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Exploring Intestinal Parasites Among Falcons in Riyadh, Saudi Arabia

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ABSTRACT

Intestinal parasites are a major threat to the health of hosts, impacting their productivity. Falcons are considered a high-value innate wealth in various parts of the world, particularly in Saudi Arabia. The current study aimed to explore the prevalence of intestinal parasites in falcons by examining 125 stool samples from different types of falcons using the direct fecal smear method. The examination revealed 87.2% of the samples were infected with intestinal parasites. The overall parasite prevalence was as follows: *serratospiculum seurati* 47.2%, *caryospora spp*. 46.4%, *strigea falconis* 6.4%, *capillaria columbae* 1.6%, *cladotaenia globifera* 5.6% *ascaridia spp*. 0.8%, and *giardia* 0.8%. Six infected samples were re-examined using the direct fecal stain and centrifugal flotation methods to compare the efficacy of these techniques. The results indicated that the direct fecal smear is one of the most effective methods for detecting parasitic infections. Chi-square tests of independence confirmed that there is no association between infection rate and the falcon's gender, age, and species and no association between falcon species and its susceptibility to infection with a particular parasite. Falcons are highly sensitive to environmental changes and can be easily affected by parasites, which can cause serious health issues and even death. Therefore, ensuring that the environment and food provided to falcons are safe and free from parasites is crucial.

KEYWORDS: Falcon, Saudi Arabia, Fecal, Intestinal parasites

INTRODUCTION

Raptors are essential to ecosystems and are seen as biological markers of environmental contamination (Rossi et al., 2021). Falcons are one of the most common raptors. They belong to the order *Falconiformes* in the family *Falconidae* (Wilcox et al., 2019) and can be found on every continent except Antarctica (Pyzik et al., 2021). These medium-sized birds breed in the Arabian Peninsula, notably in the United Arab Emirates, and in the deserts of North Africa from Eastern Libya and Egypt to Jordan, extending south-eastward along the beaches of the Red Sea and Arabian Gulf to southwestern Pakistan (Javed et al., 2012). Falcons are regarded as a highly valuable natural resource in Saudi Arabia.

There are around 9,092 captive falcons in Saudi Arabia, and this number is likely to increase due to the rising interest and investment in the sport of falconry. Falconry in Saudi Arabia holds profound cultural and historical significance, reflecting the deep-rooted traditions and values associated with the practice (Binothman, 2016). However, this sport may increase the risk of parasite transmission to humans and poultry (Bertran et al., 2012). Research on raptor pathogens offers valuable information for monitoring ecosystem health, assessing the population's health, and determining the potential role these birds may have in dispersing serious pathogens like bacteria and parasites (Rossi et al., 2021). Endoparasites are common in captive and wild raptors and can seriously threaten the birds' health (Alfaleh et al., 2020). Some protozoa and helminth species can affect a raptor's ability to fly and hunt, making them more vulnerable to secondary injuries. Additionally, bacterial infections often exacerbate lesions caused by endoparasites (Rossi et al., 2021). The most common protozoan intestinal parasites that infect falcons are *Caryospora* (Santana-Sánchez et al., 2015) and *Trichomonas gallinae* (Alrefaei et al., 2022). The most common helminth parasites are *Capillaria, Serratospiculum,* and *Strigea* (Santoro et al., 2010). Falcons are both ecologically and socially significant, but many aspects of their biology remain unknown, including the variety of parasites that infect them in the wild and captivity (Alrefaei et al., 2022). This study aims to detect intestinal parasites in falcons in the Riyadh region and identify the most effective detection methods for birds. Data has been collected and analyzed to estimate the prevalence of parasites and to observe any association between the prevalence of parasites and different groups (age, sex, and falcon species). Additionally, it recommends precautions for falconers to limit the spread of these parasites.

MATERIALS AND METHODS

Study area: This study was conducted in the Bandar Al-Daraa Falcon Care Center (24°51'54.3"N 46°50'50.5" E), Riyadh City, Kingdom of Saudi Arabia. It is the first falcon care facility in the Kingdom of Saudi Arabia. It is a specialist facility that offers falcons expert veterinary care. Its main objective is to provide exceptional veterinary care to ensure these magnificent birds' optimum health and well-being.

Sample collection

From Dec 2022 to Feb 2023, 125 fecal samples were collected from sick falcons at the Falcon Clinic Laboratory. Samples were fresh and stored. Stored samples were kept in dry, hygienic, cool, and airtight containers. Samples were from 16 male falcons and 109 female falcons. The age of falcons was among 105 adults and 20 juveniles from *F. cherrug* (*n*=69), *F. rasticolus* (*n*=28), *F. peregrinus* (*n=15*), *F. cherrug* × *F. rasticolus hybrid* (*n*=5), *and F. rasticolus*× *F. peregrinus hybrid* (*n=8*).

