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Occurrence of Gastrointestinal Parasites in Synanthropic Neozoan Egyptian Geese (*Alopochen aegyptiaca*, Linnaeus 1766) in Germany

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Abstract: Various studies have shown that the transmission and passage of alien and native pathogens play a critical role in the establishment process of an invasive species and its further spread. Egyptian geese (*Alopochen aegyptiaca*) are neozotic birds on various continents. They live not only in the countryside near fresh water bodies but also in urban habitats in Central Europe with close contact to humans and their pets. Although their rapid distribution in Europe is widely debated, scientific studies on the anthropozoonotic risks of the population and studies on the present endoparasites in Egyptian geese are rare worldwide. In the present study, 114 shot Egyptian geese and 148 non-invasively collected faecal samples of wild Egyptian geese from 11 different Federal States in Germany were examined. A total of 13 metazoan endoparasite species in 12 different genera were identified. The main endoparasites found were *Hystrichis tricolor*, *Polymorphus minutus*, and, in lesser abundance, *Cloacotaenia* sp. and *Echinuria uncinata*. Adult stages of *Echinostoma revolutum*, an anthropozoonotic heteroxenic trematode, were found in 7.9% of the animals examined postmortem. This species was additionally identified by molecular analysis. Although Egyptian geese live in communities with native waterfowl, it appears that they have a lower parasitic load in general. The acquisition of generalistic parasites in an alien species and the associated increased risk of infection for native species is known as "spill-back" and raises the question of impacts on native waterfowl. Differences between animals from rural populations and urban populations were observed. The present study represents the first large-scale survey on gastrointestinal parasites of free-ranging Egyptian geese.

Keywords: *Alopochen aegyptiaca*; synanthropic avian invasion; *Echinostoma revolutum*; anthropozoonotic risk; pathogens

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1. Introduction

1.1. Egyptian Geese (Alopochen aegyptiaca) in Europe

Many exotic species (syn. alien) benefit in a variety of ways from the human transformation and homogenisation of diverse habitats through urbanisation, large-scale monocultures in intensive agriculture, and, not least, global climate change [1,2]. There has been much research on the impact of alien species, even if the pathogens they introduce have not been studied very widely [3].

One of these beneficiaries is the Egyptian goose *A. aegyptiaca* (Linnaeus 1766). It is an alien bird not only in Europe but also in North America and the Arabian Peninsula, where these animals formed established self-sustaining populations [4]. However, the spread of this invasive bird is still ongoing. The European population is considered to present the most rapid expanding invasive waterfowl species in the Centre of Europe [4,5]. This shelgoose (Tadorninae) is originally native to Sub-Saharan Africa and along the Nile

Valley [6,7]. Originally, they were introduced as ornamental birds to parks in England and the Netherlands in the seventeenth century, but different refugees from different parks in Europe have since formed what was at first a slow, and since 1970 until the present, is now a rapidly spreading non-native population in Central Europe [1,8].

As is the case with many alien species, it is a very flexible generalist species that becomes firmly established in different habitats as well as urban and peri-urban areas [2,9]. Direct impact of this growing population on native waterfowl seem inter alia limited by high intraspecies competition [10]. However, we know very little about pathogen transmission by this alien species. Disease and organism transmission by invaders have a greater impact on native species than direct competition for resources [11]. Pathogens (i.e., parasites) play an important role in the successful establishment of an alien in an invaded area [12]. It is not uncommon for these species to carry fewer pathogens than native species in a similar niche [13]. They leave behind pathogens in their native regions or bring along specialised parasites (enemy-release/spillover) [14]. Some aliens are known to increase the infection pressure of native generalistic pathogens in the invaded areas on the native species comparatively unharmed by these pathogens (spill-back) [15,16]. The question of whether alien species decrease the fitness of direct native competitors for resources in this way in discussed here, and, through this, whether they gain an advantage in establishing themselves.

Although Egyptian geese are very flexible in adopting habitats, they prefer urban habitats in their invaded areas with open lawns, high trees for breeding, and fresh water bodies [10]. These habitats are often provided by urban parks and public swimming pools [17]. They live in synanthropic communities with mallards (*Anas platyrhynchos*), mute swans (*Cygnus olor*), and Canada geese (*Branta canadensis*), and they have frequent contact with humans and their pets. This preference for urban habitats by alien species is not uncommon [18]. Anthropogenic influences create similar urban spaces worldwide, giving generalist species an advantage [2,19].

However, unlike European native water fowl, Egyptian geese spend more time out of the water and are more often found grazing on lawns [20,21]. As a result, defecation occurs much more frequently on pavements and park meadows than with native waterfowl (Figure 1(2)). In their rural habitats, the animals prefer to graze on freshly sprouting fields. The contamination by geese droppings on fields also poses a risk for the introduction of possible anthropozoonotic pathogens into food production.

As predominantly herbivorous waterfowl, Egyptian geese feed mainly on grass, seeds, and leaves. They are dependent on protein-rich plants [10,22] and are described as engaging in anthropogenetic feeding with bread, maize, and cat food in urban areas [20]. Deformations of the wings, called angel wings or hanging wings, originating from uptake of huge amounts of energy and protein-rich food [23,24], was noticed by the authors in nearby field studies in urban populations of Egyptian geese in Frankfurt (Hesse) and Offenbach (Hesse).

Contamination with droppings in parks and swimming pools by these animals is increasingly being recognised as a widespread problem [25]. To date, only limited research has been conducted to determine whether anthropozoonotic risks emanate from the animals or their faeces. Prüter et al. examined the prevalence of some selected viral and bacterial pathogens [26]. However, the anthropozoonotic risk has not yet been investigated parasitologically. Studies on detailed composition of the endoparasitic fauna in Egyptian geese are not only missing in their invaded areas but also in their native regions in Sub-Saharan Africa. Listed in Table 1 are all the mentions/descriptions of parasite species in Egyptian geese.

Figure 1. Egyptian geese (*Alopochen aegyptiaca*) in urban habitats: (**1**) both parental birds guiding ducklings in an urban park; (**2**) Egyptian geese together with Canada geese (*Branta canadensis*) and Mallards (*Anas platyrhynchos*) in an urban public pool; and (**3**) group of subadult Egyptian geese in a urban resting site contaminated with droppings.

Table 1. References to parasites found in Egyptian geese (*Alopochen aegyptiaca*) in the literature.

Parasite Species	Family	Described by	Country Where Detected
Plasmodium spp.	Plasmodiidae	Cumming et al. [27]	South Africa, Zimbabwe
Haemoproteus spp.	Plasmodiidae	Cumming et al. [27]	South Africa
Trichobilharzia spinulata	Schistosomatidae	Fain [28]	Ruanda
Hymenolepsis biaculeata	Hymenolepididae	McDonald [29]	Sudan/South Africa
Hystrichis tricolor	Dioctophymatoidae	Avery [30]	Great Britain
Echinuria uncinata	Acuariidae	Wood [31]	Great Britain

Although Lapage [32] and McDonald [29] mentioned some ectoparasites, two tapeworm species in addition to *Fimbria fasciolaris* and *Polymorphus minutus* were found on and in Egyptian geese. Detailed descriptions are unfortunately missing.

1.2. Antropozoonotic Investiagations

The Federal Ministry for Nature Conservation in Germany assesses the invasiveness of all alien species and thus classifies its potential threat to native biodiversity and to public health concerns (i.e., anthropozoonotic risks originating from the alien). These assessments provide the basis for further action plans and long-term management. Parasitological investigations on anthropozoonotic risks originating from Egyptian goose are missing in the literature. For these reasons, we also test for potential unicellular endoparasites with an anthropozoonotic risk in Egyptian geese. Cryptosporidiosis is a globally occurring disease, caused by parasitic protozoa. Some of the subspecies pose an anthropozoonotic risk. The gastrointestinal disease is particularly dangerous for immunosuppressed persons and small children [33]. The oocysts are characterised by high tenacity in the environment [34]. Proof of *Cryptosporidium* spp. (*incertae sedis*) in geese and ducks is described, but species identification is rare. There are species of specific *Cryptosporidium* spp. that are not zoonotically relevant in waterfowl [35,36]. The unharmed passage of infectious zoonotic oocysts through the intestine of Canada geese has been demonstrated experimentally. This raises questions of the risk of spreading infectious oocysts through the intestinal passage of waterfowl by itself as mechanical vectors [35,37].

