Restricted evaluation of *Trichodectes canis* (Phthiraptera: Trichodectidae) detection methods in Alaska gray wolves

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ABSTRACT

*Trichodectes canis* (Phthiraptera: Trichodectidae) was first documented on Alaska (USA) gray wolves (*Canis lupus*) on the Kenai Peninsula in 1981. In subsequent years, numerous wolves exhibited visually apparent, moderate to severe infestations. Currently, the Alaska Department of Fish and Game utilizes visual inspection, histopathology, and potassium hydroxide (KOH) hide digestion for *T. canis* detection. Our objective was to determine optimal sampling locations for *T. canis* detection. Wolf hides were subjected to lice enumeration using KOH hide digestion. Thirty-nine of the 120 wolves examined had lice. Of these 39, total louse burdens ranged from 14 to an extrapolated 80,000. The hides of 12 infested animals were divided into 10 cm by 10 cm subsections and the lice enumerated on a subsection from each of four regions: neck; shoulder; groin; and rump. Combining the data from these 12 wolves, the highest mean proportions of the total louse burdens were found on the rump, and this section had the highest mean proportion on the neck. However, examination of the four subsections failed to detect all infested wolves. Hides from 16 of the 39 infested animals were cut into left and right sides, and each side then cut into four, approximately equal sections: neck and shoulder; chest; abdomen; and rump. Half hides were totally digested from 11 wolves, and whole hides from 5. For these 21 half hides, the highest mean proportions of total louse burdens were found on the rump, and this section had the highest sensitivity for louse detection, regardless of burden. However, removal of this large section from a hide would likely be opposed by hunters and trappers.

1. Introduction

In 1981, *Trichodectes canis* (Phthiraptera; Trichodectidae), an ectoparasite of canids, was first documented on gray wolves (*Canis lupus*) in Alaska, USA, from the Kenai Peninsula (Durdan, 2001; Schwartz et al., 1983). Observed wolves exhibited high prevalence of moderate to severe pediculosis (lice infestation). In March 1983, the Alaska Department of Fish and Game (ADF&G) initiated a management program to eradicate *T. canis* infestation of wolves. The program attempted to identify and treat all infested wolves utilizing visual examination, live-capture/release, and administration of the antiparasitic drug ivermectin (Ivomec®, Merial Limited, Duluth, Georgia, USA) (Taylor and Spraker, 1983; Zarnke, 1985). The program was unsuccessful largely due to difficulty in detection of mild pediculosis and the inability to conduct multiple treatments of infested wolves (Masteller, 2000). Since that time, the infestation has spread north of the Alaska Range (Gardner et al., 2013). At present, ADF&G uses a combination of visual inspection and skin biopsy from live wolves, histopathologic examination of representative skin samples, and KOH hide dissolution for detection of *T. canis* from deceased wolves (Gardner et al., 2013). In general, KOH hide digestion for the entire host integument appears to have the highest sensitivity and specificity for lice detection as compared to visual or histopathologic examination (Clayton and Drown, 2001). However, complete KOH hide digestion is a time-consuming procedure that destroys the entire wolf hide and lice specimen integrity. It would be advantageous if wolf hides could be inspected for *T. canis* by examining a smaller, well-defined region that is consistently infested but not of high market value. Rather than destroying the entire pelt, skin sampling could be utilized for surveillance of hunter and trapper harvested hides with limited objection to the use of this sensitive but destructive technique.

Currently, optimal sample locations for *T. canis* detection within gray wolves are undefined and it is unknown if the severity of
pediculosis influences prime sample locations. While moderate to severe pediculosis can be detected by a trained individual through visual examination, mild pediculosis can be easily overlooked. Our objective was to determine optimal sample locations for KOH hide digestion for *T. canis* detection within Alaska gray wolves.

