Survey of husbandry practices for bovidae in zoos: the importance of parasite management for reintroduction programmes

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Abstract
Animals from zoological institutions may be used for reintroductions. These individuals are considered healthy, but they are not necessarily free of parasites, despite the minimum husbandry standards required of zoological institutions as described in the European Association of Zoos and Aquaria guidelines. In this sense, parasitism has been identified as the cause of failure, or has added difficulties, in some reintroduction programmes. Here the authors attempt to summarise the risk of parasitism to animals originating from zoological institutions by analysing a questionnaire about parasite prevalence, sampling methods, treatment and control in three ungulates in European zoos. Completed questionnaires were received from 38 institutions (58.5 per cent response rate). Most of the responding institutions (97 per cent) detected the eggs of endoparasites in faeces, but only one reported ectoparasites. Most institutions followed a similar preventive schedule, with ivermectin as the preferred prophylactic treatment for parasites, commonly administered in food every six months. The frequent use of concentrating flotation techniques as the sole method to evaluate the presence of parasite eggs in faecal samples is not recommended because it fails to detect trematode and lung nematode infections, so it would be better to use flotation techniques together with sedimentation procedures or serological and molecular tests. The results suggest that parasite control in zoological institutions can be complicated, indicating the need to implement a specific management schedule for institutions involved in reintroduction projects.

Introduction
The re-establishment of threatened species through reintroduction programmes using individuals bred in zoological institutions has been a well-established conservation tool for many years. Zoos and aquaria can potentially play an important role in the conservation of biodiversity through field projects and coordinated breeding programmes.1–6 European ex situ programmes (EEPs) intensively manage animal populations in European Association of Zoos and Aquaria (EAZA) zoological institutions and are often linked to wildlife conservation.7–9 In spite of the considerable effort and resources invested in such programmes and reintroduction projects, many attempts to re-establish sustainable populations have failed.10–12 The reasons for failure are varied, but diseases, some of them parasitic, have played a significant role in the lack of success for a number of reintroduction attempts (eg, fish reintroductions (razorback sucker Xyrauchen texanus and Colorado pikeminnow Ptychocheilus lucius)13; reintroduction of elk Cervus elaphus,14 the green and golden bell frog Litoria aurea,15 black rhinoceros Diceros bicornis and white rhinoceros Ceratotherium simum,16 and wolves Canis lupus17; for a whole review see ref 18).

Parasitism poses a potential problem for reintroduction projects by three main routes:
1. Animals born and raised in zoological institutions encounter novel parasites on release into their ‘indigenous’ new habitats. For example, captive-bred Eastern bongos Tragelaphus

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institutions should be considered potential reservoirs in zoological institutions. Animal species in zoological institutions are a standard procedure before any reintroduction.36 Similarly, multiple parasites are widespread and prevalent in captive and wild populations.17 35 In this regard, screening for parasites is important to ensure that reintroduced animals do not carry contagious diseases52 and to avoid deleterious and unpredictable consequences on extant wild populations.21 22  Healthy animals associated with handling animals, has also been hypothesised as a reason for reintroduction failures.21 22 Stress-induced infection during translocation/reintroduction, associated with handling, has also been detected in ruminant faeces.24 strongyle-type eggs have been recovered from ruminant faeces.24 Similarly, multiple authors have detected the presence of ectoparasites and blood parasites in mammals (flies, lice, ticks or Haemoproteus species)26 29 held in zoological institutions. Animal species in zoological institutions should be considered potential reservoirs for parasites, although parasite–host equilibrium is a natural and essential biotic factor.10 30 In this sense, measures need to be taken to ensure that parasite egg shedding does not occur during reintroductions33 34 to avoid deleterious and unpredictable consequences on extant wild populations.27 28 In this regard, screening animals for parasites is a standard procedure before any reintroduction.36

