Diversity and prevalence of gastrointestinal parasites in two wild Galago species in Gabon

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Abstract

In this study, we characterize the diversity and estimated infection levels of gastrointestinal parasites circulating in two galago species, Galago demido and G. thomasi in two sites situated in the Southeastern forests of Gabon. Our study reveals that eleven parasites including nine helminthes (Ascaris spp., Ankylostoma spp., Dicrocoelium spp., Gongylonema spp., Oesophagostomum spp., Lemuricola spp., Strongyloides spp., Trichostrongylus spp. and Trichuris spp.) and two protozoans (Balantidium spp. and Entamoeba spp.) may infect Galago spp. with high infection rates. The results show that: a very similar parasite spectrum is found in both host species; all the taxa identified were previously observed in other Primate species and/or Man. They also show that age, gender and forest type may influence infection rates and/or parasite diversity found in a particular host and/or geographic area.

1. Introduction

Pathogens are playing a central role in ecosystems and community biodiversity due to their impact on the dynamic of populations growth and regulation (Hochachka and Dhondt, 2000; Hudson et al., 2002). A review of the literature shows that about 90% of the parasites recorded in Man include another mammal species in their cycle (Ashford and Crewe, 2003; Hugot et al., 2001). If animal’s infectious diseases and parasitosis are a major threat for the conservation of wild species they also are a potential threat for human health (Tyles and Dobson, 1993) and the increasing conquest of the rain forest environments by men heighten the probability of parasite host switching. Phylogenetic proximities between Man and other hosts like NHPs (non-human primate species) are an important factor in pathogens exchanges (Wolfe et al., 2007). Consequently, it is important to determine the diversity of pathogens circulating in wildlife, and particularly among species endangered because of human activities (Daszak et al., 2000). In this context, the data on patterns of parasitic infections in wild primate populations may provide an estimation of population health and allow to assess and manage disease risks (Gillespie, 2006).

Gabon is a central African country whose forests covers 85 of the area and are among the richest in Africa in terms of animal diversity. The estimated fauna comprises > 190 mammal species, including at least 20 species of monkeys, > 600 bird species, 86 reptile species and 100 species of amphibians (Alonso et al., 2006; Pauwels et al., 2006). Several species of nocturnal NHPs are living in Gabon including: Galago demido (G. Fischer, 1806) and G. thomasi (Elliot, 1907) (Wilson and Reeder, 2005). Galagos (bushbabies) are threatened by an increasing fragmentation of their habitat due to human activities, but last studies on these species were done long time ago and very few data are available about their parasites. In the following, using faecal samples collected in these hosts in Gabon, we examined the diversity and prevalence of gastrointestinal parasites from these two closely related species, in different study sites.

2. Materials and methods

2.1. Ethics statement

The study was conducted in Gabon. All samples were collected in
areas excluding national parks and protected areas and with the au-
thorization of the Gabonese Ministries of Water and Forestry, Higher 
Education, Scientific Research and Innovation (N’AR0031/09/MENE-
SRESI/CENAREST/CG/CST/CSAR). During faecal collections, animal 
welfare was taken into consideration and each animal was released into 
its environment after the collect of biological material.

2.2. Sites for collection of NHPs

Research of gastro-intestinal parasites was conducted from February 
2016 to January 2017 (11 months) on two different host species and 
two sites located in the province of Haut-Ogooué, Southeast Gabon 
(Fig. 1): the forest of the Primate Centre (CDP) of the “Centre Interna-
tional de Recherches Médicales de Franceville” (CIRMF) and the “Djoumou 
forest” (DJM) located between 30 and 40 km from Franceville. In CDP, 
forest habitats are divided into various relictual areas submitted to 
disturbances resulting from human activities. DJM forest covers a wider 
area than CDP and human presence is limited to traditional plantations 
and hunter’s paths. Galagos were collected using Tomahawk traps 
(Duplantier, 1989; Malcolm, 1991) placed in trees for six consecutive 
nights at different heights. All animals collected were kept in black 
room (without light) until faecal samples may be collected. Galagos 
species identification was performed using Kingdom (1997) and Wilson 
and Reeder (2005). Parameters such as host species, sex, age appraisal 
and sampling season were documented during the collections.