2. Materials and methods

Wolf hides and whole carcasses donated or purchased from harvesters and collected by ADF&G and US National Park Service were inspected for *T. canis*. Collected lice specimens were archived in the University of Alaska Museum Insect Collection under catalog number UAM_Ento83005. Detection and enumeration of *T. canis* utilizing KOH hide digestion was conducted using a modification of the methods by Welch and Samuel (1989). From each fleshed or dried hide, the limbs were removed at the elbow and hock, tail at the base, and the head immediately behind the ears. The remaining hide was cut into left and right sections along the midsagittal plane (Fig. 1). Two distinct methods of *T. canis* detection were utilized: complete hide digestion and restricted subsection sampling. For complete hide digestion, the right and left half hides were cut into four relatively equal sections using a chalk-line and numbered sequentially from the cranial to caudal region (Fig. 1). Sections were labeled on laundry tags with the corresponding wolf identification, side and section number then stored in sealed plastic bags at −20 °C. For restricted subsection sampling, a hide was subdivided into 10 × 10 cm subsections and representative subsections from the neck (subsection 1), shoulder (subsection 2), rump (subsection 3) and groin (subsection 4) regions (Fig. 2) were excised, labeled and stored as described earlier. Individual hide sections and subsections were digested separately in a 5% KOH solution in stainless steel pans for the larger hide sections and 1 L Erlenmeyer flasks for the 10 cm subsections.

The solution was composed of 2400 mL of tap water, 110.9 g KOH (Sigma-Aldrich, St. Louis, Missouri, USA), and 15 mL of liquid Dawn® detergent (The Procter & Gamble Company, Cincinnati, Ohio, USA) as a degreasing agent. Hide sections were completely submerged in the solution, incubated at 65–75 °C and stirred at least once every 30 min for 3–4 h until most of the hair and epidermis were dissolved, leaving lice exoskeletons.

Post-incubation solutions were filtered through a 180-μm sieve followed by a warm tap water rinse to remove residual KOH. A second degreasing step was conducted when fatty deposits were observed within the sieve. Filtered material was flushed into 1 L flasks, covered with tap water, sealed by Parafilm® (Structure Probe Inc., Chicago, Illinois, USA), and held at room temperature until examination within 48 h. For examination, samples were filtered and inspected under a dissecting microscope at 40× magnification and adult lice and nymphal instars were enumerated.

For complete hide digestion, pediculosis severity was classified based on parasite loads of nymph and adult life stages for each half hide. Severity of pediculosis was defined as: mild (≤200 total lice) or heavy (>200 total lice) per half hide. Additionally, a total of five wolves were subject to both right and left side complete hide digestion, and a matched pair analysis was conducted to compare left and right side lice proportions of the five completely digested wolves.

Due to the disparity between absolute numbers of lice for heavy and mild infestations, each section total was expressed as a normalized proportion of half hide using arcsine-root transformations. Parasite density was based on restricted subsection sampling and using arcsine-root transformations, was expressed as a normalized proportion of *T. canis* non-egg life stages density for all four representative subsections obtained for an individual wolf (Fig. 2). Lice density in the subsections were defined as: mild (≤0.5 lice per 100 cm²) or heavy (>0.5 lice per 100 cm²).

Analysis of subsection sample locations was conducted using a two-way analysis of variance (ANOVA) in the statistical program JMP (SAS Institute Inc., Cary, North Carolina, USA). Multiple comparisons were assessed with the Tukey honestly significant difference (HSD) test (Sall and Lehman, 1996). To determine if the degree of pediculosis influenced distribution of lice on the body, a two-way ANOVA was conducted separately for hides based on the degree of pediculosis.

3. Results and discussion

From December 2003 to February 2009, a total of 120 whole wolf carcasses were examined, 39 of which were found infested with *T. canis*. Of the 39 wolves, 16 were subject to half hide digestion, and 12 wolves were subjected to restricted subsection digestion. Eight of the 16 were classified as having mild pediculosis and eight as having heavy pediculosis.

Comparing the two half hides from a single wolf, lice proportions for each side did not differ significantly (t = 1.14, P = 0.318) and were considered identical for our analysis. Lice burdens for the 48 10 × 10 cm hide samples from the 12 wolves subject to restricted subsection sampling were analyzed to compare lice densities in animals with mild and heavy pediculosis; there was no significant difference between the two groups (F = 3.70, P = 0.0185) and they were grouped together for analysis. The highest mean proportion of *T. canis* was recorded on the rump subsection, which was significantly different only from the lowest mean proportion in the neck subsection (Table 1). None of the four 10 × 10 cm hide subsections were 100% sensitive for *T. canis* detection. Examination of the rump subsections of the 12 wolves failed to detect three wolves infested with *T. canis* and the shoulder, neck and groin subsections each failed to detect infested wolves.