Processing of samples

The veterinarian determined the falcon's species. One hundred twenty-five samples were tested using the direct fecal smear technique. Six samples were re-examined using direct fecal stain, centrifugal floatation, and direct fecal smear methods.

Direct fecal smear

The samples were combined with 0.9% normal saline solution (NaCl), covered by a coverslip, and examined at×10 magnification under a light microscope to identify the parasite genus (Broussard, 2003).

Centrifugal floatation method

Six samples (4 fresh and 2 stored) were subjected to the centrifugal floatation method by adding 3-5 grams of feces mixed with 3 ml of normal saline (0.9% NaCl) in a 5-ml-tube, then placed in the centrifuge at 2500 rpm for 5 minutes. After taking it out and filling it with normal saline, the coverslip was placed on the top of the tube for 10 minutes and examined under the light microscope at ×10-×40 magnification (Maria Pyziel-Serafin et al., 2022).

Fecal stain method

The 6 samples were prepared the same way as in the direct fecal smear. After drying the samples in the incubator for 3-4 minutes, it was fixed with 70% ethanol for 30 seconds and stained with methylene blue for 1 and a half minutes. Finally, the samples were washed, and a drop of oil was placed on the slide to be examined at ×100 magnification under the light microscope. Another method used to stain the fecal smear samples was the Ziehl-Neelsen stain. The fixation step was conducted by adding one drop of methanol for 3 min. After staining with carbol fuchsin for 10 min, the samples were washed. In the decolorization step, one drop of ethanol was added for 3 min and then washed. In the last step, the slide was covered with methylene blue for 1.5 min, then washed and left to dry (Alqarni et al., 2022).

Statistical tests

We used the Chi-square test to determine if there is an association between the prevalence of intestinal parasites and different factors (age, sex, and species). Additionally, we employed the Chi-square test to see whether there is a link between the falcon species and the frequency of particular parasite species. We used Excel to run the Chi-square test with a significance threshold of 0.05 to determine if the observed differences were statistically significant. Illustrated figures were generated using Excel.

RESULTS

In our research, 125 fecal samples from different types of falcons were examined by direct fecal smear. The parasitic infection was detected in different aged falcons of both sexes, as shown in table 1.

Table 1. The prevalence of parasitic infection in both sexes at different ages.

		No. tested	No. positive	Prevalence%
Age	Juvenile ≤1 year	20	18	90
	Adult > 1 year	105	91	86.6
Sex	Female	109	93	85.3
	Male	16	16	100
Total infection	-	125	109	87.2

We conducted chi-square tests to determine whether there are significant associations between age groups (juvenile vs. adult) and infection status, as well as between sex (female vs. male) and infection status. The chi-square test for the association between age and infection status was insignificant, χ^2 (1, N = 125) = 0.167, p = 0.683. The chi-square test for the association between sex and infection status was also not significant, χ^2 (1, N = 125) = 2.693, p = 0.101. These findings suggest no significant association between either age or sex and infection status in this sample. Thus, the likelihood of infection is independent of age and sex. A total of 109 (87.2%) samples were infected: *Serratospiculum seurati* (47.2%), *Caryospora spp* (46.4%) (Figure 1a), *Strigea*

falconis (Figure 1b,c), *Cladotaenia globifera, Capillaria columbae* (Figure 1d), *Ascaridia porrocecum* (Figure 1e), and *Giardia spp*. As shown in Table 2, *Ascaridia porrocecum* and *Giardia spp* were the least prevalent parasitic infections (0.8%). Samples were acquired from *F. cherrug, F. rusticolus, F. peregrinus, F. cherrug* × *F. rasticolus* hybrid, *F. rasticolus*×, *and F. peregrinus* hybrid, and were infected with the mentioned parasites. Compared to other falcon species, *Caryospora spp* was the most prevalent intestinal parasite observed in *F. cherrug* (62.5%), and a single instance of *Ascaridia* was detected in a sample of a falcon from the same species.