2. Materials and Methods

2.1. Sample Collection and Habitat Research

In total, 148 scat samples of *A. aegyptiaca* were collected by non-invasive techniques from February to October 2020. The sampling period therefore includes the breeding period in Germany. This is described as highly variable and was observed as such in our own field work. The main breeding season in Germany is from April to June [9]. Preferred habitats of waterfowl, which include open grasslands with high trees and freshwater bodies in urban and rural regions all over Germany, were surveyed for Egyptian geese. This survey comprised a total of 41 trips by foot and resulted in 241 h of sampling effort. Overall, 179 animals were observed in their natural habitat at eight different German Federal states and two cantons of Luxembourg (Figure 2).

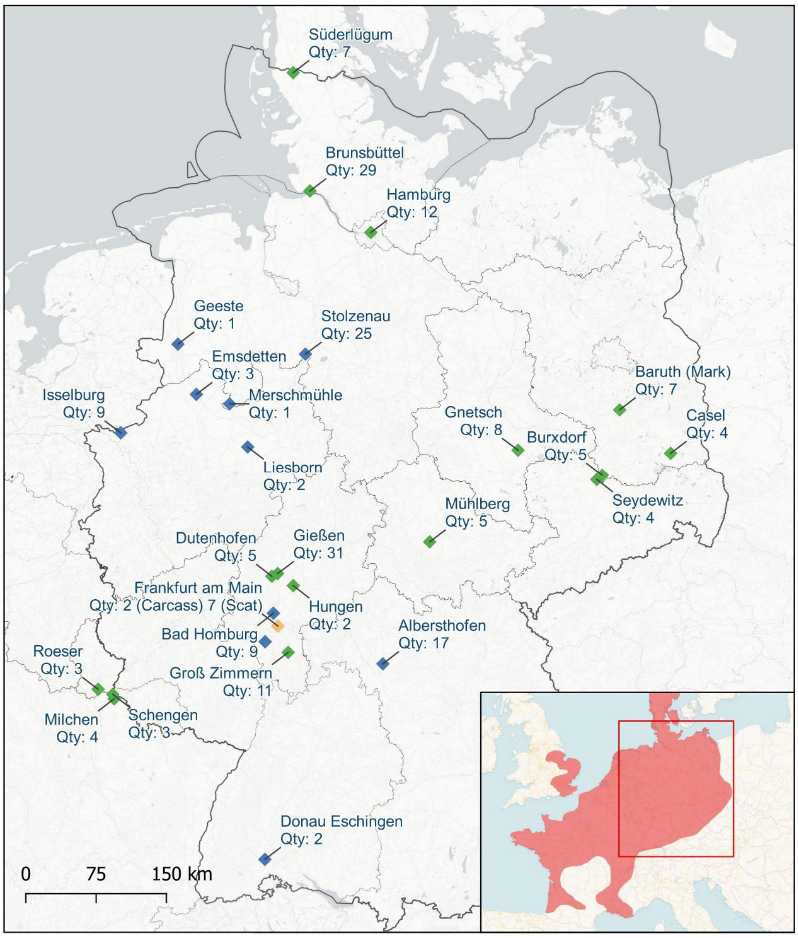


Figure 2. The map shows the origin of sampled Egyptian geese *Alopochen aegyptiaca* and the number of examined samples (scat samples marked in green, origin of carcasses marked in blue, places that offered both are marked in yellow) in Germany and Luxembourg. Both countries are within the non-native distribution area (marked red) of the non-native bird in Europe.

Especially in autumn, Egyptian geese form larger flocks with up to 70 animals. These and other groups (i.e., parents with ducklings or associations of sub-adults) with more individuals were regarded as a cluster. In these clusters, not every individual was sampled but a representative number of samples were taken. To determine the representative number of samples in the specific flock, the sample size was determined following the infinite populations correction [38]. This formula determines the number of samples needed to detect a specific pathogen in a flock.

All faecal samples were picked up immediately, whenever defecation was observed, to make sure that the sample could be allocated to an individual animal. After collection, the samples were fixed in 70% ethanol and stored until further investigation.

Furthermore, in the current study, 114 Egyptian geese carcasses, obtained from five different Federal states of Germany, were examined by necropsy. These animals were shot by hunters during the authorised hunting seasons of the respective Federal state. This was to control the *A. aegyptiaca* population or to prevent bird strikes on planes, as requested by the airport authorities, and not for research purposes or for this study. In cities, the animals were mainly shot in public swimming pools, health resorts, public lakes, and ponds. Forty-nine geese were shot in various urban habitats (parks, public pools, and airport) in Darmstadt (Hesse), Frankfurt (Main, Hesse), and Bad Homburg (Hesse) (49/114; 43. 0%). One bird was found dead, killed by a car accident in Frankfurt (Main). All other examined carcasses were obtained from rural habitats. They were mainly shot in the winter months during the hunting season on crop fields in Bavaria, Lower Saxony, and North Rhine-Westphalia (Figure 2).

The samples were sent either frozen to the Institute of Parasitology, Justus Liebig University, Giessen, or else picked up fresh from the hunters.

Using the combination of invasive and non-invasive sampling, Egyptian geese in eleven Federal states of Germany and two Cantons of Luxembourg were sampled.

2.2. Macroscopic Analyses

Pathological Analyses

Carcasses (97/114; 82.9%) and digestive tracts (17/114; 14.9%) of *A. aegyptiaca*, provided by hunters, were pathologically examined. The examination was based on the *Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds* [39]. Firstly, the bodyweights of the animals were measured. The necropsy began with an inspection of the plumage for the infestation of ectoparasites. The eyes were examined with the help of tweezers to look behind the nictitating membrane to search for *Philophthalmus* spp. (Plagiorchiida) or *Oxyspirura mansoni* (Spirurida). The beaks of the birds were sawn open at a line between the nostrils to detected any inflammation on the basis of an infection with *Trichobilharzia* spp. (Strigeatida). The pectoral muscles were sliced into 0.5 cm thick disks to detect the cyst stages of *Sarcocystis* spp. (Eimeriida) described in waterfowl by Wobeser [40]. The trachea was cut open lengthwise to detect the potential occurrence of *Cyathostoma bronchialis* (Strongylida).

The complete digestive tract was extracted and cut open lengthwise. The inner keratinoid layer of the gizzard was removed to detect *Amidostomum anseris* (Strongylida) infection. The ingesta was sieved by 150 µm and examined with a binocular microscope. If the intestinal mucosa showed petechial bleedings or redness, intestinal scrapings were taken and examined for Eimeriidae. The species of the adult parasites were determined with the three different keys to parasites in waterfowl from Malcom E. McDonald [41–43], the host-parasite catalogue of the helminths of ducks [44], the identification key to *Porroceacum* spp. [45], and an identification key for cestodes [46].

Liver, spleen, kidneys, lungs, and reproductive organs were extracted and cut longitudinally.

Macroscopic modifications passed a histological examination, in particular modifications of the kidneys to detect renal coccidiosis caused by *Eimeria truncate* (Eimeriida), described in waterfowl by Ballweber [47].

2.3. Microscopic Analyses

The 148 scat samples were prepared by the standard sodium acetate acetic formalin SAF technique, modified with ethyl acetate, and examined microscopically [48]. Eggs of trematodes, nematodes, and protozoan oocysts were identified with the help of morphological descriptions [49–53].

Additionally, a sample of the ingesta of each animal in necropsy (n = 114) was examined using the SAF technique. Moreover, 94 of 114 samples were analysed for *Cryptosporidium* spp. oocysts by performing carbol-fuchsin-stained faecal smears according to Heine (1982) [54].

