A Technique for Dissecting the Salivary Glands From the Abdomens of Deer Keds (Diptera: Hippoboscidae: Lipoptena Nitzsch, 1818 and Neolipoptena Bequaert, 1942)

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Subject Editor: Phyllis Weintraub
Received 11 February 2020; Editorial decision 19 March 2020

Abstract
Deer keds (Diptera: Hippoboscidae: Lipoptena Nitzsch, 1818 and Neolipoptena Bequaert, 1942) are hematophagous ectoparasites of cervids that occasionally bite other mammals, including humans. In recent years, a number of arthropod-borne pathogens have been sequenced from deer keds. However, it is unclear if the pathogens are just present in host blood in the gut or if the pathogens are present in other organs (e.g., salivary glands) that would suggest that keds are competent vectors. Like other hippoboscoid flies, deer keds have extensive salivary glands that extend through the thorax and into the abdomen, so simply disarticulating and sequencing the thorax and abdomen separately does not circumvent the issues surrounding whole-body sequencing. Herein, we describe a technique for dissecting the terminal portion of the salivary glands from the abdomen in order to screen the thorax and salivary glands separately from the abdomen for arthropod-borne pathogens.

Key words: deer ked, hippoboscid, dissection, salivary gland, pathogen screening

Deer keds (Diptera: Hippoboscidae: Lipopteninae: Lipoptena Nitzsch, 1818 and Neolipoptena Bequaert, 1942) (Fig. 1) are hematophagous ectoparasites of cervids that occasionally bite other mammals, including humans (Skvarla and Machtinger 2019). Thirty-two species are described worldwide, four of which occur in North America: Lipoptena cervi (Linnaeus, 1758) (northeastern United States and Canada), L. mazamae Rondani, 1878 (southeastern United States south through Brazil), and L. depressa (Say, 1823) and Neolipoptena ferrisi Bequaert, 1942 (western United States and Canada) (see Figure 3 in Skvarla and Machtinger 2019 for a range map).

Deer keds have historically been regarded as pests of only minor veterinary and medical importance. However, in Europe, reindeer (Rangifer t. tarandus) (Linnaeus, 1758); Artiodactyla: Cervidae) exhibit increased head shaking, grooming, and other stress-related signs during ked infestations (Kynkäänniemi et al. 2014) and moose (Alces alces Linnaeus, 1758; Artiodactyla: Cervidae) in northern Europe have developed hair loss and decline in condition during extremely heavy infestations (Madslien et al. 2011). Similar conditions have been less studied in North America, although Kellogg et al. (1971) observed that keds are a ‘constant annoyance to wild deer’ and Heine et al. (2017) showed that keds may be more influential on deer-grooming behavior than ticks. In northern Europe, deer keds can become so numerous that they are associated with ked bite dermatitis and considered an occupational hazard (Chistyakov 1968, Laukkanen et al. 2005, Härkönen et al. 2009). Similar ‘hotspots’ of deer ked abundance and activity have been anecdotally reported by hunters in North America but do not appear to be as widespread a phenomenon.

Deer keds have not been considered vectors of arthropod-borne pathogens historically (Skvarla et al. 2019). However, in recent years, a number of tick-borne pathogens have been sequenced from deer keds, including Anaplasma (Rickettsiales: Anaplasmataceae) (e.g., de Bruin et al. 2015, Buss et al. 2016), Bartonella (Rhizobiales: Bartonellaceae) (e.g., de Bruin et al. 2015, Izenour et al. 2020), and Rickettsia (Rickettsiales: Rickettsiaceae) (e.g., Hornok et al. 2011, de Bruin et al. 2015) (see Table 1 in Skvarla and Machtinger 2019 for a list of pathogen publications). It has even been suggested that the aforementioned ked-associated dermatitis may be caused by a ked-vectored Bartonella species (Dehio et al. 2004).

To date, every study that has screened adult deer keds for pathogens has been based on whole-body extractions (see Table 1 in Skvarla and Machtinger 2019 for a list of publications). It is thus unclear if the pathogens detected in positive samples are from host blood contained in ked digestive tracts or if they are present in other organs, such as the salivary glands (e.g., Baker 1967, Klei and Giusti 1973, Ueti et al. 2007, Lejaf et al. 2019), which might support the
idea that keds are competent pathogen vectors. One possible solution would be to screen the digestive tract, and any host blood it might contain, separately from the rest of the body, and it might seem intuitive to simply separate the head and thorax from the abdomen, which contains the majority of the digestive tract. However, deer keds have extensive salivary glands that extend through the thorax into the anterodorsal area of the abdomen (Figure 15 in Bequaert 1953), which would confound such efforts. While there are portions of the digestive tract in the thorax, they are not used to store blood meals and unlikely to contain host blood unless a deer ked was collected during or immediately after feeding—the crop is the largest section of the digestive tract in the thorax, but Bequaert (1953) found that it does not fill with or store blood in Lipoptena, unlike in the bird-feeding keds (Ornithomyinae), and the portion of the diverticulum that is used to store extra blood is confined to the abdomen (Theodor 1928, Tarshis 1957).

