

ASSOCIATION BETWEEN INFESTATION PARAMETERS OF NASAL MITES (ACARI: RHINONYSSIDAE: *TINAMINYSSUS* SPP.) AND HOST BODY CONDITION IN ROCK PIGEONS (AVES: COLUMBIDAE: *COLUMBA LIVIA*) IN MANITOBA

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ABSTRACT

Rock pigeons (*Columba livia* Gmelin) host a variety of parasites including nasal mites (Rhinonyssidae: *Tinaminyssus* spp.). While distribution and host association have been studied through surveys in Canada, there are knowledge gaps in the ecology of these parasites. We salvaged pigeons to determine the prevalence and mean intensity of nasal mites, as well as to examine the relationship between host body condition and infestation parameters (prevalence and mean intensity). Seventy-five pigeons salvaged from Wildlife Haven Rehabilitation Centre were given a body condition score (BCS) on a scale of 1–5, with 1 being emaciated and 5 being obese. Their respiratory turbinates were flushed using a curved 12 ml Monojet™ 412 syringe with soapy water. Samples were preserved in 95% ethanol until the mites were counted and identified. Data from pigeons salvaged from Wildlife Haven, Prairie Wildlife Rehabilitation Hospital, and Pembina Vet Hospital (2000-2011) were pooled with recent data and analyzed using Quantitative Parasitology (QPweb). Pigeons were infested with nasal mites, *Tinaminyssus melloi* (Castro) and *T. columbae* (Crossley). Prevalence and mean intensity were 52.4% and 14.9 mites per bird, respectively (n=615). Co-infestations of *T. columbae* and *T. melloi* were present in 78 (33%) infested birds (n=236). The prevalence of infestation in emaciated birds (BSC 1-2.5) was significantly greater than for birds in better condition. There were no differences in intensity based on host body condition.

KEYWORDS: pigeon parasites, respiratory mites, host-parasite ecology, body condition score, co-infestation, prevalence, mean intensity

INTRODUCTION

Birds are widely admired by citizens and scientists alike. Birds have at least 40 families (around 2500 species) of closely associated symbiotic mites (Proctor & Owens 2000). Mites have taken advantage of the diversity of habitats available over the landscape and immediate environment of

birds, including the nest, skin, feathers, and respiratory tract (Walter & Proctor 2013). Five families of mites live in the respiratory tracts of birds, a life history strategy which independently evolved three times (Fain 1994). The most diverse and widely documented are the haematophagous endoparasites of the family Rhinonyssidae (Mesostigmata), with fifty documented species in Manitoba alone (Knee & Galloway 2017). Rhinonyssids are robust, slow-moving mites, well adapted to living within the nasal passages of birds and feeding on blood from these highly vascularized tissues (Bell 1996). Nasal mites infest birds on every continent, including Antarctica. *Rhinonyssus schelli* (Fain & Hyland) infests gentoo (*Pygoscelis papua* (Forster)) and Adélie penguins (*Pygoscelis adeliae* (Hombron & Jacquinot)) (Vanstreels *et al.* 2020). Nasal mites are occasionally found in the tracheae and lungs (Porto *et al.* 2022). Direct transmission potentially occurs between birds in proximity, for example, during courtship or feeding offspring (Amerson 1967; Porter & Strandtmann 1952). Indirect transmission may occur via shared perches or communal water sources (Bell 1996). Rhinonyssids are not generally considered pathogenic; however, feeding activity can damage the nasal passages, leading to rhinonyssidosis avium disease (Dimov 2011). *Sternostoma tracheacolum* (Lawrence) can be highly pathogenic to canaries (*Serinus canaria* (Linnaeus)) and Gouldian finches (*Erythrura gouldiae* (Gould)). This species colonizes the deep respiratory passages, leading to pneumonia and sometimes death (Bassini-Silva *et al.* 2019).