Scanning Electron Microscopy (SEM) Analysis

Two adult *Polymorphus minutus* (Polymorphida) specimens and one adult specimen of *Echinostoma revolutum* (Echinostomida) were recovered from an infected *A. aegyptiaca*, obtained from a crop field near Albertshofen (Bavaria) and were transferred onto 10 mm glass coverslips (Thermo Fischer Scientific, Schwerte, Germany). These specimens were chosen for their clearly identifiable and well-preserved external features. The glass coverslips were pre-coated with 0.01% poly-L-lysine (Merck, Darmstadt, Germany) for 10 min. at room temperature (RT). Subsequently, the acantocephalans and the trematode were fixed in 2.5% glutaraldehyde (Merck, Darmstadt, Germany), post-fixed in 1% osmium tetroxide (Merck, Darmstadt, Germany), and washed in distilled water before dehydration and critical point drying with CO_2. Lastly, the specimens were gold labelled by sputtering and viewed on a Philips XL30 scanning electron microscope at the Institute of Anatomy and Cell Biology, Justus Liebig University Giessen, Germany.

2.4. Molecular Analyses

2.4.1. Quantitative PCR on Cryptosporidium spp.

To confirm the identification of *Cryptosporidium* spp. and determine its genotype to evaluate the anthropozoonotic risk, a real time PCR was performed. Thus, different DNA-extraction methods described for *Cryptosporidium* spp. were tested for Egyptian geese droppings inter alia the Qiagen stool kit, semi-purification of oocysts, and a water-ether concentration of oocysts [55–58]. The best method was used to test the 94 samples using the commercial real-time *Cryptosporidium* spp. kit "VIASURE Cryptosporidium Real Time PCR Detection Kit" (CerTest, Biotec, Spain) with an internal inhibition control.

The DNA-Extraction was performed as followed: a swab saturated with sample material was washed in 1× phosphate buffered saline solution. After vortexing and being centrifuged at 184× g and discarding the supernatant, the pellet was re-suspended with 400 µL 0.1% Diethyldicarbonat water. The tubes were incubated in 100 °C for 20 min, and then at −20 °C for 10 min. The supernatant was used for the PCR-protocol after vortexing and centrifuging the tubes again.

2.4.2. Coproantigen Analyses

Furthermore, the 94 faecal samples were examined with coproantigen-ELISAs on *Cryptosporidium* spp. (ProSpecTTM, Thermo ScientificTM, Schwerte, Germany), according to the manufacturer's instructions.

2.4.3. PCR and Sequencing of Echinostoma spp.

Morphological identification on a species level is described as difficult in the so-called *Echinostoma* '*revolutum*' group [59]. Therefore, a molecular level species' identification was performed. A segment of the mitochondrial cytochrome c oxidase subunit 1 gene (*cox-1*) and the mitochondrial NADH-ubiquinone oxidoreductase chain 1 gene (*ND1*) were amplified and sequenced to determine the species of the adult trematodes from the *Echinostoma-revolutum*-group.

DNA was extracted from three specimens, obtained from an infected bird, originating from Darmstadt, Hesse. DNA extraction was performed using the DNeasy Blood and Tissue kit (Qiagen), according to the manufacturer's instructions. The used primers were described by Morgan and Blair [59]. A PCR was performed to generate the segment of the ND1 gene, using the primers JB11 (5′AGATTCGTAAGGGGCCTAATA3′) and reverse JB12 (5′ACCACTAACTAATTCACTTTC3′). A second PCR was performed for the generation of a segment of the CO1-gene using the forward primer JB3 (5′TTTTTTGGGCATCCTGAGGTTTAT3′) and reverse JB13 (5′TCATGAAAACACCTTAATACC3′).

Both PCRs were performed in 45 µL reaction volume. They included 5 µL of the isolated template DNA, 5 µL 10x PCR buffer S (Peqlab, VWR, International GmbH, Erlangen, Germany), 1 µL of each primer, 1 µL dNTPs, and 1 µL 5U/µL taq Polymerase (Peqlab, VWR, International GmbH, Erlangen, Germany). The PCR protocol for the ND1 gene started with an initial denaturation for 5 min. at 94 °C followed by 35 cycles at 94 °C for 30 s., annealing at 48 °C for 45 s. and extension for 45 s. at 72 °C, with the final extension for 5 min. in 72 °C. The PCR-protocol for the amplification of the cox-1 gene started with the same initial denaturation, but it was followed by 35 cycles at 94 °C for 20 s., annealing at 48 °C for 30 s. and extension for 30 s. in 72 °C. The cycles were also followed by a final extension for 5 mins in 72 °C.

The resulting PCR product was finally visualised. Therefore, a gel-electrophoresis on 1,5% agarose gel with Midori Green was conducted. The final extraction was performed by using the HiYield GelPCR DNA Extraction kit. Sanger Sequencing was performed by LGC genomics, Berlin.

The obtained sequence was deposited to the NCBI database, with the number OQ466599.

2.4.4. Phylogenetic Analyses

Highly matching sequences of Echinostomatidea were obtained from BLAST searches in the GenBank database (GenBank release 245, 08/2021). Since Morgan and Blair showed that the ND1-gene is the most rapidly diverging gene and thus the most selective region for species diagnosis in a 37-collar-spine group of *Echinostoma* [59], these sequencing results were further investigated. Alignment was performed with the sequence alignment program MegAlignPro, with the ClusualW method and phylogenetic trees constructed using a Maximum Likelihood algorithm with a 1000 bootstrap replication [60].

3. Results

3.1. Observation of the Egyptian Geese in Germany

The animals were found in two principal habitats in Germany. The first were the urban areas, predominantly in parklands with high trees and freshwater bodies. In rural areas, they were found on freshly growing young fields and at a greater distance from water bodies. Most of the birds were found paired. This observation is confirmed by the Dachverband Deutscher Avifaunisten Database [8].

The origin of the scat samples and the shot birds, which were examined in necropsy, are visualized in the map (Figure 2). The distribution of sampling in Germany is influenced by the west-east gradient in the population density of Egyptian geese in Central Europe [8,9]. Overall, 44% of the shot Egyptian geese were from urban areas (50/114), so there is an approximately equal distribution of the examined carcasses between two comparable groups—urban and periurban living animals.

The synanthropic birds were sharing resting and feeding sites with mallards, mute swans (*Cygnus olor*), Canada geese (*Branta canadensis*), and sometimes greylag geese (*Anser anser*) (own studies). In rural habitats, as well, Egyptian geese were often found together with greylag geese flocks.

3.2. Body Weights

The average weight of the male birds examined in the postmortem was 2.6 kg ($\sigma = 0.5$) and the female birds 2. 1 kg ($\sigma = 0.5$). Of all ($n = 114$) the examined birds, 98 were complete (46 female and 39 male) to be able to include them in the weight statistics. The graphical distribution tests, such as the histogram and a quantile-quantile diagram as well as the analytical Kolmogorov–Smirnov test, indicated a normal distribution of the values. The 34 geese from urban habitats in Hessia weighed on average 2.4 kg (σ = 0.4). Rural, shot Egyptian geese had an average weight of 2.3 kg (σ = 0.5), which was 50 g less compared to the bodyweight of birds originating from urban habitats. A comparison of the two groups with the *F*-test showed no significant differences between the weights of the urban and peri-urban animals (F (62, 34) = 1.30, n = 98).

Parasite species-oriented comparisons of infected and non-infected animals showed a noticeable difference in only one species. Birds from urban habitats, infected with *Hystrichis tricolor* (Enoplida) (12/114), weighed on average 2.3 kg (σ = 0.5). Only 16.7% of the birds with hystrichiosis (2/12) carried another detectable species of endoparasite in their gastro-intestinal tract. These were low-grade infections with *Echinostoma revolutum* and *Polymorphus* spp. Compared to the examined birds of the same habitat, which showed no signs of *H. tricolor* infections, the infected Egyptian geese had on average 149 g (6.1%) less bodyweight. A statistical comparison of the two groups with the *F*-test showed no significant differences between the weights of the infected and non-infected animals (F (22, 11) = 1.47, n = 35). Thus, the number of infected carcasses was not big enough to provide statistically reliable results.

3.3. Macroscopic Findings

46% of the examined Egyptian geese in necropsy were males (52/114) and 54% were females (62/114).