2.3. Faecal samples collection and examination

A total of 147 bushbabies: G. demidoff (n = 83) and G. thomasi 
(n = 64) were examined. Faecal samples were analyzed via sodium 
nitrate flotation and faecal sedimentation following the protocol of 
Gillespie (2006). Detection and counting of parasite eggs, larvae, and 
cysts was performed using a compound microscope (objective ×10). 
We used the ×40 and ×100 objectives to take pictures (Fig. 2). Iden-
tification of parasites is based on eggs shell and nucleus morphology 
according to Chitwood et al. (1950), Golvan (1990), Ashford and Crewe 
books were used to obtain information about the parasites biology and 
their host spectrum.

2.4. Statistical analyses

Statistical analyses performed using R software. Infestation rates 
between species, sexes and age groups calculated and compared using the Chi-square ($\chi^2$) method. Differences were considered statistically 
significant at 0.05 confidence level.

3. Results

Here after we consider successively the different parasite taxa in 
relation to the importance and variations of their prevalence in dif-
ferent hosts (Fig. 2 and Tables). We also try to relate these variations to 
what we know about the cycle, host specificity and taxonomic status of 
the parasites. The identification of the parasites collected during our
study is based on the light microscope observation of the eggs or cysts found in the faecal samples and by comparison with similar documents found in the literature. This method doesn’t allow a precise determination of the parasite at the species level and in the following we identify the positive samples at the generic level only. The genus name used is that of the parasite of the same type (order, suborder, family) also found in other Primates, including Man. When several names were available we chose the most probable of them in relation to the particularities of the parasite cycle and the systematic position of the hosts. For most of the parasites, we also had to consider the possibility that each of the taxonomic units distinguished in this study may include different parasite species; for instance following which locality and/or host species is considered. This is the reason why we are systematically using here after the abbreviation “spp”.

### 3.1. Ancylostoma spp.

The prevalence values are comprised between 7.5 and 10 and are twice as high in CDP than in DJM for both host species (Table 1). The prevalence is higher in females than in males (5.1 vs 13.2) and more than ten times higher in the young than in the adults (22.2 vs 2.1) (Table 3). After Anderson (2000) infection with hookworms is due to the penetration of larvae (L3) through the skin or by direct ingestion of dirt or fresh vegetables containing filariform larvae. They are parasite of the small intestine of mammals. Such parasites where identified as Necator spp. in both gorillas and chimpanzees by Graber and Geyrey (1981) in Congo. Kouassi et al. (2015) also signal hookworms, identified as Necator sp. or Ancylostoma sp., in several different guenon species in the Tai forest (Ivory Coast) sometimes with a high prevalence value (> 70). Because Malekani et al. (2014) signal Ancylostoma spp. in G. demidoff and G. gabonensis in the Democratic Republic of Congo with a high prevalence: 25.3 and 50 respectively; also because Golvan (1990) remarks that Ancylostoma eggs contain 4 blastomers only when laid, whereas Necator eggs contain 8, the parasites observed in our work must more likely be identified as Ancylostoma spp.

### 3.2. Ascaris spp.

This parasite exhibits one of the highest prevalence values observed in our study: 63.2 in G. thomasi and in CDP. For both host species the prevalence is higher in CDP than in DJM (Table 2) and in the males than in the females (Table 3). The Ascaridoidea are parasite for vertebrate hosts: contamination results from ingesting food (vegetables or fruits) soiled in contact with earth or by drinking water. Genus Ascaris spp. are the single taxa in this group recorded in Primate hosts and, within the NHPs, they were observed in apes (gorillas and chimpanzees) (Graber and Geyrey, 1981) and in G. demidoff by Malekani et al. (2014) with a prevalence of 37.04. In both publications the authors identify the parasites collected in Primates as A. lumbricoides, the parasite of Man. However this identification looks dubious because A. lumbricoides is generally described as highly specific and unable to maintain itself in another host than man (Anderson, 2000; Ashford and Crewe, 2003).

