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Research Article

Estimation of Ectoparasites in an African Ground Squirrel

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Abstract

Studying the parasites of wildlife necessitates the accurate estimate of ectoparasites of free-ranging animals, often in a field setting. The objective of this study was to test the relative accuracy of ectoparasite estimate in a rodent species, the Southern African ground squirrel (Xerus inauris). *Estimates of ectoparasites using combing were compared to total counts of ectoparasites on sacrificed animals. Results suggest that our combing method and visual inspection was a reliable method to estimate flea and lice intensity and abundance for* Xerus inauris *species. However, differences were found in prevalence of these parasites between estimated and total collected, as the total was 1.5 times that of the estimates. These results demonstrate successful estimation of parasites in a live small mammal species without requiring anaesthesia.*

Keywords: Ectoparasites, Field Techniques, Fleas, Lice, Squirrels

1 Introduction

Parasites can have high impacts on hosts in terms of fitness (survival and reproduction) and more directly on their behaviour and physiology[1]. Host parasite infestations are usually extremely variable, with a high proportion of parasites concentrated in a few host individuals[2, 3]. Infestation includes a minimum of one parasite per host. To understand the factors that can influence infestation and ultimately variances in the fitness of hosts, it is important to have accurate parasite estimates[4]. Absolute and mean values can be of limited use in most parasitology studies due to the over-dispersion of parasite distributions[2]. The most common measures to assess parasites are estimates of prevalence (percentage or proportion of hosts with the parasitic infestation) and intensity (quantity of parasites on infested hosts[3]). However, these measures rely on accurate estimates of the parasites, which are often difficult to assess under field conditions where sacrificing the host is not an option[4].

In mammals, a common technique to quantify parasites include live-animal combing to estimate the relative infestation of ectoparasites[5, 6]. The accuracy of the combing method is controversial due to parasite specific differences such as duration of attachment, parasite size and mobility, as well as variances in host size, age, skin type, immune status, ectoparasite density, and even differences in the time spent examining hosts by the researcher[7]. This controversy requires methodological studies to assess the accuracy of the combing technique in difference species to assess parasitic infestation.

Ectoparasite estimation can be influenced by the species, morphology, and behaviour of both the host and the parasite, as well as magnitude of infestation, time available for inspection, and experience of the investigator[7]. Due to these variations, studies have addressed potential parasite sampling errors by using subsampling. Subsampling techniques aim to standardize the estimation process by either decreasing the time spent examining individuals, using a predefined sampling time, or to decrease the surface area that is examined. Thus, by examining certain parts of the individual, subsampling can be used as a procedure to evaluate relative quantities of parasites with less chance of sampling errors[7]. Previous studies have found samples of ectoparasites by paying specific attention to the ears, face and genital areas of small mammals after combing for ectoparasites[8]. Another study in gerbils (*Gerbillus andersoni*) demonstrated aggregation patterns of mites and ticks to the mouth, ears, nose, and hind legs[9].

The objective of this study was to assess the relative accuracy of parasite estimates in a small mammal, the Cape ground squirrel *Xerus inauris*, in a field setting. We compared these field estimates of parasites to the number of parasites collected after subsequent sacrificing of the animal. Hitherto, we will refer the number of ectoparasites found on the sacrificed animal as the "total collected" ectoparasites. If combing is a good relative estimate of ectoparasite infection, then the total ectoparasites collected will positively correlate with combing estimates.

2 Materials and Methods

2.1 Study Species, Trapping and Handling

Cape ground squirrels are a highly social, semi-fossorial species of squirrel living throughout the arid regions of southern Africa [10]. The ectoparasites recorded on Cape ground squirrels include fleas (*Ctenocephalides connatus, Echidnophaga bradyta, Echidnophaga gallinacea, Synosternus caffer, Chiastopsylla rossi, Demeillonia granti, Pulex irritans*), lice *(Neohaematopinus faurei)*, and ticks *(Rhipicephalus theieri)*[11,12]. Only ectoparasites large enough for visual observation without the need of a microscope were collected during this procedure. The study was conducted on two farms near Bultfontein, Free State Province, South Africa (28.28°S, 26.15°E) in July 2013, where squirrels are routinely removed as control measures to reduce crop damage. We handled all squirrels that had been captured in live traps (Tomahawk 15x15x50cm) using techniques described in Hillegass et al.[6], where squirrels were immediately placed in a cloth handling bag.