In total, from the identification of eggs in scat samples and the identification of adult specimens in necropsy, 13 different species of metazoan endoparasites were detected (Table 2). Urban Egyptian geese were infected by 5 different species of endoparasites, and 11 different species were found in living rural animals. In total, 42.0% of birds shot in urban habitats (21/50) harboured a minimum of one adult endoparasite. In contrast, only 20.3% of the animals from rural habitats (13/64) were infected with at least one parasite. A statistical comparison of these two groups with a χ 2 -Test showed a significant difference c^2 (1, n = 114) = 6.71, p = 0.001. The differing species composition of the endoparasites obtained from birds of urban and rural habitats is visualized in Figure 3. A significantly more frequent occurrence of nematodes in urban areas was also proven by a chi-square test (c^2 (1, n = 114) = 7.26, p = 0.007). The most frequently found parasite species in total were adult stages of *Hystrichis tricolor* (10.5%; 12/114), *E. revolutum* (7.9%; 9/114)), and *Polymorphus minutus* (7.9%; 9/114). The total worm burden varied between one and seven parasites per bird for *P. minutus* and one and six for *E. revolutum*. In the case of *E. revolutum*, single specimens of the trematode in the host was found most of the time. Nematodes were by far the most detected phylum in the carcasses (14.0%; 16/114) followed by Trematodes (8.8%; 10/114) and Acantocephalans (7.9%; 9/114). Only 3.5% (4/114) birds were infected with more than two endoparasitic species when shot. Only in a single Egyptian goose could three different parasite species have been identified in the gastrointestinal tract.

Table 2. Calculated prevalence of metazoan endoparasites in Egyptian geese (*Alopochen aegyptiaca*) generated by postmortem examinations and examination of collected scat samples.

Species	Prevalence of Adult Parasites in Necropsy (n = 114)	Prevalence of Eggs in Collected Faeces (n = 148)
Nematodes		
Hystrichis tricolor	10.5%	1.4%
Echinuria uncinata	2.6%	0.7%
Porrocaecum spp.	0.9%	0.7%
Ascardia galli	1.8%	0.7%
Capillaria spp.		2.0%
Hetrakis dispar		1.4%
Syngamus trachae		0.7%
Amidostomum anseris		0.7%

Table 2. *Cont.*

Species	Prevalence of Adult Parasites in Necropsy (*n* = 114)	Prevalence of Eggs in Collected Faeces (*n* = 148)
unknown nematodes		1.4%
Tapeworms		
Cloacoteania spp.	2.6%	-
Acantocephalans		
Polymorphus minutus	7.9%	-
Trematodes		
Echinostoma spp.		6.8%
Echinostoma revolutum	7.9%	
Echinostoma grandis	0.9%	
Notocotylidae		0.7%
unknown trematodes		1.4%

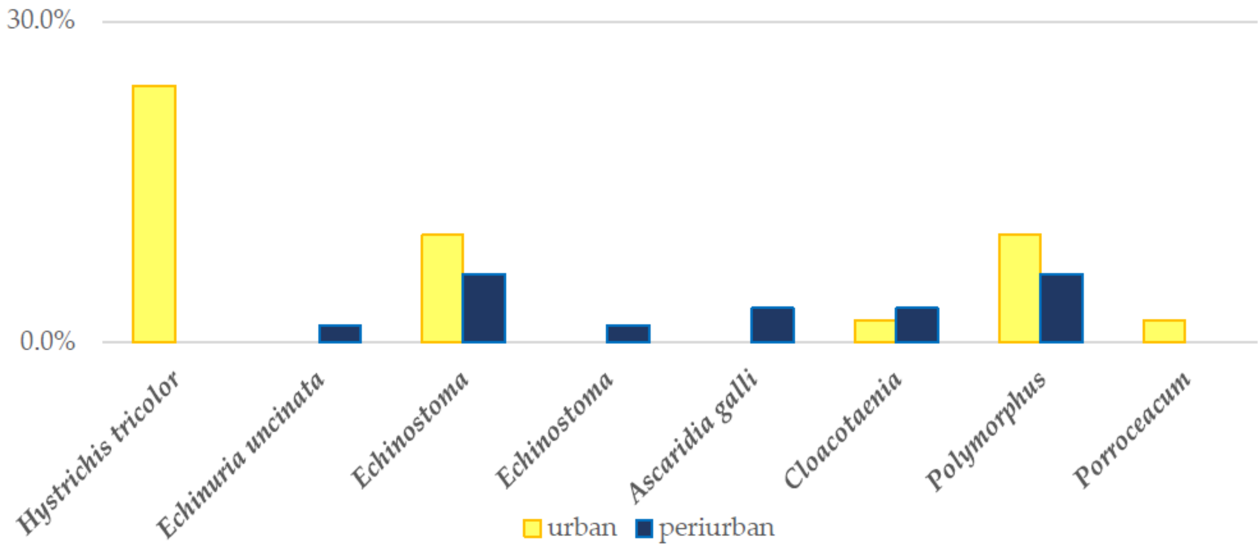


Figure 3. Adult metazoan endoparasite species detected in Egyptian geese (*Alopochen aegyptiaca*) in carcasses from urban and peri-urban habitats. This figure represents both an extract of the composition of parasite species and their abundance (urban Egyptian geese were infected with five different species of endoparasites and eleven different species were identified in rural animals) in urban and peri-urban areas.

By relating positive samples to their origin, we identified one cluster of hystrichiosis in the metropolitan area of Frankfurt Main (including Frankfurt, Bad Homburg, and Darmstadt) with a local prevalence of 24.5% (12/49).

One female was in recognisably poorer health externally and had 10.4% less body weight on average than other sampled female birds. In the necropsy, apart from a high-grade infection of *Ascaridia galli* (Ascaridida) and a low-grade infection of *Cloacotaenia* spp. (Cyclophyllida), no abnormalities were documented.

Nevertheless, local accumulations of parasite occurrences could be recognised in the necropsy results—-for example, in the local occurrence of *H. tricolor* in the metropolitan area of Frankfurt am Main (Hesse) or in the only identifications of *E. uncinata* (Spirurida) near Stolzenau (Lower Saxony).

3.4. Microscopic Findings

3.4.1. Coprological Findings in Ingesta

Although it was not always possible to detect adults and eggs in the same host individually, it was possible to prove for the following species that they not only infect these avian alien species but also cause patent infections in them (patent infection of a host = the parasite multiplies in the host and secretes an infectious form (i.e., eggs or oocysts)). These species are: *H. tricolor*, *E. uncinata*, *Porrocaecum* spp., *A. galli*, and *Echinostoma* spp. (Figures 4 and 5).

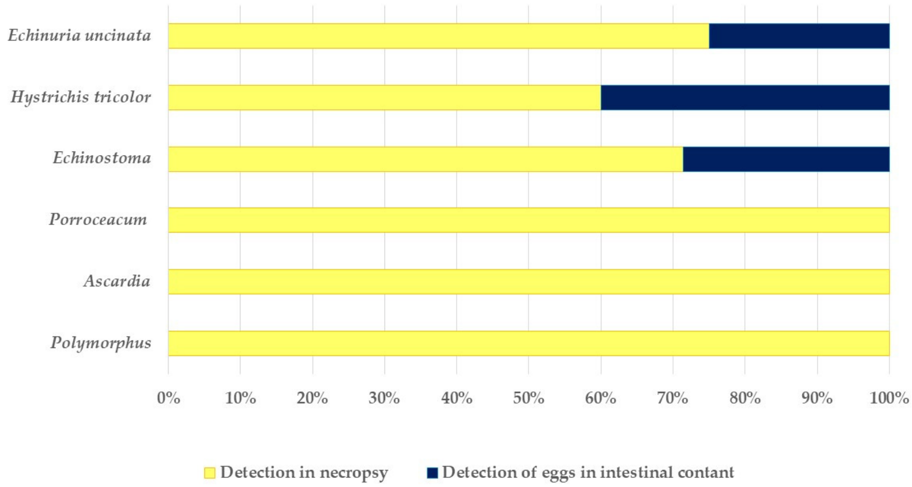


Figure 4. Types of verification of different endoparasitic parasites in carcasses of *Alopochen aegyptiaca* adults in the intestine vs. excreted eggs in the intestine in the same animals. Depending on the parasite species, a different patency can be recognised in this alien host. Patent infections clearly show that the parasite can not only infect this alien bird but also reproduce in it.

Oocysts of *Eimeria* spp. were found in 2.4% (2/114) of the samples of ingesta, but these findings were never related with petechial bleeding or thickening of the intestinal wall or other indications of inflammation.