Rock pigeons (*Columba livia* Gmelin) in Manitoba are infested by two species of rhinonyssids, *Tinaminyssus melloi* (Castro) and *T. columbae* (Crossley) (Grossi & Proctor 2021; Knee *et al.* 2008; Knee & Galloway 2017). In some situations, rock pigeons are considered nuisance animals and referred to by many as “rats of the sky” (Jerolmack 2008). They are synanthropic, often associated with farmsteads and ubiquitous in urban landscapes. Most Western Hemisphere populations are descended from released and escaped domestics. Domestic pigeons are kept for meat, as pets, and for entertainment, including racing and being ceremoniously released at events (Lowther & Johnston 2020). Rock pigeons are an important component of urban ecology and serve a variety of roles, including as prey for urban predators (Capoccia *et al.* 2018). As rock pigeons have become established in new environments, they have brought many of their parasites with them, including *T. melloi* and *T. columbae* (Grossi & Proctor 2021). Rock pigeons and their associated parasites have established populations in sensitive environments where they can impact the conservation biology of related species such as the laurel pigeon (*Columba junonia* Hartert) in Tenerife (Canary Islands) (Foronda *et al.* 2004). *Tinaminyssus* is known primarily from columbid hosts, with the cosmopolitan nature of rock pigeons allowing for potential transfer to novel columbid species, as was the case where one Eurasian collared dove (*Streptopelia decaocto* (Frivaldszky)) was infested with *T. columbae* (Veiga *et al.* 2021).

The habitat of *Tinaminyssus* within the nasal cavities of hosts makes observations of live mites difficult. As a result, there is a lack of knowledge on the host-parasite ecology of these cryptic organisms. Rock pigeons are common and easily obtainable for scientific study. Even so, the impacts of nasal mites on pigeons and other avian hosts have not been adequately explored. Studying the interactions between rock pigeons and their nasal mites provides an opportunity to infer the impacts of rhinonyssids on other species of rare and endangered birds. Preliminary

research on *Tinaminyssus* spp. has revealed microbial symbionts (Osuna-Mascaró *et al.* 2020, 2021), raising further questions about the potential impact of these mites on host health.

Body condition scoring (BCS) is a system used by veterinary professionals and the agricultural industry to assess animal health quickly by palpating the muscle and fatty tissue deposits and can be applied to birds (Wenker *et al.* 2022). BCS typically scales from one to five, with one being emaciated and five being obese. Populations of feral rock pigeons are variable in size, making BCS a more appropriate tool than body mass to assess health and fitness. We found that pigeons assigned a specific score (BCS 3.5, n=20) displayed weights ranging from 260.5 g to 448.2 g, with a mean of 318 g. This justifies the use of BCS for this system because each BCS class includes the range of body sizes present in rock pigeon populations.

Our preliminary objective was to establish infestation parameters for *T. melloi* and *T. columbae* infesting rock pigeons in Manitoba. This is the first attempt to extend the understanding of the host-parasite relationship of rhinonyssids and to assess potential negative health impacts on hosts based on BCS. We provide novel evidence documenting an association between the presence of nasal mites and a marker of poor host condition, as the prevalence of *Tinaminyssus* spp. was higher among emaciated rock pigeons. This information may be of particular interest to pigeon enthusiasts. Inferences may also be made on the impacts of other species of rhinonyssids on rare, endangered, or economically important species of birds.

MATERIALS AND METHODS

Rock pigeons were salvaged from Wildlife Haven Rehabilitation Centre, September 2016 to August 2022. Permits are not required to salvage pigeons in Manitoba. The birds came from Winnipeg and surrounding areas. Rock pigeons for this study were triaged on or shortly after arrival at the rehabilitation hospital, assessed as unsuitable for rehabilitation, and euthanized. The primary reasons pigeons were admitted to Wildlife Haven Rehabilitation Centre were for traumatic injuries assumed to be from window strikes or predator attacks based on the presence of obvious injuries. Postmortem, hosts were labelled with a unique case number and date of death, individually bagged, and frozen at -20°C, which subsequently killed any arthropod symbionts. Hosts were individually bagged upon death to reduce the risk of cross-contamination and loss of arthropod ectosymbionts to the environment. The pigeons remained frozen in chest freezers until the day of processing.

All hosts were weighed on a digital scale after being removed from the freezer. Serial washes were performed to remove the arthropod parasites. Rock pigeons were washed in a 12 L plastic bucket with warm water and Sunlight™ dish detergent. The typical process involved two repeated washes with soapy water, followed by a rinse with clear water to ensure that as many parasites as possible were being removed. In cases of extreme infestation, an extra wash with soap was required. Following each wash, the contents of the bucket were passed through a 90 µm sieve. The nasal passages of each bird were flushed with warm soapy water using a 12 mL Monojet® 412 curved-tip plastic syringe. Each nare was flushed once, allowing the water to run out of the mouth and onto the sieve. Following the nasal flush, the filtrate was rinsed into a

container and preserved with 95% ethanol. Labels with host information were retained with the container of preserved host filtrate. Each rock pigeon, once thawed, was palpated over the keel to assess muscle and fat deposits to assign a body condition score (BCS) (Figure 1, DeVoe & Reininger 2006). For the purposes of analysis based on BCS, hosts were sorted into two categories: BCS 1.0-2.5 (emaciated) and BCS 3.0-4.5 (healthy). Hosts sampled in this study never exceeded a BCS of 4.5. To overcome the variation in assigned scores in 2022, two people (MD and MK), each assigned a score to every host. When the scores differed, an average score was assigned to the host.