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) are threatened ungulates listed in the International Union for Conservation of Nature (IUCN) Red List,37 and they are managed in zoos through EEPs. On December 31, 2012, 54 institutions participated in the management of 417 (133/284 (♂♂/♀♀) scimitar-horned oryx, 12 institutions held 193 (87/106) Mohor gazelle and six institutions had 186 (74/112) Cuvier’s gazelle. Reintroductions/translocations are recommended by international bodies and range-states authorities (eg, IUCN) as appropriate tools for the conservation of these three species. A number of reintroduction projects have been carried out since the 1980s in Senegal (scimitar-horned oryx and Mohor gazelle), Tunisia (scimitar-horned oryx, Mohor gazelle and Cuvier’s gazelle) and Morocco (scimitar-horned oryx and Mohor gazelle).18 40 At the time of writing, ongoing projects include reintroduction of the scimitar-horned oryx42 and Cuvier’s gazelle in Tunisia.53 While some of the projects seem to have succeeded,41 42 others have failed and parasites have been identified as the likely cause for lack of success in some cases.45 46 Some studies have assessed parasitism in bovidae in zoological institutions which provide the source animals for reintroduction or translocation conservation actions (scimitar-horned oryx in two Belgian zoos38 and Cuvier’s and Mohor gazelle at one Spanish institution67–50) or other taxa and projects.51 This information is crucial to minimise the risk of reintroducing animals with contagious diseases52 and to provide prophylactic treatment to the selected founder stock for reintroductions.53

Materials and methods
In July 2010, a modified version of the questionnaire by Isaza et al38 was sent to 65 EAZA member zoological institutions holding any of the three study species (ie, scimitar-horned oryx, Mohor gazelle and Cuvier’s gazelle). Questions were asked on the presence of ectoparasites, blood parasites and other endoparasites that shed eggs in the faeces for animals housed in those institutions, as well as their husbandry and veterinary practices for detection and control of parasites. Responses were received from 38 institutions (58.5 per cent) between August 2010 and July 2011. Not all institutions answered every question, so responses were relatived to the number of answers received to each specific question rather than to the total number of institutions answering the questionnaire. The sample size for each question is provided in tables 2 and 3. Some institutions had two host species, and in all these cases responses reported for both species were always the same. Zoological institutions are treated as the sample unit, and the prevalence of each item category was defined as the percentage of institutions reporting a given answer in the questionnaire. Confidence limits (CI) of 95 per cent were calculated.

Results
Thirty-eight completed questionnaires were received from 12 European countries and Israel (table 1). Most housed scimitar-horned oryx (86.8 per cent), while Mohor and Cuvier’s gazelles were present in nine (23.7 per cent) and four (10.5 per cent) institutions,
respectively. Most institutions considered the control of endoparasites that shed eggs in the faeces and blood parasites (79 per cent; CI ±13) as well as the control of ectoparasites (82 per cent; CI ±12) to be important for the health of the host species; however, only 66 per cent (n=25) routinely took blood samples from the ground to detect the eggs of endoparasites, with concentrating flotation technique as the preferred method (82 per cent; CI ±12).

Up to 25 institutions (66 per cent; CI ±15) routinely took blood samples, most of them (24 out of 25) from some (unknown number) individuals rather than from all members of the herd. Detailed, individual examination for ectoparasites was carried out in 33 of the respondent institutions (87 per cent; CI ±11).

The responses related to chemoprophylaxis protocols are summarised in table 3. While 66 per cent (CI ±15) of the institutions had regular prophylactic treatments for endoparasites, only 21 per cent (CI ±13) routinely used preventive treatments for ectoparasites. Ivermectin is the most commonly used medication to prevent parasitism. It is usually administered in medicated feed every six months when used for endoparasites, but only once a year via medicated feed, topically or by parenteral injection, in similar dosages, for prevention of ectoparasites. A third of the institutions (32 per cent; CI ±11) take additional measures to control parasitic diseases, including the use of an insecticide spray in the enclosures and buildings.

**Discussion**

The zoological institutions surveyed in this study indicated that endoparasites, blood parasites and ectoparasites are perceived to be important threats to the health of ungulates, and preventive treatment and control were considered to be a key aspect of husbandry protocols. The three surveyed ungulates were regularly treated with ivermectin against sensitive endoparasites (generally every six months), and yet prevalence was
still high (97 per cent of the institutions). Parasite anthelmintic resistance has been suggested as a cause of treatment failure, but the prevalence recorded could also be attributed to the timing and frequency of the prophylactic treatment, the route of administration, or a combination of these. The uneven uptake of medication by individuals when sprinkled over food, as most institutions prefer to do, is a well-recognised problem that limits treatment success as well (63 per cent; CI ±15). Asymptomatic animals may act as a reservoir of parasites that can result in rapid infection of wild populations at the release site. Additionally, these animals may become symptomatic as a result of the stress associated with translocation and release.