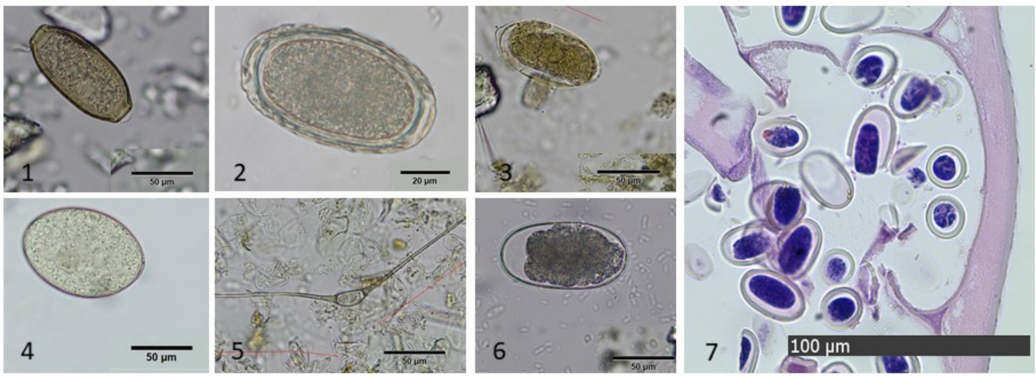


Figure 5. Detected eggs of nematodes and trematodes of Egyptian geese (*Alopochen aegyptiaca*) in Germany and Luxemburg by using the SAF technique: (**1**) *Capillaria bursata*, (**2**) *Hystrichis tricolor*, (**3**) *Syngamus trachea*, (**4**) *Echinostoma* spp., (**5**) *Notocotylus* spp., (**6**) *Amidostomum anseris*, and (**7**) *Echinuria uncinata* in the wall of a proventriculus of an Egyptian goose, HE-stained.

3.4.2. Coprological Findings in Scat Samples

The faecal samples were collected almost equally from urban and peri-urban areas. 41.2% (61/148) of them were from birds in urban areas, and 58.8% (87/148) were collected in rural habitats occupied by Egyptian geese. Eight different nematode eggs were identified (Table 2).

Although nematode eggs were detected almost twice as often in urban samples than in rural areas, this observation could not be confirmed statistically.

Trematodes (especially *Echinostoma* spp. and Notocotylidae (Plagiorchiida)) (Figure 5(4,5)) are noticeable and found in locally increased clusters. For example, of a family stock with eight birds sampled in Süderlügum at the border to Denmark (Schleswig-Holstein), seven excreted either eggs of *Echinostoma* spp. or Notocotylidae or both. A comparison of the frequency of detection of at least one trematode between the sampled animals in Süderlügum and other animals in Schleswig-Holstein (e.g., Brunsbüttel) showed significant differences with the Fisher's exact test (c^2 (1) = 15.61, n = 29, p < 0.001).

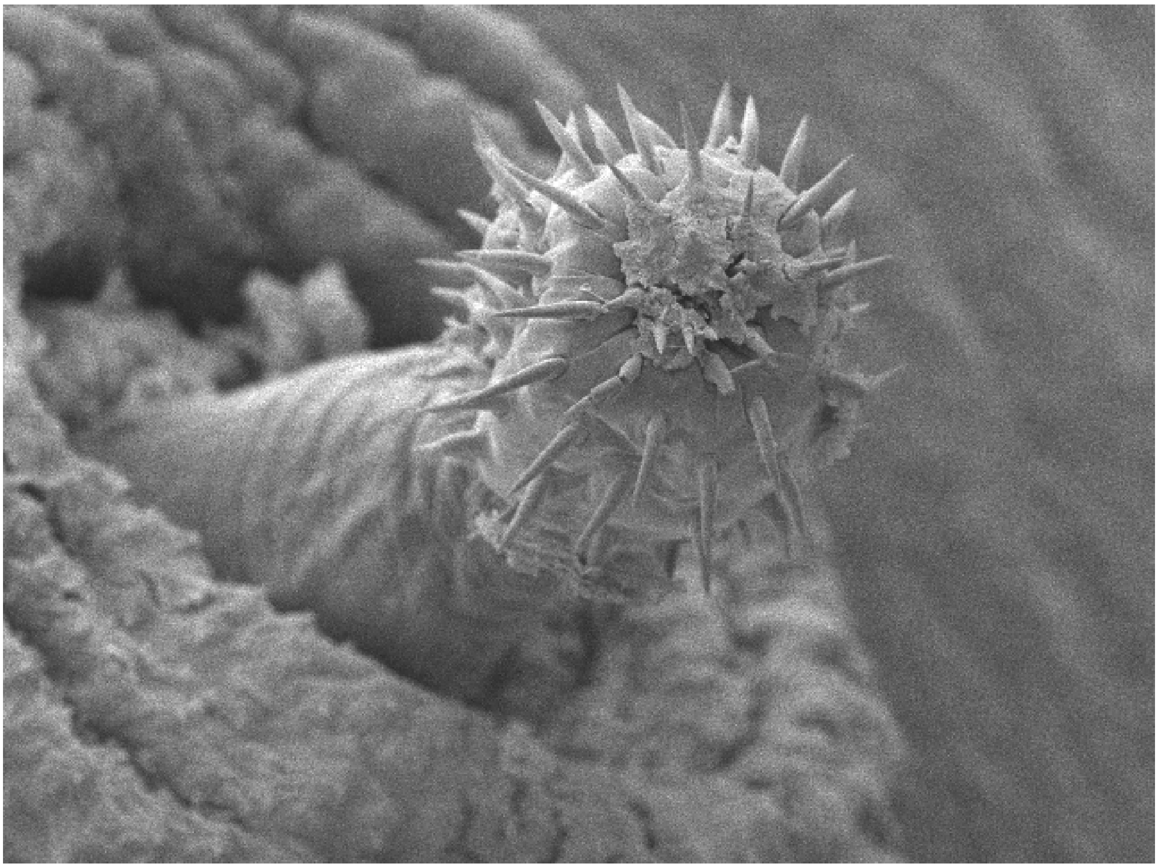
1.4% (2/148) of all collected and examined scat-samples were positive on the nematode *H. tricolor* (Figure 5(2)).

Different genera of Eimeriidae were seen. These were morphologically assigned to inter alia *Tyzzeria perniciosa*, *Tyzzeria parvula*, and different *Eimeria* spp. with descriptions from Gajadhar et al. [61] and Berto et al. [62]. The frequency per sample varied between one and twelve oocysts per sample, but most of the samples contained only one single oocyst.

A total of 8.8% of the stained and microscopically examined faecal samples from Egyptian geese contained single oocysts of *Cryptosporidium* spp. (9/94).