### 3.3. Balantidium spp.

For both host species the prevalence for this parasite is not very different (19.3 vs 15.6) (Table 1). The prevalence is markedly highest in CDP than DJM, relatively close between males and females (15.2 vs 20.6), and very different between adults or young (24.7 vs 4.0) (Tables 2 and 3). Balantidium coli is a cosmopolitan parasite of the large intestine of the pig, but it is also found in vicarious hosts: Primates (macaques, guenons), Rodents (especially rats), dogs, cats and cattle. The cysts resist > 3 months in the wild and can be carried by flies (Golvan, 1990). This parasite has been identified in several species of African guenons (Kouassi et al., 2015) living in the rain forest, in which it appears to be relatively frequent and abundant. These characteristics, the indisputable ubiquity of this parasite and its proven presence in the same habitats make its presence in the galagos very likely.

### 3.4. Dicrocoelium spp.

For both host species the prevalence values are similar and relatively weak (< 10) (Table 1); the contrast between the two sites is marked: prevalence is two to eight times higher in CDP (Table 2); the males are twice as parasitized as the females; the prevalence values are identical in adults or young: around 8 (Table 3). Dicrocoelium spp. live mainly in the bile ducts and gall bladders of domestic and wild ruminants but occasionally parasitize primates including Man. They use insects, especially ants, as intermediate hosts (Kouassi et al., 2015). Ingesting infected ants or food contaminated by ants infects monkeys. This way of contamination is accessible to galagos, which are in contact with earth or by drinking water. Genus Ascaris spp. are the single taxa in this group recorded in Primate hosts and, within the NHPs, they were observed in apes (gorillas and chimpanzees) (Graber and Geyrey, 1981) and in G. demidoff by Malekani et al. (2014) with a prevalence of 37.04. In both publications the authors identify the parasites collected in Primates as A. lumbricoides, the parasite of Man. However this identification looks dubious because A. lumbricoides is generally described as highly specific and unable to maintain itself in another host than man (Anderson, 2000; Ashford and Crewe, 2003).
Table 2
Prevalence of parasite taxa collected in Galago demido or G. thomasi during the period of study following the site of collection. Comparison of prevalence between the two sites computed using the chi-square test. (N = number of positive specimens; P = prevalence; S = +, test significant; CDP = S.1. Primatology Center forest; DJM = S.2. Djoumou forest; + higher prevalence in CDP or DJM).

<table>
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<tr>
<th>Galagoides demido</th>
<th>CDP</th>
<th>DJM</th>
<th>X2</th>
<th>df</th>
<th>Comparison test</th>
<th>S</th>
<th>CDP+</th>
<th>DJM+</th>
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<tr>
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<td>N/42</td>
<td>P (%)</td>
<td>p-Value</td>
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<td>1</td>
<td>0.73</td>
<td>−</td>
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<td>4</td>
<td>9.5</td>
<td>2.58</td>
<td>1</td>
<td>0.11</td>
<td>−</td>
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<td>14.6</td>
<td>3</td>
<td>7.1</td>
<td>0</td>
<td>1</td>
<td>1.00</td>
<td>−</td>
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<td>12.2</td>
<td>4</td>
<td>9.5</td>
<td>0</td>
<td>1</td>
<td>1.00</td>
<td>−</td>
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<td>1</td>
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<td>1</td>
<td>0.63</td>
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<tr>
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<td>9.5</td>
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<th>df</th>
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<tbody>
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<td>N/45</td>
<td>P (%)</td>
<td>p-Value</td>
<td></td>
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<tr>
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<td>3.22</td>
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<td>8.38</td>
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<td>0.00</td>
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<td>6</td>
<td>14.3</td>
<td>0.08</td>
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<td>0.70</td>
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<td>3.22</td>
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<td>0.07</td>
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<td>2</td>
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<tr>
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<td>4.0</td>
<td>9.5</td>
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</tbody>
</table>

3.5. Entamoeba spp.

For both host species the prevalence values are similar and relatively weak (< 10) (Table 1); the prevalence is very similar in CDP or DJM and higher in females or young (Tables 2 and 3). Following Golvan (1990) about ten different species of Entamoeba have been identified in Man. A part of them are apparently non pathogenetic. Infection occurs orally, from drinking water, food, etc. E. histolytica is the most frequent, predominantly infecting humans and other primates and distributed worldwide. E. coli is identified as a parasite of chimpanzees and baboons by Howells et al. (2011); the same species was recorded in Eu-lemur fulvus E. Geoffroy, 1796 from Madagascar, by Clough et al. (2010). If according to the above, finding Entamoeba spp. in galagos is not surprising more investigations are needed to precise which species is/are present in these particular hosts.