The 11 wolves subject to digestion of a half hide, together with the 5 subject to digestion of the whole hide, resulted in the examination of 21 half hides which generated 84 samples, each one-eighth of a hide. Lice proportions differed significantly between the

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**Fig. 1.** Divisions of wolf half hides designated for lice proportion analysis utilizing potassium hydroxide digestion. Wolf hides were cut in half and the right and left subdivided into four relatively equal sections and numbered sequentially from the neck to the tail base.

**Fig. 2.** Divisions of wolf hide into 100 cm² subsections for lice density analysis utilizing potassium hydroxide digestion. Each hide subsection square represents 10 cm by 10 cm, one representative section from each region was examined: 1 from the neck; 2 from the shoulder; 3 from the groin; 4 from the rump.
Currently, it is unknown why the groin and rump region of the wolf is favored by *T. canis*, whereas the neck area possesses the lowest proportion of adult and nymphal instars of lice. The pelage of the groin is relatively thin as compared to the mane of the wolf, which possesses long erectile guard hairs (Mech, 1970). It is possible that these areas possess different microclimates and could affect the survival of *T. canis*. Currently the range of environmental conditions favored by *T. canis*, and how the progression of clinical signs of pediculosis affects lice distribution and mortality is unknown.

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References

Clayton, D.H., Brown, D.M., 2001. Critical evaluation of five methods for quantifying chewing lice (Insecta: Phthiraptera). J. Parasitol. 86, 1291–1300.
Darden, L.A., 2001. Lice (Phthiraptera). In: Samuel, W.M., Pybus, M.J., Rocan, A.A. (Eds.), Parasitic Diseases of Wild Mammals. Iowa State University Press, Ames, Iowa, pp. 3–17.
Gardner, C.L., Beckmen, K.B., Pamperin, N.J., Del Vecchio, P., 2013. Experimental treatment of dog lice infestation in interior Alaska wolf packs. J. Wildl. Manage. 77, 626–632.
Masteller, M., 2000. Unit 14 wolf management report. In: Hicks, M.V. (Ed.), Wolf Management Report of Survey and Inventory Activities 1 July 1996–30 June 1999. Alaska Department of Fish and Game, Juneau, Alaska, pp. 88–112.
Mech, D.L., 1970. The wolf itself. In: Mech, D.L. (Ed.), The Wolf: the Ecology and Behavior of an Endangered Species. University of Minnesota Press, Minneapolis, MN, pp. 1–37.
Sall, J., Lehman, A., 1996. JMP Start Statistics: a Guide to Statistics and Data Analysis Using JMP and JMP in Software. SAS Institute Inc., Duxbury Press, Belmont, CA, 628 pp.
Schwartz, C.C., Stephenson, R., Wilson, N., 1983. *Trichodectes canis* on the gray wolf and coyote on Kenai Peninsula, Alaska. J. Wildl. Dis. 19, 372–373.
Taylor, W.P., Spraker, T.H., 1983. Management of a biting louse infestation in a free-ranging wolf population. In: Fowler, M.E. (Ed.), Annual Proceedings: American Association of Zoo Veterinarians. American Association of Zoo Veterinarians, Atlanta, Georgia, pp. 40–41.
Watson, D.W., Lloyd, J.E., Kumar, E., 1997. Density and distribution of cattle lice (Phthiraptera: Haematopinidae, Linognathidae, Trichodectidae) on six steers. Vet. Parasitol. 69, 283–296.
Welch, D.A., Samuel, W.M., 1989. Evaluation of random sampling for estimating density of winter ticks (*Dermacentor albipictus*). Int. J. Parasitol. 19, 691–694.
Zarnike, R.L., 1985. Experimental investigations of *Trichodectes canis* louse infestation in wolves. Alaska Department of Fish and Game. Federal Aid in Wildlife Restoration. Research Final Report. Grants W-22-3, W-22-4. Job 18.9. Juneau, Alaska, 4 pp.