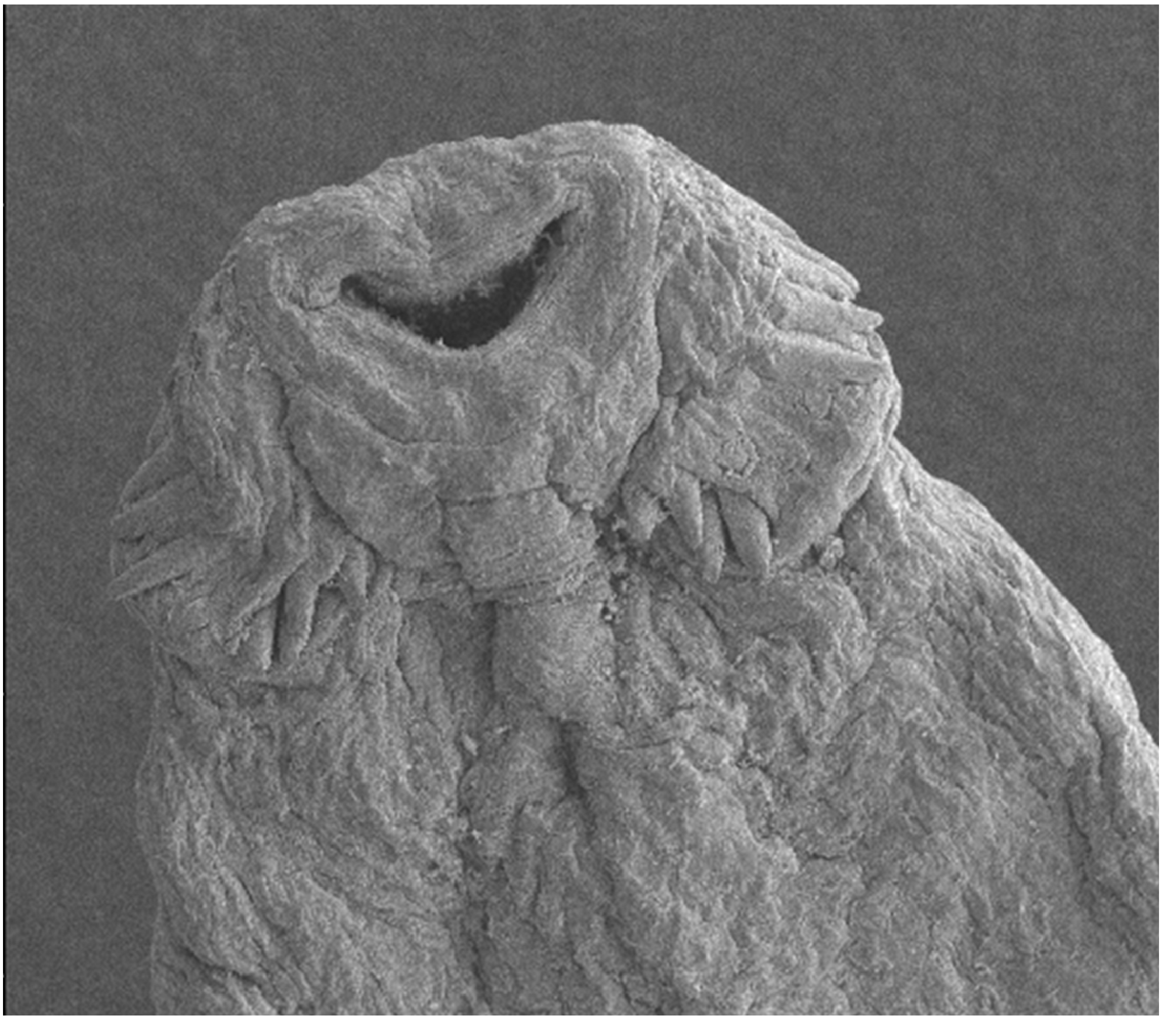
3.4.3. SEM Findings

The external specifications of the parasite highlighted by the electron micrographs could unfortunately not contribute to an accurate species diagnosis in the case of the 37-collar-spine-group of *Echinostoma* (Figure 6). Therefore, a species diagnosis on the molecular level followed.



(1)

Figure 6. *Cont.*



(2)

Figure 6. Electron micrographs of (**1**) the mouth opening and collar spines of *Echinostoma revolutum*, and (**2**) of the ovoid proboscis of *Polymorphus minutus*.

3.5. Molecular Findings

3.5.1. *Cryptosporidium* spp.

The findings of *Cryptosporidium* spp. in microscopy could neither be confirmed in ELISA nor in a real-time PCR. PCR and karbol-fuchsin stained smears are described as being comparable in sensitivity [58,63]. Quantitative PCR is described as somewhat more sensitive, and the manufacturer of the "VIASURE *Cryptosporidium* Real Time PCR Detection Kit" states the detection limit of ≥100 DNA copies per reaction.

3.5.2. Sequencing of E. revolutum

A molecular species diagnosis of *Echinostoma* spp. was successful. The sequencing of the cox-1 gene and the nd-1 gene provided the unambiguous species diagnosis *E. revolutum* (Figure 7. This parasite is distributed worldwide, has a three-host life cycle, and a low host specificity. Intermediate hosts are fresh water snails, particularly *Lymeneae* spp. Adult stages also infect the small intestine of humans and are able to cause serious intestinal echinostomiasis [59,64,65].

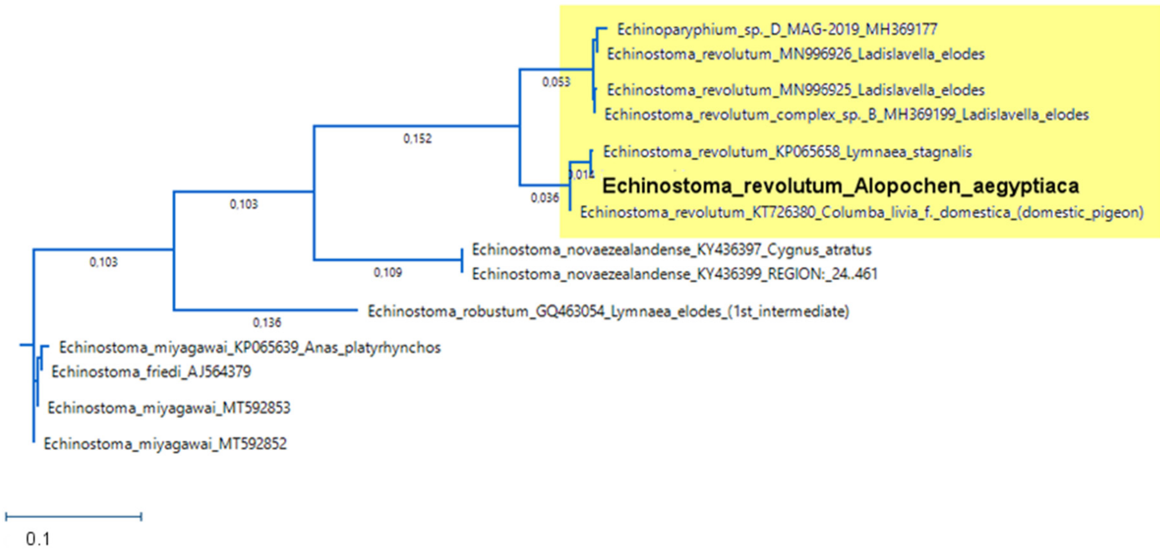


Figure 7. Species diagnosis of the specimens of Echinostoma revolutum obtained from Egyptian geese (Alopochen aegyptiaca), based on the 475-nt fragment of mitochondrial nad1 gene. The species *Echinostoma revolutum* is highlighted by a yellow box. The GenBank number of the obtained sequence is OQ466599. The highest similarity in the generated sequence is to a European cecarial isolate of *Echinostoma revolutum* (*sensu stricto*) from a freshwater snail (*Lymnaea stagnalis*) in Hamru (Czech Republic). Isolated by Georgieva et al. [66]. (ClustualW-Alignment, Maximum Likelihood tree).

4. Discussion

Postmortem examinations of numerous Egyptian geese from very different parts of Germany provide very accurate data on local as well as on the total parasite prevalence in this alien population. Examination of the ingesta of these animals for parasite eggs provide indications of the probability with which an actual infection of a certain species may also be detected in faecal samples. In this study, for the first time, numerous indigenous European endoparasite species were detected in Egyptian geese. Furthermore, it was also demonstrated that an additional ecological factor is that this invasive alien bird species is firmly established in Central Europe and can successfully cope with indigenous pathogens.

Consideration of host-parasite interaction at the population level starts with determining the prevalence of the parasite in the host population. A conclusion about the distribution of the endoparasites in the host population, for example, is only possible if the animals examined are randomly culled. Hudson showed in 1968 that parasitological investigations of deceased bird carcasses or birds killed by predator attacks suggest a higher parasite prevalence than the examination of shot animals [67]. The reason for this is mainly that the fitness of pathogenic parasites is only guaranteed if they only aggregate in a few host individuals [68]. These weakened individuals are selected by natural causes. The more virulent parasites are forming stable equilibriums with the host population through accumulation in less individuals than less virulent parasites. Through this, they also influence the development of the host population, because these infected individuals have a shorter lifespan and/or have less offspring [68,69].

In order to be able to classify the generated prevalence, a comparison with native waterfowl species is necessary. For this purpose, studies on greylag geese and mallards were referenced, because, as described above, both species are often found in coexistence with Egyptian geese. Intermediate stages of heteroxenic parasites might be transmitted when feeding together and sharing the same areas to rest, especially when feeding Egyptian geese spend more time on land then mallards, and, in this regard, they are more similar to Greylag geese [21], although phylogenetically and therefore also physiologically, this shelgoose is more related to Anatidae than to Anserinae [70]. For this reason, the main focus is on the mallard for the comparison of parasite prevalence in native waterfowl.