We immediately estimated ectoparasite numbers by combing three strokes on the left, middle and right plane of the animals back, from the shoulders to the base of the tail with a metal flea comb. Any collected ectoparasites (fleas, ticks, and lice) were placed into a petri dish with 70% ethanol and counted. We modified Hillegass et al.'s[6] procedure by also including a careful examination of the groin and inner thighs of the squirrel and collecting any visible ectoparasites from these areas using forceps. Upon completion of ectoparasite combing, squirrels were given to local farmers and we received carcasses from the farmers following euthanization by chloroform placed on cotton pads, the best method to collect ectoparasites[13,14,15]. The carcasses were then held by the tail or foot over a white paper in an enamel tray and the entire body was rubbed to remove the remaining ectoparasites[16].

We also searched all parts of the body, handling bag and tray and removed any remaining visible parasites. All collected fleas, lice and ticks were counted and stored in 70% ethanol. As ticks are rarely found on *Xerus inauris*[6], we found only four ticks in total. These ticks were excluded in this analysis. Mites were also excluded from this study as they are not collectable by combing methods. All trapping and handling was in accordance with the American Society of Mammalogists' guidelines[15], and the University of Manitoba Animal Care Committee (Protocol #F10-030).

The abundance (mean number of parasites found on all individuals), prevalence and intensity of the parasites, were calculated using Quantitative Parasitology 3.0 web version[17,3]. We used exact unconditional 95% confidence intervals to estimate prevalence of infestation[18] and sign tests to compare our estimates and total counts. A 2000 replication bootstrap with replacement two-sample t-test was used to compare abundance and mean intensity and for all analyses we reported 95% confidence intervals (CI) using Clopper-Pearson estimates, unless otherwise specified[3]. We used JMP© 10.0 (SAS Institute Inc., Cary, North Carolina, USA) for our sign, Spearman's correlation, and Wilcoxon signed ranked tests.

3 Results

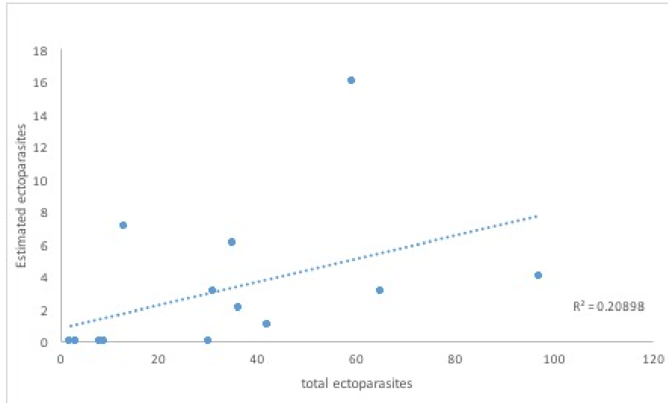

Figure 1: *Estimated ectoparasites from 15* Xerus inauris *hosts using a combing method were positively correlated with total number of ectoparasites (Spearman's correlation, $P < 0.05$).*

A total of 15 ground squirrels were collected, including eight females and seven males. The confidence intervals of overall ectoparasite prevalence from before and after euthanasia overlapped (Table 1), but prevalence of all species was higher in the euthanized estimates (sign test: $M = 3$, $P = 0.03$). Differences in the prevalence of lice were also significant (sign test: $M = 6$, $P = 0.0005$) while fleas were close to significantly different (sign test: $M = 2.5$, $P = 0.063$). As expected, median intensities of live estimated ectoparasites were lower than the median intensities of total ectoparasites (mean intensity was only 6.5±2.6% of total intensity; Table 1) and we found similar lower intensities in estimates of fleas (21.7±0.07%) and lice (0.64±0.46%).