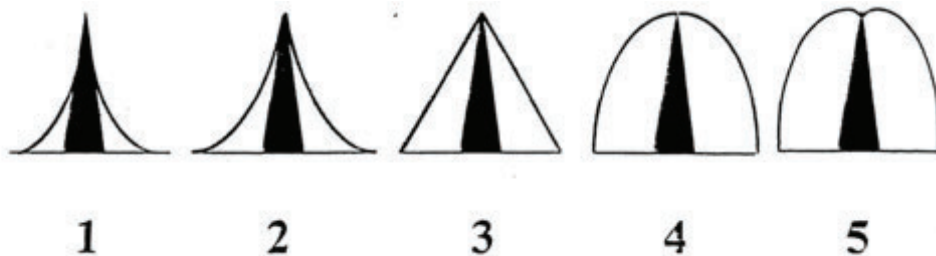


Figure 1. Body condition scoring chart for generic bird species. The drawings represent a body cross section with the black section representing the keel and the lines on either side indicating pectoral muscle. Healthy birds usually score from 3.5-4.5 with scores under 2.5 indicating emaciation. From <https://nagonline.net/3877/body-condition-scoring/> 2022, reproduced with permission.

Containers of filtrate were sorted using a stereomicroscope to obtain the associated arthropods. The number of nasal mites per host was recorded. Nasal mites were identified to species and the number of each species per host recorded. Representative specimens of adults of each species were cleared at room temperature in lactophenol for one to four hours, mounted in Hoyer's medium, and cured in a slide warmer at 45-50 °C for four days. Voucher specimens of both species of nasal mite were deposited in the J.B. Wallis/R.E. Roughley Museum of Entomology in the Department of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada.

Archived data (2000-2011) from T.D.G and W.K. were pooled to generate historical infestation and coinfection parameters. The following data analyses were done using Quantitative Parasitology (QPweb) (Reiczigel *et al.* 2019). Confidence limits (95%) for prevalence were calculated using Sterne's exact method (new algorithm) and for mean intensity using the Bootstrap BCa method with 2000 replications. Bootstrap two sample t-tests with 1000 replications were used to compare mean intensities among selected variables. Prevalence of the two species of mites was compared using Fisher's exact test. We compared prevalence of mite infestations across BCS classes with Chi-squared in R v.4.3.1 (R Core Team 2023).

RESULTS

During the 2022 sampling period, 33/43 examined hosts (76.7%) had obvious traumatic injuries. General infestation parameters of *Tinaminyssus* spp. infesting rock pigeons (n=615) were determined. Of these, 322 birds (52.4% [95% C.I. 48.4-52.4]) were infested with one or more nasal mites. Among infested birds, the mean intensity was 14.9 (95% C.I. [12.9-17.6]) mites per

infested host. The greatest intensity of 178 mites occurred in a pigeon collected in 2004. Four other hosts had ≥ 100 nasal mites. Among the 75 birds sampled by M.D. and M.K., 38 birds or 50.7% (95% C.I. [(39.3-62.1)]) were infested with *Tinaminyssus* spp. at a mean intensity of 14.5 (95% C.I. [10.5-21.4]) mites per bird.

Mean intensity of *T. melloi* was greater than the mean intensity of *T. columbae* (bootstrap T-test: BCa method, $p = 0.014 < \alpha = 0.05$) (Table 1). Although prevalence of *T. melloi* was greater when compared to *T. columbae*, it was not statistically significant (Fisher's exact test, $p=0.0586 > \alpha=0.05$).

Table 1. Infestation parameters of *Tinaminyssus melloi* and *T. columbae* in rock pigeons (*Columba livia*, n=75) in southern Manitoba, collected 2016–2022.

	Infested	Prevalence % (95% C.I.)	Mean Intensity (95% C.I.)
<i>T. melloi</i>	32	42.7 (31.9-54.0)	13.6 (9.97-21.6)*
<i>T. columbae</i>	20	26.7 (17.8-37.9)	5.6 (3.4-10.6)*

* bootstrap T-test: BCa method, $p = 0.014 < \alpha = 0.05$

Out of the 38 recently sampled (2016-2022) and infested hosts (n=75), co-infestations of *T. melloi* and *T. columbae* were present in fourteen birds (36.8%). When recent samples were pooled with archived data (n=236), co-infestations were found in 78 infested hosts (33.1%).