Only one institution (3 per cent) reported that ectoparasites were detected in their gazelle or oryx herds, which may be a true reflection of the low parasite burden in the studied ungulates. Alternatively, this may be the result of the challenge of detecting ectoparasites, which requires manual or chemical restraint of the host species. Such procedures are often limited to specific health checks (opportunistic sampling), and institutions only treat against ectoparasites when clinical signs are observed. The advantage of the relatively high use of ivermectin as the preferred treatment against endoparasites might prevent ectoparasites in the hosts as well (table 3).

While most institutions (65.8 per cent) collected blood samples for parasite screening, none reported the presence of haemoparasites. The occurrence of blood parasites in antelope populations in zoological institutions would be a concern given their documented impact on the taxa. Asymptomatic animals may act as a reservoir of parasites that can result in rapid infection of wild populations at the release site. Additionally, these animals may become symptomatic as a result of the stress associated with translocation and release.

**Table 3** Responses to questions related to chemoprophylaxis protocol used in the surveyed zoos (n is the number of zoos that responded to the specific question)

| Question                                                                 | Answer                          | Zoos (n) | % Relative to the total zoos included in the study (38) | 95% Confidence limits |
|--------------------------------------------------------------------------|---------------------------------|----------|----------------------------------------------------------|-----------------------|
| Does your endoparasite control programme include routine preventive treatments for the host species? (n=36) | Yes 25 66 51–81                  | 25       | 66            | 51–81                |
|                                                                         | No 11 29 15–43                  | 11       | 29            | 15–43                |
| What types of antiparasitic drugs are routinely used at your institution for endoparasites? (n=27) | Ivermectin 25 66 51–81          | 25       | 66            | 51–81                |
|                                                                         | Benzimidazoles 11 29 15–43      | 11       | 29            | 15–43                |
|                                                                         | Levamisoles 4 11 1–21           | 4        | 11            | 1–21                 |
|                                                                         | Organophosphates 0              | 0        | 0             |                      |
|                                                                         | Other 0                         | 0        | 0             |                      |
| What is the frequency of treatment? (n=25)                              | Once a month 1 3 1–8            | 1        | 3             | 1–8                  |
|                                                                         | Once every two months 1 3 1–8   | 1        | 3             | 1–8                  |
|                                                                         | Once every three months 7 18 6–30 | 7      | 18            | 6–30                 |
|                                                                         | Once every four months 5 13 2–24 | 5      | 13            | 2–24                 |
|                                                                         | Once every five months 0        | 0        | 0             |                      |
|                                                                         | Once every six months 8 21 8–34 | 8      | 21            | 8–34                 |
|                                                                         | Once a year 2 5 1–12            | 2        | 5             | 1–12                 |
|                                                                         | Other 1 3 1–8                   | 1        | 3             | 1–8                  |
| What is the route of administration? (n=26)                             | Oral/Medicated feed 24 63 48–78 | 24       | 63            | 48–78                |
|                                                                         | Parenteral (injection) 11 29 15–43 | 11    | 29            | 15–43                |
| Does your ectoparasite control programme include routine preventive treatments for the host species? (n=34) | Yes 8 21 8–34                  | 8        | 21            | 8–34                |
|                                                                         | No 26 68 53–83                  | 26       | 68            | 53–83                |
| What types of antiparasitic drugs are routinely used at your institution for ectoparasites? (n=11) | Ivermectin 8 21 8–34            | 8        | 21            | 8–34                |
|                                                                         | Organophosphates 2 5 1–12       | 2        | 5             | 1–12                 |
|                                                                         | Other 2 5 1–12                  | 2        | 5             | 1–12                 |
| What is the frequency of treatment? (n=9)                               | Once a month 3 8 1–17           | 3        | 8             | 1–17                 |
|                                                                         | Once every three months 3 8 1–17 | 3      | 8             | 1–17                 |
|                                                                         | Once every six months 4 11 1–21 | 4      | 11            | 1–21                 |
|                                                                         | Once a year 6 16 4–28           | 6        | 16            | 4–28                 |
| What is the route of administration? (n=11)                             | Oral/Medicated feed 4 11 1–21   | 4        | 11            | 1–21                 |
|                                                                         | Parenteral (injection) 5 13 2–24 | 5      | 13            | 2–24                 |
|                                                                         | Topical 4 11 1–21               | 4        | 11            | 1–21                 |
|                                                                         | Other 0                         | 0        | 0             |                      |
| Do you carry out any additional sanitary prophylaxis for parasite control? (n=32) | Yes 12 32 17–47                | 12       | 32            | 17–47                |
|                                                                         | No 20 53 37–69                  | 20       | 53            | 37–69                |