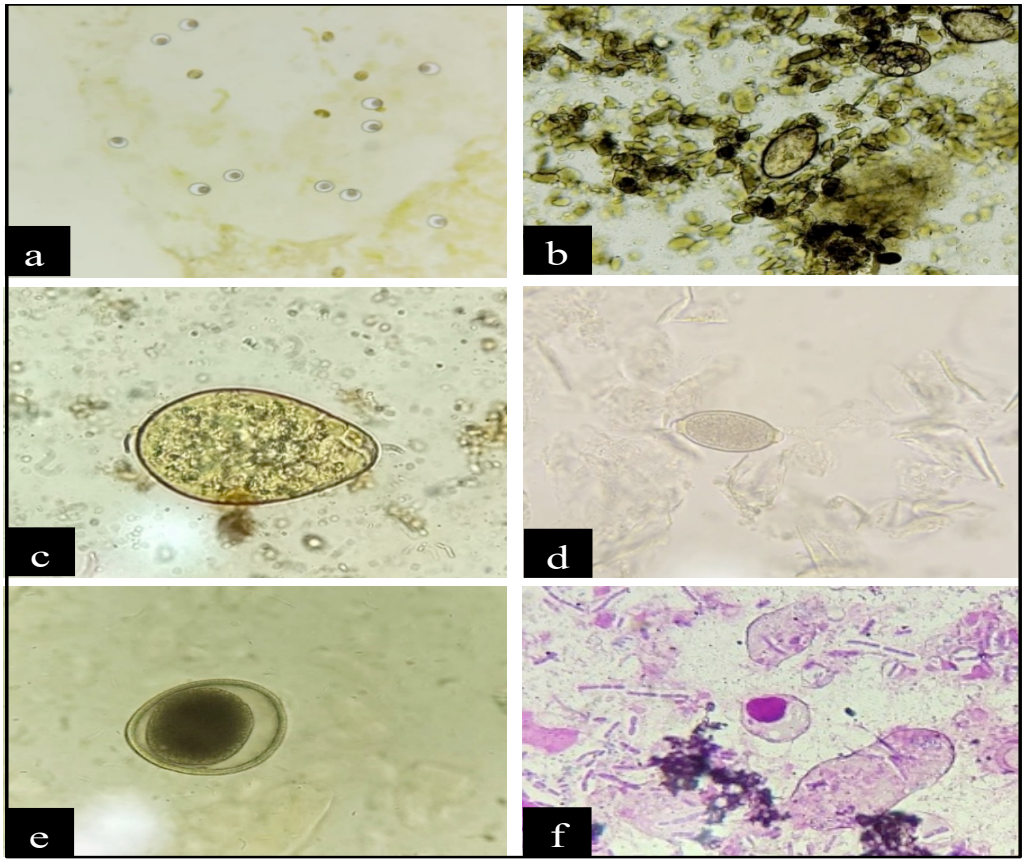

Figure 1. (a) *Caryospora* spp. was isolated from a female Gyrfalcon fecal sample by direct fecal smear (x100). (b) Sporulated ovum of *Strigea falconis* from a female saker detected by direct fecal smear (X400). (c) An unsporulated egg of *Strigea falconis* from a female saker falcon by direct fecal smear(x400). (d) An egg of *Capillaria columbae* from a male peregrine falcon (x400). (e) An egg of nematode *Ascaridia* spp. from a juvenile female saker falcon (x 400). (f) An egg of *Caryospora spp*. from a fresh female gyrfalcon sample using the Ziehl–Neelsen staining method (x1000).

The approximate prevalence of *S. seurati* and *Caryospora spp.* in *F. rusticolus* was close to that in *F. cherrug*. In contrast, the prevalence of *Cladotaenia globifera* was nearly similar to that of *F. peregrinus*. The greatest percentage of *S. seurati* (64.2%), *Strigea falconis* (14.2%), *C. columbae* (14.2%), and *Cladotaenia globifera* (14.2%) were found in *F. peregrinus*. *Caryospora* and *Serratospiculum* were the only parasites identified in the two-hybrid falcons, with no detection of other parasites except *Giardia spp* in one sample from *F. rasticolus*× and *F. peregrinus hybrid.* The rest of the prevalence of the parasite in each falcon species is present in Table 3.

Table 2: The prevalence of parasitic infection in falcons.