However, the relative abundances of ectoparasites were reflected in our estimates. We found a significant relationship between the total estimated mean abundance of ectoparasites and the total ectoparasite abundance of sacrificed squirrels (Spearman's correlation; $\rho = 0.666$; $P = 0.007$; $n = 15$; Figure 1). The estimated mean abundance of fleas (2.5; CI = 1.13-4.87) was correlated with total flea abundance (Spearman's correlation: $\rho = 0.702$; $P = 0.0035$; $n = 15$) and the estimated lice abundance was also correlated with total lice abundance ($\rho = 0.504$; $P = 0.055$; $n = 15$).

Table 1: *Estimated and total prevalence, intensity, and abundance results of ectoparasites on Cape ground squirrels (n=15).*

n	Prevalence total (%)	Prevalence estimated	Mean Intensity total	Mean Intensity estimated	Median Intensity total	Median Intensity estimated	Mean Abundance total	Mean Abundance estimated
Total	100.0	60.0	39.8	4.8	34.0	3.0	39.8	2.9
15	(78.2-100.0)	(32.3-83.7)	(24.6-61.0)	(2.7-8.9)	(8.0-68.0)*	(1.0-7.0)**	(23.5-60.9)	(1.4-5.9)
Fleas	93.3	60.0	12.6	4.1	6.5	3.0	11.7	2.5
15	(68.1-99.8)	(32.3-83.7)	(7.4-21.2)	(2.2-7.2)	(2.0-16.0)	(1.0-7.0)	(6.5-19.4)	(1.1-4.9)
Lice	100.0	20.0	27.4	1.3	25.0	1.00	27.4	0.3
15	(78.2-100.0)	(4.3-48.1)	(14.5-47.0)	(1.0-1.7)	(1.0-31.0)***		(14.7-48.2)	(0.0-0.6)

Parentheses indicate 95% confidence intervals unless otherwise specified by an asterisk (*96.5-96.9% CI; **97.0-97.1%CI; ***98.2% CI).

4 Discussion

Our estimates via the combing method correlated positively with the overall parasite amounts collected post-mortem and may have accurately predicted the total counts up to a point, but with an associated error rate. As well, the abundance of fleas and lice between estimated and total measurements were significantly correlated. In this study, we were only concerned with parasite species visible to the naked eye as in field estimation of parasites, equipment is not always available. We do acknowledge the limitations of the combing method, as it does not accurately detect very small ectoparasites, and data collection requiring finer ectoparasite counts or a smaller error rate should avoid the combing method.

As well, previous ectoparasite collection from *Xerus inauris* was performed by combing the individuals from the shoulders to the base of the tail in the two lateral and one medial planes of the back[19]. Thus, we used the modified Hillegass et al.[6] combing technique as previously stated with special attention to the groin and inner thigh of the individuals in attempts to produce the most accurate estimation of the parasites. Heckenberg et al.[20], using a similar combing technique (without our direct observation of the groin and thigh) on dogs (*Canis lupus familiaris*), recovered between 67 and 75% of the total flea burden.

In comparison, Eads et al.[21] used a combing technique on anesthetized prairie dogs (*Cynomys ludovicianus*). They combed for three fifteen second intervals including combing the dorsal, lateral and ventral surfaces without using direct observation and detected a 5.4% error in prevalence if only one 15 second comb was used. There are other studies that also suggest an accurate estimation method of lice may be one that involves anesthetizing the animals and using combing with visual estimation or the use of a fumigant insecticide powder[22, 13, 16]. However, while these methods may allow for more thorough checking of the animal, they also require more handling and stress to the subject as well as the risks and prolonged recovery times of anesthetic use (including numerous undesirable side effects such as regurgitation, aspiration and hypothermia[23]). Our results suggest that combing and visual inspection result in good estimates of relative parasite prevalence, intensity and abundance for both fleas and lice without a need to use any anesthetic.

One of the reasons why tick abundance and prevalence is low in this study could be the seasonal pattern of ticks occurring on this mammal, as the study was done only in winter. Also most of the tick's lifecycle is not on the host, thus those mammals with a larger body mass (more surface area) and those that travel long distances (more chances to become infected with ticks), are more likely to be parasitized by ticks. Ticks are also most often found on vegetation and the larger the host species, the more likely it is to come into contact with the surrounding vegetation and become infested by ticks[24]. *Xerus inauris* is a small species, which may relate to the low density of ticks found on their bodies.