Because deer keds are small, highly sclerotized, dorsoventrally flattened ectoparasites and their salivary glands are soft, white organs attached to the mouthparts by a thin duct, it was not immediately obvious how to best separate the salivary glands from the abdomen. We found that attempting to access them through the dorsal abdominal cuticle was often not successful and devised the method for separation presented here, for which we provide step-by-step instructions and photomicrographs.

Fig. 1. Deer keds (L. cervi). Top row: dorsal. Bottom row: ventral. (a) Unfed ked with small abdomen. (b) Ked that has fed at least once with an expanded abdomen. (c) Ked with mature larva visible through the ventral abdomen.
Experimental Design

The tools we used to dissect deer keds are listed in Table 1. Manufacturer/vendor information is provided for each item; however, the exact materials listed do not need to be used. The first author learned how to create the dissection tools described here from Dr. Don Steinkraus at the University of Arkansas, who incorporated techniques previously suggested by Smith (1923), Dade (1962), and others.

Wax-bottomed Petri dishes (Fig. 2a) were made by melting dental wax in a double-boiler (an empty food can in a pot of boiling water) on a kitchen stove and pouring the hot wax into a 90 × 20 mm glass Petri dish, which was then allowed to cool overnight. Unlike pure paraffin wax, dental wax was used because it is stiff but not brittle. If dental wax is unavailable, a 1:1 mix of paraffin and beeswax can also be used.

Microscalpels and dissection probes (Fig. 2b) were manufactured using bamboo skewers, which were cut to a comfortable length (15–20 cm), microblades, and insect pins. Microscalpel blades were manufactured by securing a utility knife blade in a vice and breaking a corner from the blade using lineman’s pliers. A small split was made in a bamboo skewer using a utility knife and the scalpel blade was pushed into the split using lineman’s pliers and affixed with epoxy. This technique resulted in somewhat unusual microscalpels but was easily done with materials available at hand. If more uniform blades are desired, breakable scalpel blades and blade breakers are available.

Table 1. Materials used to dissect deer keds

| Item                              | Manufacturer/vendor     | Catalog/item number |
|-----------------------------------|-------------------------|---------------------|
| Fine-point forceps                | Bioquip                 | 4535                |
| Insect pins, #2                   | Bioquip                 | 1208B2              |
| Insect pins, #1                   | Bioquip                 | 1208B1              |
| Wax-bottom Petri dish             | Custom made             | N/A                 |
| Petri dish, glass, 100 × 20 mm    | VWR                     | 75845-514           |
| Dental wax                        | N/A                     |                     |
| Microscalpel                      | Custom made             | N/A                 |
| Dissection probe                  | Custom made             | N/A                 |
| Utility knife replacement blade   | Workpro (Amazon)        | N/A                 |
| Breakable scalpel blade           | Fine Science Tools      | 10050-00            |
| Blade breaker                     | Fine Science Tools      | 10052-11            |
| Bamboo skewer                     | Amazon                  | N/A                 |
| Lineman’s pliers                  | Home Depot/Husky        | 48057               |
| Diagonal cutting pliers           | Home Depot/Husky        | 48056               |
| Epoxy resin (ClearWeld)           | Home Depot/JB Weld      | 50114H              |
| 70% ethanol (diluted from 100%)   | Koptec                  | V1001               |
| SZ-61 stereomicroscope (0.75–13.5 magnification) | Olympus                | SZ-6145             |
| Iris scissors                     | Bioquip                 | 4715                |

Protocol

The protocol described here is best performed on wingless deer keds that have fed at least once (Fig. 1a) and have been stored in 70–80% ethanol. Such keds are most easily collected from host animals. Keds that have not fed, such as winged specimens that land on people, have much smaller abdomens (Fig. 1b). Because there is no risk of sequencing pathogens from host blood in unfed keds, the dissection outlined here is unnecessary for pathogen screening; however, unfed keds are more difficult to collect in large quantities. Specimens stored in higher percentage ethanol or isopropanol can become brittle and break easily during dissection.

Begin by adding enough 70% ethanol to the wax Petri dish to fully submerge a deer ked, but not so much that it splashes if you move the dish. Transfer a ked to the Petri dish and remove the middle and hind legs using iris scissors or a microscalpel. Once the

Dissection probes were made by cutting off the apical 1–2 cm of an insect pin using diagonal cutting (or side-cutting) pliers and pushing the cut pin into a bamboo skewer using lineman’s pliers. Cutting different sized pins (#0–3) and creating multiple probes of various sizes is recommended. Probes manufactured using #0 pins were found to dull after several dissections, so if many dissections are planned, it may be worthwhile to use sharpenable tungsten wire probes (for materials and methods, see Brady 1965).
legs are removed, secure the ked to the wax ventral-side up using two #2 or #3 pins across the thorax just behind the front legs (Fig. 3a). Once the ked is secured in position, hold the abdomen in place with forceps and cut the cuticle along the lateral edge; the cuticle of the first ventrite is more sclerotized, but can be carefully cut with a microscalpel. The juncture of the thorax and abdomen is thin, so be sure not to cut completely through the abdomen when working that area. The entire venter can be cut away and removed or a small flap left along the posterior so that it can be pulled back like a flap (Fig. 3b).