There was a significantly greater prevalence of *Tinaminyssus* spp. in pigeons that were emaciated (BCS 1.0-2.5) when compared to healthy individuals (BCS 3.0-4.5) (Chi-squared test, $X^2=5.3788$, df=1, $p=0.02038 < \alpha=0.05$) (Table 2). There were no differences in mean intensities between BCS classes (bootstrap T-test: Bca method, $p=0.268 > \alpha=0.05$).

Table 2. Infestation parameters of *Tinaminyssus* spp. in rock pigeons (*Columba livia*) in Manitoba classified by body condition score as emaciated (BCS 1.0-2.5) or healthy (BCS 3.0-4.5).

	N	Infested	Prevalence % (95% C.I.)	Mean intensity (95% C.I.)
BCS 1.0-2.5	27	19	70.4 (50.0-85.3)*	14.32 (9.37-22.7)
BCS 3.0-4.5	48	19	39.6 (26.7-54.3)*	14.79 (8.95-28.4)

* Chi squared test, $X^2=5.3788$, df=1, $p=0.02038 < \alpha=0.05$

DISCUSSION

Nasal mites have been documented across a broad array of host species from many geographic regions worldwide (Knee 2018; Knee *et al.* 2008; Maa & Kuo 1965; Pence 1975; Spicer 1987). The taxonomic focus of these studies is diverse, with some focused on host diversity, while others target just a few host species. Rhinonyssids of rock pigeons have been studied in many other regions (Crossley, 1951; Foronda *et al.* 2004; Porto *et al.* 2022; Veiga *et al.* 2020) and

sample sizes have varied in examinations of epidemiological parameters for *Tinaminyssus* spp. For example, Veiga *et al.* (2020) (n=250), Porto *et al.* (2022) (n=202), and Grossi & Proctor (2021) (n=162) sampled relatively large numbers of pigeons, while in other studies, sample sizes are much smaller (*e.g.*, Crossley 1951 (n=60), Foronda *et al.* 2004 (n=50)). The cumulative infestation parameters in our study are based on the largest host sample size (n=651) available in the published literature.

About half (52.4%) of Manitoba pigeons were infested with *Tinaminyssus* spp. with an average intensity of 14.9 mites per infested host. Porto *et al.* 2022 determined that *Tinaminyssus* spp. infested rock pigeons in Brazil at a prevalence of 25.5% and mean intensity of 9.9 mites per infested host. Variation among infestation parameters of the Brazil study and the current study could be that hosts in Brazil were collected randomly rather than being salvaged from wildlife rehabilitation centres. Salvaging hosts from rehabilitation centres is an ethical source of hosts for parasitological research, even if it technically limits randomization of experimental units (Galloway 2023). Although, when the salvaged hosts from this study were examined, many of them had obvious injuries, suggesting a more random process than if the birds had been unwell for a period prior to their euthanasia.

Intensity of infestation with *Tinaminyssus* is variable, with many hosts having one to few mites and some hosts infested with more than 100. The reason certain individuals presented with such large infestations is unknown. Nasal mites are blood-feeders, so perhaps immunocompromised individuals are unable to mount an adequate defense, and mite populations rise precipitously. The highest mean intensity reported in the literature was by Foronda *et al.* (2004), 218.3 *T. melloi* per infested host (n=50 ± 117.3) which is much higher than that observed in Manitoba. Their study showed exceptionally high mean intensity compared to other reports in the literature, such as Veiga *et al.* (2020) with 14.4 *T. melloi* per infested host, and Mascarenhas *et al.* (2022) with 14.8 *T. melloi* per infested host. Regional differences among populations may reflect a combination of internal and external conditions in host accommodation of nasal mites. When sampling pigeons from major Canadian cities, hosts from Vancouver (British Columbia) had the highest prevalence of nasal mites and Edmonton (Alberta) the lowest (Grossi & Proctor 2021). Climatic data, including humidity and temperature extremes, were attributed to driving variation in the mite assemblage of rock pigeons (Grossi & Proctor 2021).