This parasite is present in both host species with a relatively low prevalence: 4.7 and 6 in G. thomasi and G. demido respectively (Table 1). It is absent from CDP and more frequent in females or young (Table 3). Members of the genus occur embedded in the mucosa of the anterior region of the gut of birds and mammals (Anderson, 2000). The eggs are evacuated in the outside environment. If ingested by coprophagous insects (cockroaches, dung beetles, etc.) the larvae continue their development in the haemocoeel of insects or become encapsulated in their muscles (Quentin et al., 1986). Definitive hosts are contaminated by accidentally swallowing these insects (Golvan, 1990). G. pulchrum is the most frequent and cosmopolitan species. This nematode is usually a parasite of the digestive tract of cattle and swine but Man infestations have been reported in most parts of the world. The high frequency of this parasite in tropical areas, its ubiquity including Man and the fact that insects are intermediary hosts makes plausible its presence in galagos. More investigations are needed to precise which species is/are present in these particular hosts.

3.7. Lemuricola spp.

The samples collected in G. thomasi are negative all of them. Three individuals of G. demido only, out of 83 animals examined, were found positive and all of them were captured in DJM. An Oxyurid identified as Enterobius sp. is recorded by Malekani et al. (2014) in a free-living galago; G. demido in Congo with a prevalence of 11.1. In a previous work (Hugot et al., 1995) oxyurids were found and described in a closely related host: G. senegalensis E. Geoffroy. But this animal was dead in captivity in the Faculty of Medicine of Paris where it was living in close proximity to a colony of Microcebus murinus (Miller) in a breeding lab. The parasites collected from the galago were morphologically very similar to Lemuricola microcoeli, Hugot et al., 1995 considered to be specific for M. murinus. Finally because of the low numbers of parasites available for examination, it was decided impossible to choose between: either a transfer from the microcebes to the galago or the possibility that the two samples actually belong to two different species. Until more material or information could be obtained, it was proposed to refer to the parasites of the galago as Lemuricola sp. The results exposed above are giving some consistence to the existence of one or several particular and new parasite species in the galagos.
3.8. Oesophagostomum spp.

With a prevalence of 39.1 for *G. thomasi* this parasite reach the highest value after *Ascaris* spp. for this host and one of the highest values observed for a parasite in DJM: 19 (Tables 1 and 2). For both host species the prevalence is higher in CDP than in DJM (Table 2) and in the young than in the adults (Table 3). After Golvan (1990): several Oesophagostomum spp. are present in African Primates like macaques, baboons, guenons and gorillas; all of them may contaminate Man who is considered a substitution or abnormal host; the oesophagostomiasis is considered resulting from absorption of plants soiled by droppings of domestic animals (Munene et al., 1998). This modality of infection is eliminated in the stools and the faeco-oral contamination of Primates looks unlikely or almost impossible. It seems perfectly plausible a contamination by human parasites. A precise identification of the parasite(s) observed in this study result of contamination by monkey or ape parasite(s) or are host specific for the galagos.

3.10. Trichostrongylus spp.

The prevalence for this parasite is much higher in *G. thomasi* (23.4 vs 1.2) and it is observed in DJM, only. The prevalence is about three times higher in the females than in the males (16.2 vs 6.3), and in the young than the adults (22 vs 5.2) (Table 3). According Golvan (1990), *Trichostrongylus* spp. are cosmopolitan and above all parasites of Ruminants, Lagomorphs and Rodents sometimes also from Equidae. In Africa these parasitosis are known in Man and monkeys: the eggs are eliminated in the stools and the faeco-oral contamination of Primates seems perfectly adapted to Man and can therefore be maintained outside the presence of monkeys. It should be noted that in these cases of human infestation, eggs and not larvae are found in stools. Future investigations will have to determine if the parasite(s) observed in this study result of contamination by monkey or ape parasite(s) or are host specific for the galagos.
3.11. *Trichuris* spp.