More than one option could be ticked in most questions.
procedure most often employed among the ones used to detect the eggs of endoparasites. However, this method fails to detect trematode and lung nematode infections. Concentrating sedimentation technique was used by only one-third of the institutions in this study (34 per cent) and Baermann was employed by 24 per cent (table 2). Consequently, subclinical trematode and lung nematode infections may not be diagnosed. Therefore, animals involved in future reintroductions should be carefully investigated for parasites using a combination of faecal concentrating techniques (sedimentation, floating and Baermann) and even molecular or serological diagnosis tests, according to standard procedures of good veterinary practice.

The results of these survey suggest that it may be difficult to control parasites (mainly the ones that shed eggs in animal faeces) for scimitar-horned oryx, Cuvier’s gazelle and Mohor gazelle in zoological institutions, despite the preventive measures implemented and the administration of treatments when parasites are detected. Precise management practices need to be enacted to ensure that novel parasites from captive populations are not introduced to naive wild populations by translocations or reintroductions. Furthermore, animals from zoological institutions need to develop resistance to parasites to ensure that they are not completely naïve when released into their historical range. This balance is difficult to achieve in zoological institutions. A recent study has shown that ‘dewormed and vaccinated’ and ‘non-dewormed and unvaccinated’ Cuvier’s gazelles at an institution had similar health and fitness results (eg, bodyweight, blood parameters related to immune system).

Some reintroduction programmes have failed, partly or totally, as a result of parasitism. Most of the Cuvier’s gazelles released in Tunisia in 1999 and their offspring died from parasitism. In contrast, all the reintroduced populations of scimitar-horned oryx as well as the population of Cuvier’s gazelle reintroduced in 2016 have persisted and increased in size, and parasites have not been associated with mortalities.

Faecal analyses carried out in Dghoumes National Park, Tunisia, suggest the oryx appear to be free of parasite burden (T. Gilbert, unpublished data).

The presence of parasites in captive animals may have a contradictory importance for reintroduced animals and reintroduction programmes. It is possible that captive animals are more susceptible to parasites than the wild ones (see, for instance, ref 76). Conversely, regular deworming of animals to avoid parasite transmission in captivity may reduce their immunity to parasites when released, especially if such pathogens are present in the wild. However, captive-born animals that are released could also harbour parasites that could become invasive to naïve wild populations. Therefore, the treatment of captive individuals must be specific to the particular situation of each reintroduction programme.

Our results show that parasites are widely distributed in zoological institutions, although these institutions carefully apply the recommended procedures of good veterinary practices. This could be a critical point when reintroduction/translocations of animals are planned. In this sense, management practices should be improved, such as the association of coprological diagnosis with more sensitive methods (eg, molecular techniques and serological tests) when possible, or alternatively the design of particular treatment strategies for each situation. Reintroduction practitioners should be aware of the parasite diversity and parasitic load affecting source populations and the impact they may have on the health of individual animals. A subclinical parasite burden could stimulate the immune system benefiting animals that are released into the wild where parasites are present in extant wildlife or livestock populations. In this sense, further research should be performed to facilitate the work of zoos in their role as essential components for the conservation of threatened species.

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