The infestation parameters for *T. melloi* and *T. columbae* in the present study are like those found by Veiga *et al.* (2020). Prevalence and mean intensity for *T. melloi* in Manitoba were greater than for *T. columbae*, although the difference in prevalence was not statistically significant (Table 1). Veiga *et al.* (2020) found similar results, with the principal difference being the greater prevalence of *T. melloi* compared to *T. columbae* (Fisher test, $p < 0.001$). Differences in reproductive capacity and/or survival could explain differences in infestation parameters of the two species. Most likely due to their cryptic nature, little is known about the life history and reproduction of rhinonyssids. It is possible that the prevalence and intensity of these mites are undervalued due to mites occupying regions of the respiratory tract that are not accessed through nasal flushing. For example, *T. columbae* occasionally occupies the tracheae. In some studies, host heads were dissected to expose mites (Crossley 1951; Knee *et al.* 2008; Mascarenhas *et al.*

2022). Porto *et al.* (2022) found three of 202 hosts with *Tinaminyssus* in their tracheae. One host was infested by one *T. melloi* and the other hosts by 1–13 *T. columbae* in their tracheae. However, it is unlikely that a significant portion of the hosts in our study were infested by mites that could not be collected using a nasal flush. Wilson (1964) found dissection and nasal flushing to be comparable techniques with the former being more thorough and the latter more efficient.

Co-infestations of *T. columbae* and *T. melloi* were common in our study. Among the recent (2016–2022) sample group (n=75), 14 out of 38 pigeons were co-infested. Infestations of both *T. melloi* and *T. columbae* have been previously documented in Manitoba (W. Knee, archived data). Rock pigeons infested with both *T. melloi* and *T. columbae* (8.8% of infested birds) have also been reported in Seville, Spain (Veiga *et al.* 2020). In contrast, coexisting infestations of *T. melloi* and *T. columbae* were not detected in rock pigeons in Texas (n=60), despite both species being present in the population of birds sampled (Crossley 1951). Co-infestation has been reported among other rhinonyssids, such as those infesting brown-headed cowbirds (*Molothrus ater* (Boddaert)) (Hilario-Pérez & Dowling 2024). Hilario-Pérez & Dowling (2024) reported 84 out of 764 infested hosts to be infested by two species and 11 additional hosts infested with three. Broader surveys of avian hosts by Knee *et al.* (2008) and Knee & Galloway (2017) in both cases, found four species of birds, including rock pigeons, co-infested by two species of nasal mites.

Emaciated pigeons are 30.8% more likely to be infested by *Tinaminyssus* spp. than their better-conditioned counterparts (Table 2). However, there was no difference in mean intensity between emaciated and well-conditioned hosts (Table 2). There was a concern that hosts salvaged from a rehabilitation centre would be biased towards emaciation. This did not seem to be the case in our study, since the average BCS was 3.0 and ranged from 1.0–4.5 out of 5. If the mites were the cause of the host's poor condition; the expected result would be elevated mean intensities among infested birds. Emaciated birds may be more likely to become infested by nasal mites if host immunity is compromised. Another unknown is the unexplored vector potential of rhinonyssids, with metagenomic analyses of *T. melloi* finding evidence of a bacterial endosymbiont in the family Bartonellaceae (Osuna-Mascaró *et al.* 2020, 2021). Along the same lines, 5% of splenic samples from feral pigeons taken by Ebani *et al.* (2016) (n=84) were infected with *Bartonella* spp. (family: Bartonellaceae) a genus where many species are transmitted by arthropods (Billeter *et al.* 2008). While the presence of a pathogen in a potential vector and host does not prove transmission this vector hypothesis could account for the lack of association between mean intensity and poor host body condition.

Other variables such as the age of the host could be independently associated with increased prevalence and with poor body condition; potentially driven by internal factors such as immunity. Increased prevalence of nasal mites with increased age has been previously documented among herring gulls (*Larus argentatus* Pontoppidan) by TerBush (1963) and sooty terns (*Onychoprion fuscatus* (Linnaeus)) (Amerson 1967). Among rock pigeons, Mascarenhas *et al.* (2022) found higher prevalence of *T. melloi* among sexually mature adults when compared to immatures; supporting the hypothesis that there is higher prevalence of nasal mites among older

birds as they have had higher probability of acquiring them through social behaviours suggested by TerBush (1963) and Amerson (1967).

This work may interest pigeon fanciers, ornithologists, acarologists and parasitologists. Infestation parameters of *Tinaminyssus* spp. were established with a robust sample size (n=615) with a prevalence of 52.4% and mean intensity of 14.93. Infestation parameters and co-infestations of *T. melloi* and *T. columbae* were determined (n=75), with *T. melloi* being present in higher levels and similar prevalence between species. Statistically greater prevalence among emaciated hosts is reported for the first time. These findings may be useful to infer health effects of Rhinonyssidae on rare and endangered bird species. Furthermore, we demonstrated how body condition scores are an efficient and informative variable to include when conducting studies pertaining to avian health.

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