The prevalence for this parasite is one of the lowest in both host species: 3.6 vs 9.4 (Table 1). Its prevalence is higher in CDP and quite identical in males, females, and adults or young (= 6) (Tables 2 and 3). The eggs of this parasite localized in the small intestine are evacuated in the external environment with the stool. The eggs are extremely resistant to atmospheric and chemical agents and must stay in the ground during 6 to 12 months before to be infective. Man may be infested with fruits or vegetables growing on ground and raw (Golvan, 1990). About 60–70 species of whipworms have been recorded from mammals (primates, artiodactyls, rodents, lagomorphs, shrew, felids and foxes) and they are cosmopolitan (Anderson, 2000). *T. trichura* is a parasite of humans and other primates and is regarded as the second most common parasitic infection in humans in the tropics. Ooi et al. (1993), described specimens from *Macaca fascata* and *Papio papio* as *T. trichiura*. Future investigations will have to determine if the parasite(s) observed in this study result of contamination by Man, monkey or ape parasite(s) or are host specific for the galagos.

4. Discussion

Recently several works were published regarding the parasitic fauna of African NHPs (Landosoud-Soukate et al., 1995; Clough et al., 2010; Nath et al., 2012; Kaloousova et al., 2014) but only one of them concerned the galagos: Malekani et al. (2014). Unfortunately, if this work is providing a first sight on the diversity of the gastro-intestinal parasite fauna in two galago species, the observations are founded on a relatively low number of specimens (N = 30) captured in not least than seven different stations of captures. Logically considering this, the positive results are not identified following their localization and no mention of the gender or age of the animals examined is given. This makes difficult a comparison with our own results. Two Cestoda and one Digena recorded in this work are absent of our observations. However, several parasite taxa identified in the present work are also present in Malekani et al. (2014): *Ascaris* spp., *Lemuricola* spp., *Ancylostoma* spp., *Trichostrongylus* spp. and *Strongyloides* spp. Because of the characteristics of the cycle of the first two taxa (see discussion above), it seems likely that the parasites observed here may belong to species specific to their hosts and still undescribed.

4.1. Diversity and prevalence of gastrointestinal parasitoses by host species (Table 1)

Data analyses revealed the presence of nine helminth parasites and two protozoa parasites. With the exception of *Lemuricola* spp., present in *G. demidoff* only, the same parasite taxa are recorded in both host species. For both host species the arithmetic means of the prevalence values are very close: 14.7 for *G. demidoff* vs 15.5 for *G. thomasi*. However, the prevalence values observed for each parasite vary markedly. Two parasites only exhibits a high prevalence whatever which host is considered: *Ascaris* spp. (31.3 vs 40.6) and *Balantidium* spp. (19.3 vs 15.6). The differences in prevalence are particularly marked and statistically significant for *Strongyloides* spp. (62.7 vs 10.9) and *Oesophagostomum* spp. (9.6 vs 39.1).

4.2. Difference in prevalence according to collection sites and host species (Table 2)

Three parasites taxa are never represented in CDP site, whatever which host species is considered: *Gongylonema* spp., *Lemuricola* spp. and *Trichostrongylus* spp. For the other eight parasite taxa the prevalence is systematically higher in CDP than in DJM site, whatever which host species is considered: for *G. demidoff* the mean prevalence is 18.3 vs 11.9 and 20.4 vs 8.6 for *G. thomasi*. Proportionally, for the same parasite, the prevalence in CDP is 1.5 to 4 times its value in DJM for both host species. Finally, if the parasite diversity appears to be higher in DJM, several parasites reach their highest prevalence value in CDP; for *G. demidoff* *Strongyloides* spp. (53.7), *Ascaris* spp. (46.3); for *G. thomasi* *Strongyloides* spp. (73.7), *Ascaris* spp. (63.2). CDP forest is more fragmented and impacted by human activities than DJM. Previous studies have shown that these factors may influence the diversity and prevalence of parasites, particularly because forest fragmentations reduce food availability and increase host densities (Gillespie and Chapman, 2008).

4.3. Difference in prevalence according to the age or gender of the host species (Table 3)

In our data set the effective of males or females are quite equivalent: 79 vs 68; conversely the contrast between the representation of adults or young is high: 97 vs 50.