	No. infected	The prevalence (%)
Serratospiculum seurati	59	47.2
Caryospora spp	58	46.4
Strigea falconis	8	6.4
Capillaria columbae	3	2.4
Cladotaenia globifera	7	5.6
Ascaridia porrocecum	1	0.8
Giardia spp.	1	0.8

Table 3: The prevalence of various parasites in different falcon species

Species	No. tested	No. positive / %	*Serratospiculum seurati* / %	*Caryospora Spp* / %	*Strigea falconis* / %	*Capillaria columbae* /%	*Cladotaenia globifera* /%	*Ascaridia porrocecum* /%	*Giardia spp* /%
F. cherrug	69	56 / 81.1	31/55.3	35/ 62.5	3/ 5.3	1/ 1.7	2/ 3.5	1/ 1.7	-
F. rusticolus	28	22 / 78.5	11/50	13/ 59	2/ 9	-	3/ 13.6	-	-
F. peregrinus	15	14 / 93.3	9/64.2	6/ 42.8	2/ 14.2	2/ 14.2	2/ 14.2	-	-
F. rasticolus× F. peregrinus hybrid	8	8 / 100	4/50	3/ 37.5	-	-	-	-	1/ 12.5
F. cherrug × F. rasticolus hybrid	5	5 / 100	2/40	3/ 60	-	-	-	-	-

The chi-square test for the association between bird species and infection rate was not significant, χ^2 (4, N = 125) = 4.477, p = 0.345. This result indicates no significant association between the species of the birds and their infection rate in this sample. Also, The chi-square test examining the relationship between falcon species and the presence of particular parasites was not significant, χ^2(24, N = 137) = 32.894, p = 0.106. Therefore, there is no significant association between the bird species and the presence of the parasites listed in the table. Besides direct fecal smear, 6/125 (4 fresh and 2 stored) were examined using two other techniques: centrifugal floatation and direct fecal staining. In fresh samples, 3/4 show no detection of parasitic infection in the centrifugal floatation ,while 2/4 of the stained samples gave negative results, the other half of the stained samples were positive (Figure 1f) contrary to the direct fecal smear where the infection was detected in all samples as shown in Table 4. All stored samples in direct fecal smear (Figure 2a, b) and staining methods (Figure 2c, d) showed positive results, unlike the centrifugal method

DISCUSSION

Despite the appearance of a convergence in the prevalence of infection between adults and juvenile brids, as the Chi-square test revealed, the infection rate is higher as the bird's age increases, as reported by Juárez et al. (2020). The most common parasite was *Serratospiculum seurati* (47.2%). Ibarra et al. (2019) and Tarello (2006) also determined that it was the most prevalent parasite in falcons of the Middle East. We found

Caryospora spp (46.4%) to be a common parasite in falcons, as was previously identified by Alfaleh et al. (2020) and Santana et al. (2015).

Table 4. Detection of parasitic infection in the six samples using various techniques.

Sample No.	Sample type	Direct smear method	Centrifugal floatation	Staining method
1	Fresh	*Caryospora* spp	-	-
2	Fresh	*Strigea falconis*	-	+
3	Fresh	*Caryospora* spp	+	+
4	Fresh	*Caryospora* spp	-	-
5	Stored	*Caryospora* spp	-	+
6	Stored	*Strigea falconis* & *Serratospiculum seurati*	-	+

