

The zoos that participated in this survey indicated that gastrointestinal parasites, particularly nematode and protozoa species, were the most frequent parasites detected in coprological analyses, which have also been widely reported in captive ungulates both in our study areas and in other regions.[16,17] The questionnaire demonstrated that most institutions collected the faeces from the ground (92%).[9] However, for the future, individual sample collection from animals involved in reintroduction programmes should be recommended to avoid potential sample contamination. Similarly, flotation techniques were the only diagnostic method used by most institutions (82%)[9] limiting diagnostic capability, but a combination of faecal concentrating techniques and serological tests, or even molecular analyses when possible, would help provide more accurate results.[8,9] Parasitic infections described in this study are usually asymptomatic in ungulate hosts, but they may cause severe clinical signs and lesions in animals with a poorly developed immune status, such as juveniles, stressed individuals or those naïve to a parasite species.[18] Inbreeding also has an effect in some gazelle species, for example, inbreeding coefficients are positively related to trichostrongylids and *Trichuris* spp. infections in Cuvier's gazelle.[19] Clinical signs are generally associated with diarrhoea, dehydration, cachexia or weakness, which could be complicated in some cases, such as nervous or cardiorespiratory lesions in *Strongyloides* spp. infections, anaemia by *Haemonchus* spp. (trichostrongylid nematode) or haemorrhagic discharge by *Trichuris* spp. and *Eimeria* spp.[18,20–23] All parasitic groups reported in the present study have been frequently linked to other concomitant pathogens and diseases in wild ungulates.[24–26]

The optimal development of parasite biological cycles described in this study are carried out at temperatures between approximately 20–30°C with high humidity (>%80–90%), except for *Eimeria* spp., which requires drier conditions.[27–32] Although temperature and humidity are the climatic variables most frequently considered in the studies of nematode and protozoa biology, factors as UV radiation, wind or precipitation, among others, should also be examined.[32,33] The presence of these parasites in theoretically unfavourable climatic regions, as in some of our study areas, might be due to the existence of microclimates in zoos (e.g., animal shelters, heating systems, pasture irrigation, etc), that probably play an important role in becoming hotspots for gastrointestinal parasites of captive ungulates. Alternatively, infection may be facilitated by grazing access, for example, the presence of parasitic species belonging to the Trichostrongylidae family, which depend on infection through grass consumption to complete their biological cycle, has previously been reported in captive conditions where ungulates have been fed grass but do not have access to natural grazing.[34] Moreover, survival strategies of detected parasites in adverse climatic conditions (e.g., temporal larval inhibition of Trichostrongylidae nematodes, heterogonic cycle of *Strongyloides* spp. and egg or oocyst resistance of *Trichuris* spp. and *Eimeria* spp., respectively) can account for their large geographical distribution in both wild and captive environments.[27,32,35,36] However, all parasites have been reported at freezing temperatures in this study, indicating that they do not use their survival strategies in zoo enclosures, since artificial microenvironments can keep them under optimal conditions for their development.

It seems evident that the widespread distribution of gastrointestinal parasites in EEP zoos could compromise the success of reintroduction programmes in varied environments with non-immunocompetent animals. To the authors' knowledge, this is the first study covering the relationship between parasite presence and climatic factors in captive wildlife.

Our results indicate that the parasite management in captive animals must address a broad range of parasites across all seasons, regardless of the application of preventive treatments or the geographical location of animals. Most institutions involved in the present survey detected endoparasite eggs in faeces in spite of applying regular prophylactic treatments, mainly ivermectin-based (66%), which are generally administered biannually in medicated food.[9] Problems related to anthelmintic resistance particularly for the Trichostrongylidae family, timing and frequency of prophylactic treatment, route of administration or drug dosage could be operating in zoos.[9,37] Consequently, parasite management programmes implemented for *ex situ* ungulate populations should be revised to counter the effects of anthelmintic resistance. Aside from climatic features, other factors including stress, food-borne infection, environmental contamination or close association of animals in zoos could favour the role of captive individuals as reservoirs of gastrointestinal parasites.[10,11,17,18]

Parasite management in zoos must include the parasitic species described in this study and others with a direct biological cycle, since they are the most common in captive environments.[11] Proactive screening for parasites are critical to avoid their introduction in naive wild populations through conservation translocations or in captive populations where individuals are exchanged between zoos to meet programme (EEP) goals.[14] Similarly, in-depth assessment of parasitofauna in the recipient environment should be conducted to ensure the survival of founders from zoos.[38]

In conclusion, this study helps to understand the complex network of factors that influence the presence of parasites in captive ungulates managed within EEPs. Hereafter, ecological features (mainly temperature and relative humidity) of artificial microenvironments in zoos, as well as their epidemiological implications, must be considered in future breeding programmes to maximize the reintroduction success.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

ETHICS APPROVAL

This study was questionnaire based, and as such ethical approval was not required.

AUTHOR CONTRIBUTIONS

M.G., E.M., J.O., F.V. and M.R.R.Y. developed the core idea and designed the study. J.O., G.E. and B.I. collected the data and M.G., P.P-C., J.B. and B.I. prepared the database and analysed output data. M.G., E.M., P.P-C and M.R.R.Y analysed the resulting information. M.G. and M.R.R.Y. wrote the initial draft of the manuscript. M.G., E.M., P.P-C., T.G., J.O., F.V. and M.R.R.Y. reviewed and edited the manuscript, and all authors contributed substantially to revisions. F.V. acquired the funding.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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