A postmortem study of 60 mallards in Austria in 2020 by Jirsa et al. presents numerous parasite species, such as *Polymorphus* spp., *Porroceacum* spp., *E. revolutum*, and *E. grandis*, which were also found in the present study in Egyptian geese [71]. They found in total eleven different species of metazoan endoparasites. Compared to the results of the necropsies in the current study, they detected nearly the same number of different species in half of the examined animals. Three species were found in more than 20% of the mallards (*Notocotylus attenuatus* 23.3%; 14/60, *P. minutus* 30. 0%; 18/60, *Diorchis* spp. 31.7%; 19/60). The highest determined parasitic prevalence in Egyptian geese carcasses was 10.5% (12/114).

The Shannon–Wiener index was used to compare the parasitic diversity in the different hosts. This index provides information about the diversity of a biotic community. This was determined for the gastrointestinal parasites of the Egyptian geese as well as in the study from Jirsa et al. Since the detection of parasite eggs in a faecal sample does not indicate how many adult stages of the parasite species are in the bird's intestine, each positive faecal sample was counted as "one adult stage", regardless of how many eggs it contained. According to this metric, there is a slightly higher diversity (1.89) than determined for endoparasites in mallards (1.6) in Austria by Jirsa et al., but a near high distribution (or evenness) of the parasite species (0.73 in mallards and 0.76 in Egyptian geese). However, it should be noted that the current study involved a much larger number of examined samples of Egyptian geese (i.e., 114 carcasses and 179 non-invasive sampled birds). With a higher number of samples examined, statistically more parasite species are found. In addition, if the birds originate from many different habitats, more potential intermediate hosts' habitats are examined. Thus, the comparability of these indices might have been distorted.

Another study in Central Europe on mallards (2018) examined 100 shot birds from several locations in Germany for endoparasites. Almost two-thirds of the endoparasites detected were cestodes, while a nematode prevalence of 1.9% (2/100) was determined. In total, they identified 19 different endoparasite species in fewer examined animals than in the current study [72]. Both studies do not describe any findings of *H. tricolor* or *E. uncinata*.

In contrast, Kavetska et al. report 51 different Helminths in mallards (n = 178) in Poland, and also mention findings of *H. tricolor* and *E. uncinata* [73].

Surveys on 175 greylag geese over five years in Rheinland-Pfalz (n = 175) showed that about one-third of the birds were infected with *Amidostomum anseris*. Tapeworms and *Ascaridia* spp. were detected on a much smaller scale [74]. An egg of *Amidostomum anseris* was detected in only one scat sample of Egyptian geese in the present study. The parasite was not found in any postmortem examination. The positive scat sample was obtained from a pair of Egyptian geese in the company of greylag geese, and it cannot be excluded that this egg is an intestinal passant. Nevertheless, this important point should not be neglected, and it is discussed below.

A comparison of the parasites found in Egyptian geese showed that these parasite species were also detected in native mallards, but a direct comparison of species-specific prevalence turned out to be difficult. Even studies within Europe on the same waterfowl species come to very different results via consideration of the parasitic prevalence. The reason for this is discussed, and explanatory approaches hint at the differently structured ecosystems in which the birds live. Species-rich areas in Poland, which are less influenced by humans or agricultural activities, could harbour a higher diversity of intermediate hosts [71], and thus make a successful transmission from an infected definitive host to the next much easier.

Even if comparisons between the different studies, as mentioned above, are difficult, one fact is clear from all comparisons. Each of the studies listed discovered higher overall parasite prevalence in native waterfowl species. This observation is particularly impressive as the mallard is also a generalist species, benefiting from anthropogenic influences throughout the Northern Hemisphere. In the Southern Hemisphere, it is even an invasive species in many areas [75].

These parasitological observations allow conclusions to be drawn regarding the alien host. In total, there is always a lower parasitic load in *A. aegyptiaca* than in the described native waterfowl. The role of the invader's immune system by successful establishment in new biotic communities is increasingly discussed [76–78]. Invaders must be able to handle a range of pathogens that are new to them either as well as or else better than native species in order to establish themselves successfully in new areas. Repeated emphasis is placed here on the energetic costs of immune defence. Successful avian invaders appear to have stronger T-cell-mediated immunity and are thus more able to cope with exotic pathogens and parasites [2]. Thus, they are multiplying the indigenous pathogen and outcompete native bird populations. In this way, inter alia their fitness is more affected by parasite infestation [2]. It has been observed, for example, that native parasites impact native species more than exotic species in a comparable niche [78]. The results presented in this study, moreover, support such a hypothesis. For example, greylag geese in Western Germany seem to be infected by *Amidostomum anseris* quite often [74]. Although Egyptian geese live in similar habitats as Greylag geese, sometimes even sharing feeding and resting sites in the immediate proximity, only one infection has been detected in Egyptian geese. It is even possible that the egg detected is only an intestinal passant and *A. anseris* is not able to infect *A. aegyptiaca*.

We do not know whether Egyptian geese take up fewer intermediate parasite stages due to their feeding behaviour or whether their immune system is better able to resist endoparasites.

There are similar observations with other alien vertebrates and invertebrates. The parasite load is not only lower in aliens compared to native animals, but also compared to their conspecifics and their native ranges [13]. Torchin et al. interpret the lower parasite load as an undisputed advantage that allows the aliens to establish and disperse in new habitats [14].

Torchin et al. also observed in several alien species that individuals in their non-indigenous areas more often attain higher bodyweights and larger body sizes than their conspecifics in the native areas [13,79]. The current study was also able to confirm these observations.

The measured bodyweights of the birds were on average much higher than the described weights of the European and African Egyptian geese populations in the literature [3,7,9,80]. The weight of Egyptian geese fluctuates over the year. During the molting period, for example, the birds potentially lose about 19% of their body mass [81]. The examined birds were shot over the period of one year and most of them outside the molting period, though, so this potential distortion can be excluded.

In order to obtain an overview of the potential infection pressure for other animals (birds and mammals) in the surrounding Egyptian geese, consistent attempts were made to detect the patency of the adult parasites. Rates of egg production can not only vary on the age of the parasite (pre- and post-patency) but are also species specific on the individual host-parasite interaction [82].

A sample from the intestinal content of each dissected animal was examined for parasite eggs. This not only provides information on whether the adult species detected is actually competent to infect an Egyptian goose patent, but also provides guidance on the evaluation of the faecal samples collected.

Some of the species detected, such as *Cloacotaenia* sp. or *Polymorphus* sp., are described as very rarely detected in scat samples [83]. Certain reasons may cause this: one species, for example, *Polymorphus minutus*, is only present in the gastrointestinal tract of Anseriformes for a relatively short time (approx. 1.7 months). Prepatency and patency have almost the same length. This results in almost all eggs being excreted in a very short phase. Detection of *P. minutus* eggs under field conditions is described as "virtually impossible" [83]. In contrast, infections with *Echinostoma* spp. seem to be patent over a longer time, because the detected prevalence in scat samples and in necropsy are on the same scale.

An advantage of the combination of necropsies and the examination of ingesta for parasite eggs supplemented by large-scale collected faecal samples is that it can be shown with certainty for some parasite species, such as *Echinostoma* spp., *Porrocaecum* spp., *A. galli*, and *E. uncinate*, that these species are not only present in the gastrointestinal tract of Egyptian geese but are also capable of reproduction and excrete eggs in this species. This is important proof of the parasite´s potent infections in Egyptian geese, which also means that they are able to infect their conspecifics and other waterfowl species or mammals via intermediate hosts.

In attempting to detect parasite eggs in non-invasively collected specimens or in samples of the ingesta, the amount of excreted faeces in waterfowl should also be considered. As predominantly herbivorous and relatively small birds, they are dependent on high-quality food in comparatively higher quantities than carnivorous or fructivorous birds [22]. This also means larger amounts of faeces, in which parasite eggs are diluted and found relatively less often.