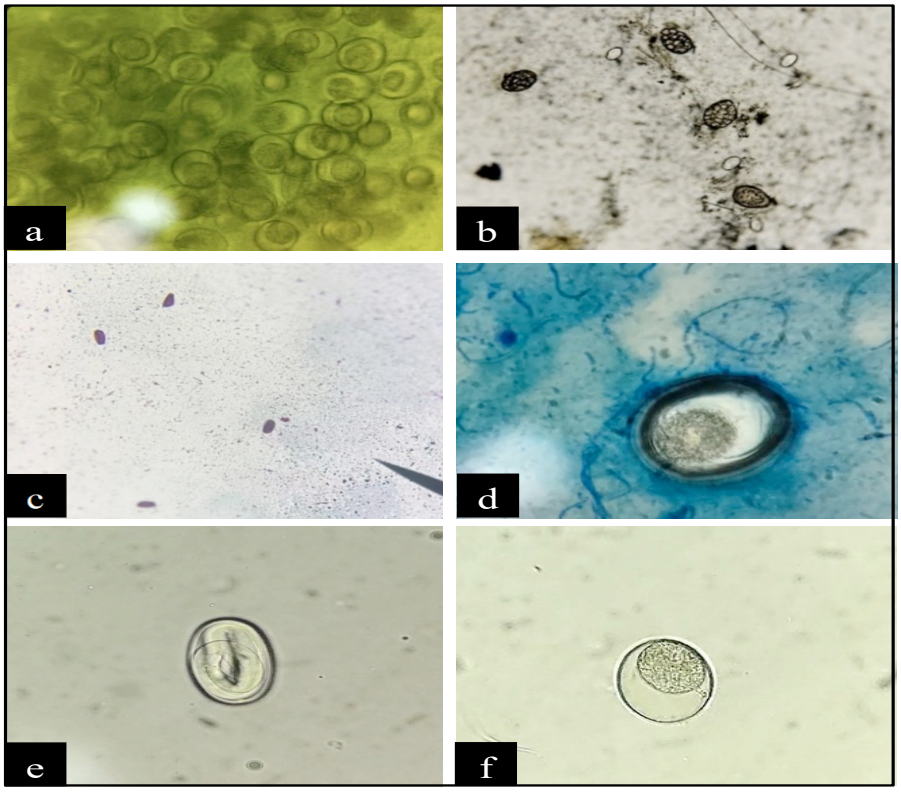

Figure 2. (a) Intensive infestation of *Caryospora spp.* In a stored male saker falcon sample (x400). (b) Co-infection with *S. seurati* and sporulated and unsporulated S. *falconis* investigated in a stored female saker falcon sample examined by direct fecal smear (X100). (c) *S. seurati* isolated from a stored female saker falcon sample using *the Ziehl–Neelsen staining method(x100).* (d) Unsporulated oocyst of *Caryospora spp.* from a stored male saker falcon sample using a direct fecal stain technique (× 1000). (e) Embryonated egg of *S. seurati* from a stored female saker falcon sample (x400). (f) *Caryospora spp.* oocyst from a stored male captive saker falcon obtained from the middle of the centrifugal floatation tube (x 400).

Similarly to other published results, we found no zoonotic parasites except Giardia. *Giardia duodenalis* (also known as *Giardia lamblia* or *Giardia intestinalis*) can infect a wide range of hosts, including humans, and cause the gastrointestinal disease called giardiasis (Maestrini et al., 2022; Shu et al., 2022). Furthermore, our results indicate a variation in the effectiveness of the three identification methods (direct fecal smear, staining, and centrifugal floatation method) used to detect the presence of parasites in the samples, where it was concluded that the direct fecal smear method is the most efficient in detecting parasites compared to other methods.
In the staining method, 2/4 of the fresh samples gave negative results due to the breakage of the parasite oocyst at one of the staining steps. In the centrifugal floatation method, *Strigea falconis* did not appear in any of the two samples (1 fresh & 1 stored) due to its high specific gravity density, which causes it not to float, so it is preferable to detect it by sedimentation method as discussed by Inês et al. (2016). We tried a simple flotation method in one of *Strigae's* samples (fresh) without waiting for 10 minutes and received a positive result. In the second sample (stored), we found *Strigea falconis* with *S. seurati* in the direct fecal smear, but *Strigea* did not show up for the reasons discussed earlier. At the same time, *S. seurati* appeared after taking part of the floatation solution from the middle of the tube to ensure that the *S. seurati* eggs were not broken due to the solution used (Figure 3e). Hence, the negative result in Table 4 can be explained. *Caryospora* is one of the protozoans easily detected in the centrifugal floatation method due to its low density compared to the floatation solution. As claimed by Inês et al. (2016), it appeared insignificantly in the examined samples (2 fresh & 1 stored), which might be due to several reasons, one of which is the low amount of parasites in the fresh sample. The second explanation could be due to the increase in the specific gravity of *Caryospora* occurring during its storage. To ensure that the *Caryospora* was not broken, we took part of the floatation solution from the bottom of the tube as we did with *the S. seurati* sample, and the parasite was found in a healthy condition (Figure 3f).

CONCLUSION

Wildlife plays a significant role in Saudi Arabia's Vision 2030, aligning with the goals of sustainable development and the objective of achieving a diverse and sustainable economy. Our study has revealed that falcons, an important species in the Kingdom, have an alarming prevalence of intestinal parasites, with an overwhelming 87.2% of the sampled birds being affected. The most common parasites identified in the study were *Serratospiculum seurati* and *Caryospora* spp., which can be attributed to their life cycles, environmental exposure, and specific host interactions. The study recommends using the direct fecal smear method to detect bird parasitic infections due to its effectiveness, simplicity, cost-efficiency, and time-saving benefits. Maintaining and improving the hygiene of areas where falcons reside is crucial, as some parasite species' oocysts thrive in unsanitary environments. Ensuring the safety and nutritional quality of the falcons' food is essential, including incorporating anticoccidial agents to prevent specific parasitic infections. The establishment of multiple care facilities is necessary to ensure the health of raptors and protect them from infections and the risk of extinction. Given the popularity of falconry in the Middle East, conducting routine examinations frequently is vital to ensure the continued health and parasite-free status of these birds.

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