We hope future studies will focus on estimation of different species of ectoparasites to see if there is a pattern amongst or between species, and if there is a correlation amongst parasites that are too small to be estimated without using specialized equipment. In addition, it would be interesting to repeat this study in different seasons to account for varying parasite patterns throughout the year. To conclude, our data suggest that the combing method was a reliable method to estimate fleas and lice in Cape ground squirrels.

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References

1. Folstad, I. & Karter, A. J. 1992. *The American Naturalist*, 139: 603–622.
2. Neuhauser, M. & Poulin, R. 2004. *Journal of Parasitology*, 90: 689–691, doi:http://dx.doi.org/10.1645/GE-256R.
3. Rozsa, L., Reiczigel, J., & Majoros, G. 2000. *Journal of Parasitology*, 86: 228–232.
4. Heinzmann, D. & Torgerson, P. R. 2008. *Parasite*, 15: 477–483.
5. Gooderham, K. & Schulte-hostedde, A. 2011. *Behavioral Ecology*, 22: 1195–1200, doi:10.1093/beheco/arr112.
6. Hillegass, M., Waterman, J., & Roth, J. 2008. *Behavioral Ecology*, 19: 1006–1011, doi:10.1093/beheco/arn070.
7. Wilson, M. 1994. *Ecological Dynamics of Tick-Borne Zoonoses*. Oxford University Press, New York.
8. Guerra, A., Eckerlin, R., Dowling, A., *et al.* 2016. *Journal of Medical Entomology*, 53: 851–860.
9. Hawlena, H., Abramsky, Z., & Krasnov, B. R. 2006. *Oecologia*, 148: 30–39, doi:10.1007/s00442-005-0345-4.
10. Skurski, D. A. & Waterman, J. M. 2005. *Mammalian Species*, 781: 1–4.
11. De Graaff, G. 1981. *The rodents of southern Africa*. Butterworths, Pretoria.
12. Segerman, J. 1995. *South AFrican Institute for Medical Research*, 264.
13. Clayton, D. H. & Drown, D. M. 2001. *Journal of Parasitology*, 87: 1291–1300.
14. Manjerovic, M. B., Kinahan, A. A., Waterman, J. M., *et al.* 2008. *Journal of Zoology*, 275: 375–380, doi:10.1111/j.1469-7998.2008.00449.x.
15. Sikes, R., Care, A., & of the American Society of Mammologists, U. C. 2016. *Journal of Mammology*, 97: 663–688, doi:10.1093/jmammal/gyw078.
16. Barnett, A. & Dutton, J. 1995. *Expedition Field Techniques: Small mammals (excluding bats)*, volume 44.
17. Reiczigel, J., Rozsa, L., Reiczigel, A., *et al.*, Quantitative Parasitology (QPweb).
18. Reiczigel, J., Abonyi-toth, Z., & Singer, J. 2008. *Computational Statistics and Data Analysis*, 52: 5046–5053, doi:10.1016/j.csda.2008.04.032.
19. Scantlebury, M., Waterman, J. M., Hillegass, M., *et al.* 2007. *Proceedings of the Royal Society B*, 274: 2169–2177, doi:10.1098/rspb.2007.0690.
20. Heckenberg, K., Costa, S. D., Gregory, L. M., *et al.* 1994. *Veterinary Parasitology*, 53: 153–157, doi:10.1016/0304-4017(94)90027-2.
21. Eads, D. A., Biggins, D. E., Doherty, P. F., *et al.* 2013. *International Journal for Parasitology: Parasites and Wildlife*, 2: 246–256, doi:10.1016/j.ijppaw.2013.09.002.
22. Perez-orella, C. & Schulte-hostedde, A. I. 2005. *Canadian Journal of Zoology*, 83: 1381–1385, doi:10.1139/Z05-126.
23. Flecknell, P. 2015. *Laboratory Animal Anaesthesia*. 4th edition, Academic Press.
24. Sponchiado, J., Melo, G. L., Martins, T. F., *et al.* 2017. *Parasitology*, 144: 475–483.