Despite the proven patents of Egyptian goose infections, the risk of native waterfowl infections seem to vary. In our own field observations, Egyptian geese were found to live in two different habitats in Germany, showing the adaptability of this alien. Urban animals are living in parks and swimming pools, often in the company of mallards and mute swans. These animals probably have a low flight distance (Figure 1(3)) and, on the basis of their urban circumstances, smaller areas for feeding, but they are fed more often by humans [20]. According to local reports, they are residential and are limited to short-range movements.

In comparison, Egyptian geese inhabit larger areas in rural areas, have a high flight distance, and are less often seen with native waterfowl but are sometimes seen with groups of greylag geese. It is assumed that differing feeding behaviour, sharing different feeding sites with different native species, and different food supplies could have an influence on the endoparasitic fauna.

The parasitological comparison of these two groups confirms the hypothesis and showed more than the differences in parasitological fauna. Almost twice as many animals from urban areas were infected with at least one parasite more than animals from rural areas. In the urban environment, five different parasite species were identified; in rural areas, twice as many. There are different possible explanations for this result.

The occurrence of certain parasites could be due to local occurrences of this parasite species or their intermediate hosts. Urban animals have, as stated above, comparatively smaller areas for feeding. If these are contaminated with parasite eggs once, reinfection, infection of conspecifics, or, in the case of heteroxenic parasites, infection of other birds occurs more quickly. This would promote the emergence of local clusters with a locally increased prevalence of one parasite species, and would also lead to a higher parasite load of the hosts. These proven patent infections of Egyptian geese with an endemic parasite increases the infection pressure not only for conspecifics but also for native waterfowl, such as mallards. This phenomenon of increasing the pathogen pressure of an indigenous pathogen by an alien has already been described in the context of the phenomenon known as "parasite spill-back" [15,84]. It describes the increase in pathogen pressure on native species. In most cases, the alien species multiplies the pathogens but they are less affected in their fitness than infected native species. This phenomenon should not be neglected, especially with virulent parasites such as *H. tricolor* and *E. uncinata*.

All *H. tricolor* positive carcasses originated from the metropolitan area of Frankfurt (Main). The local prevalence in Frankfurt (Main) is 24.5% (12/49). This comparatively high prevalence is best demonstrated in comparison with an investigation from Kavetska et. al., which presents a parasitological study of waterfowl over ten years. Out of 1052 birds of 17 different species, 6 specimens of *H. tricolor* were found in 2 mallards (0.2% (2/1052) [85]. Not only was the detection of adult parasite stages successful, but the eggs of *H. tricolor* were found in two scat samples originating from one other metropolitan area (Hamburg).

The thesis that non-indigenous Egyptian geese are short-range migrants is underpinned by the fact that even in rural areas, despite the lower total parasite load, two clusters of increased prevalence were found. These local clusters would also mean that not just urban Egyptian geese repeatedly visit the same feeding sites. This result suggests most of the Egyptian geese are usually non-migratory in Germany. They are described as "limited to short-distance ranges" in Europe in the literature [10,86]. This assumption is confirmed by ringing observations in Germany, where the ranging behaviour of different bird species is observed via ringing of the animals and the civil reports of recoveries [87]. In their native regions, there is a different migratory behaviour, where undirected movements of the animals up to 1000 km are described [80]. This seems to be different in their synanthropic non-native ranges where there is no need to follow the food seasonally [10,87,88]. For example, nine Egyptian geese examined from the health resort park in Bad Homburg were not infected with *H. tricolor*. The town is a similar urban habitat and only 50 km away from Darmstadt. This could indicate a slight change of location of the birds from Darmstadt or Frankfurt (Main) to Bad Homburg. Egyptian geese in the south of the United Kingdom show less movement [17] than birds in Lower Saxony (according to reports from various hunters), which can also be explained by their high demand for protein-rich plants. A more balanced climate favours year-round plant growth [89].

It is not only local clusters that offer approaches to explain the increased parasite load in urban-living Egyptian geese. The diet of the birds in these habitats could also be considered. As mentioned before, herbivorous birds, such as Egyptian geese, depend on fresh protein-rich sprouts from plants [10,22]. Green areas in parks usually offer only a limited food ration for a high population of waterfowl. Nevertheless, our own observations showed that birds from urban habitats are slightly heavier (on average, 50 g) and the angel-wing syndrome is shown in up to 20% of the birds. The predispositions for the occurrence of angel-wing syndrome are discussed in many ways. A very high energy and high protein intake during growth as well as a lack of vitamin E are considered to be the main causes, along with other promoting factors [24]. Thus, these observations together with the higher weight of the animals could indicate a deficient diet. Links between good host nutrition and high parasite load have already been shown [90]. However, not only the quantity of the host's food plays a role in parasitic colonisation. Experiments on canaries, *Serinus canaria*, showed that animals that were provided with additional minerals and proteins, i.e., received better quality food, were more resistant to parasites [91]. The increased load of parasites in the postmortems of urban animals could therefore be explained by the non-optimal diet of the animals, too. Baked goods, food waste, and toxic substances are ingested more by animals in urban areas. This unbalanced diet for herbivorous water fowl could lead to a compromised immune system.

Not all parasite species occurred in clusters with a comparatively increased prevalence. There is also a parasite species that has repeatedly appeared in both rural and urban counterparts in both necropsies and scat samples with a more or less even distribution. Only one cluster of *E. revolutum* with an increased prevalence was discovered. This may be due to the low host specificity of *E. revolutum*. It infects both birds and mammals and many common snail species as intermediate hosts, such as *Radix* spp. Even in comparison with other *Echinostoma* spp., *E. revolutum* seem to have the widest range of potential intermediate hosts [66]. A similar spread of the parasite was found in studies of the intermediate host by Georgieva et al. [66]. They also investigated the occurrence of *E. revolutum* in different places in Germany, but, in addition, they also examined freshwater snails, such as *Radix* spp. and *Stagnicola* spp., for the presence of cercariae. As in the present study, they found a low prevalence of *Echinostoma* cercariae at all locations examined and also at a locally elevated prevalence.

Anthropozoonotic Parasites in Egyptian Geese

The potential transmission of pathogens to humans from alien species is important for an invasiveness assessment [92], especially for an alien species that also spreads synathropically. In this study, the potential transmission of *Cryptosporidium* spp. was investigated. According to the literature, zoonotic *Cryptosporidium* spp. appear to be transmitted by waterfowl only vectorially [35,37]. Individual oocysts of *Cryptosporidium* spp. were found microscopically, but a species diagnosis by PCR was not possible. In the case of very small concentrations of oocysts, it is possible that the concentration was under the detection limit of the PCR. This means that the risk of a possible transmission of zoonotically relevant *Cryptosporidium* spp. seems to be present but very low.

Eggs and adult stages of an anthropozoonotic parasite *E. revolutum* were detected frequently. They were identified by molecular biological methods as an anthropozoonotic parasite subspecies. This parasite occurs across a wide range in Egyptian geese in both urban and peri-urban regions of Germany. Humans can become infected by ingesting infected intermediate hosts. Due to aspects of German culture, there is only an accidental risk of picking up a freshwater snail. This is a greater risk for small children in parks, where they could potentially pick up these snails and become infected by echinostomiasis. However, as studies on mallards and fresh-water snails have also shown, it is an endemic parasite to Central Europe [71]. Egyptian geese do not significantly increase the risk of echinostomiasis in the local population.

5. Conclusions

Egyptian geese are parasitologically integrated into the native fauna in Central Europe. They carry the same parasite species that are described in mallards. Overall, though, they have a lower parasite load than native waterfowl and higher bodyweights than in their conspecifics in their native ranges, which confirms parasitological investigations of alien organisms. Thus, besides other aspects, the immune system could be an important reason why this avian alien has spread so successfully not only in the center of Europe but also in parts of North America and the Arabian Peninsula. Further studies should focus on the unspecific immune defense of Egyptian geese in comparison to a native non-generalistic waterfowl species and the increased infection pressure of virulent parasites such as *H. tricolor* and *E. uncinata* in the context of a parasitic spill-back on native waterfowl. From a parasitological point of view, the potential of anthropozoonotic pathogen transmission seems to be limited.

It is only if we continue to investigate and record as much information as possible of the framework surrounding invaders that we will be able to protect native ecosystems from possible displacement by invaders and subsequent ecological depletion.

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