4.3.1. Gender

For both sexes the highest prevalence values deal with the same parasite taxa: *Ascaris* spp., *Balantidium* spp. and *Strongyloides* spp. The mean prevalence values are higher for the males than the females: 14.7 vs 11.0. However the females have a relatively stronger prevalence than the males for four parasites: *Ancylostoma* spp. (13.2 vs 5.1), *Trichostrongylus* spp. (16.2 vs 6.3), *Gongylonema* spp. (7.4 vs 3.8) and *Entamoeba* spp. (13.2 vs 3.8). Within the males the contrast between the highest vs the lowest prevalence values is higher (median = 6.3) than within the females (median = 10.3). These differences may be related with the particular behavior of the galagos where matriarchal groups are the stable units, with a family defending a territory during nomadic males are travelling over several female territories every night (Kingdon, 1997).

4.3.2. Age

The mean prevalence values are relatively close when the adults are compared to the young: 13.0 vs 15.3; for both class the highest prevalence values deal with the same parasite taxa: *Ascaris* spp. (35.1 vs 36.0) and *Strongyloides* spp. (49.5 vs 20.0). The young have a markedly stronger prevalence (two to ten times higher) than the adults for six parasite taxa out of 11: *Ancylostoma* spp. (22.0 vs 2.0), *Dicrocoelium* spp. (12.0 vs 6.2), *Entamoeba* spp. (16.0 vs 4.1), *Gongylonema* spp. (8.0 vs 1.0), *Oesophagostomum* spp. (16.0 vs 3.1) and *Trichostrongylus* spp. (22.0 vs 5.2). However, the differences in prevalence are statistically significant ($\chi^2$ test) only for *Ancylostoma* spp., *Balantidium* spp., *Entamoeba* spp. and *Strongyloides* spp. Within the adults the contrast between the highest vs the lowest prevalence values is higher (median = 7.2) than within the young (median = 14.0). Mbito et al. (2010) and Mbuya and Udendeye (2011) have shown the existence of a link between age and gastrointestinal parasites infestation. The fact that young are more infected could be explained by their higher susceptibility to helminthisis due to a lack of premunity (acquired immunity through exposure). The highest values observed in adults for some parasites may be related to the phenomenon of surinfestation (increasing population of a parasite in one host due to successive reinfections).

4.4. Conclusion

Our work reveals very similar parasite spectrum in both host species and it is notable that all the taxa identified here were found previously in other Primate species, including Man. Because the hosts are closely related and living in sympathy a questions arise for each identified parasite taxon: is it specific for the Primates as a whole, for the *Galago* spp., or is it strictly host specific? To answer to this question it is needed to be able to identify precisely the parasites at the species level.

This point also deals with a hypothetical role of some parasites as zoonotic agents. Because the galagos are strictly arboreal and nocturnal and are not considered to be game animals, the contamination of Man...
by galagos parasite(s) or the contaminations of galagos directly by Man looks unlikely. Obviously this observation is not valid for zoonotic parasites having a ubiquitous host spectrum. However and whatever which host is considered a majority of parasites have a higher prevalence in CDP site. For this reason the influence of a closer cohabitation with Man including a fragmentation of the galagos habitats may be considered.

When compared with females, males generally exhibit higher prevalence values for 7/11 parasite taxa; males also reach the highest prevalence values observed during our study for several parasites. This may be related with the behavioral differences between galago males or females.

The young have a higher prevalence value for 6/11 parasite taxa and an equal prevalence for two of them. This may be interpreted in the context of a higher sensibility to parasitism and diseases probably partially linked with an immature immune system.

Measures to protect the environment and endangered species oblige parasitologists to change their method of study. It is no longer possible to dissect numerous animals in order to collect their internal parasites. The determination of the parasites may possibly be improved through the use of molecular analysis methods. Unfortunately these methods were not usable in the context of the present study. We therefore used available data on geographical distribution, host spectrum and host specificity of the parasitic taxa in an attempt to identify them. For these reasons, this work must be considered preliminary and, in order to try to answer to the questions it asks, additional observations are needed. This includes predominately the identification and if possible the description of the parasite taxa.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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