Next, use two probes to gently loosen and remove the posterior half of the digestive tract. If the digestive tract is empty, it should

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Fig. 3. Abdominal and salivary gland dissection process. (a) Ked with mid- and hind legs removed. (b) Ventral abdomen removed and pulled back. Specimen is a male, note the aedeagus and extensive accessory glands. (c) Male specimen with accessory glands removed. Note the blood-filled portion of the anterior digestive tract. (d,e) Female keds with a developing larva (indicated by arrow) and a fully mature larva. (f) Abdomen after the digestive tract has been removed. Salivary glands are indicated by arrows. (g) Ked post-dissection after salivary glands (indicated by arrows) have been isolated and abdomen has been removed.
remain malleable and come out intact (Fig. 3b). If the ked fed before being collected, there may still be partially digested blood in the digestive tract. If a bloodmeal is present (Fig. 3c), the digestive tract may be difficult to remove intact; however, if it breaks apart and blood is released, the blood should appear as solid clumps that can be pushed aside with a probe.

Because deer keds reproduce via adenotrophic viviparity, eggs and partially to fully developed larvae may be encountered (Fig. 3d and e). Fully developed larvae can often be seen through the cuticle prior to dissection (Fig. 1c). Eggs and larvae can be dissected from the uterus for separate pathogen screening if desired. Mature larvae are especially easy to remove as they are large and the membrane surrounding them is thin. However, because mature larvae take up so much space in the abdomen and consequently compact the surrounding viscera, it is often difficult to find and isolate the salivary glands without destroying the glands.

Once any eggs or larvae, testes and accessory glands, and most of the digestive tract have been removed, slowly tease apart the remaining parts of the anterior digestive tract. The two salivary glands should appear as white sac-shaped organs attached to thin ducts that enter the thorax (Fig. 3f). The glands are often appressed to the remaining parts of the anterior digestive tract. The two salivary glands intact. Other methods that were attempted (e.g., cutting around viscera, it is often difficult to find and isolate the salivary glands without destroying the glands.

With steady hands, patience, and a bit of luck, the salivary glands should now be free of the abdomen and majority of the digestive tract but still attached to the thorax. The dissected parts of the abdomen and head, thorax, and salivary glands can be transferred to separate containers for pathogen screening or other work. Occasionally one or both glands may be broken off so only the ducts remain. When this happens, it is sometimes possible to find the broken salivary gland amongst the digestive tract and extract it so that it can be processed separately from the digestive tract.

Discussion

With practice, the lead author was able to perform the described dissection in 8–10 min per specimen and consistently recover the salivary glands intact. Other methods that were attempted (e.g., cutting through the dorsal abdomen) may be slightly faster, but did not reliably produce intact salivary glands.

Tarshis (1955) described and illustrated a similar method for dissecting bird keds (Stillbometopha impressa (Bigot, 1885) and Icosta bursuta (Ferris, 1927)), which we independently arrived upon while dissecting out the salivary glands of deer keds. Much like how replication of experimental studies is needed to confirm the results of those studies, this ‘convergent evolution’ of techniques suggests that the techniques described by Tarshis (1955) and here are the most optimized way to dissect keds.

Bird keds are generally larger than deer keds, and the methods described by Tarshis (1955) are for finer, more precise dissections that likely take longer to perform. Because we are only interested in separating the salivary glands from the rest of the abdomen, the method presented here can be performed faster. Pairing the directions in the protocol section with the step-by-step photos will reduce the learning curve of those who attempt the described dissection for the first time.

Acknowledgments

We thank Jessica Brown, Carley Lionetto, Taylor Miller, Alex Pagac Hannah Tiffin, and our field volunteers for their assistance with collecting deer keds, deer processors who let us sample deer, and hunters who submitted deer keds through the PA Parasite Hunters community science program. This project was funded by the Penn State Extension Multistate and Integrated Program Grant.

References Cited

Baker, J. R. 1967. A review of the role played by the Hippoboscidae (Diptera) as vectors of endoparasites. J. Parasitol. 53: 412–418.

Bequaert, J. C. 1953. The Hippoboscidae or louse-flies (Diptera) of mammals and birds. Part I. Structure, physiology and natural history. Entomol. Am. 32: 1–442.

Brady, J. 1965. A simple technique for making very fine, durable dissecting needles by sharpening tungsten wires electrolytically. B. World Health Organ. 32: 134–144.

de Bruin, A., A. Doctors van Leeuwen, S. Jahnari, W. Takken, M. Földvári, L. Dremmel, H. Sprong, and G. Földvári. 2015. Vertical transmission of Bartonella schoenbuchensis in Lipoptena cervi. Parasites Vectors 8: 176.

Buss, M., L. Case, B. Kearney, C. Coleman, and J. D. Henning. 2016. Detection of Lyme disease and anaplasmosis pathogens via PCR in Pennsylvania deer ked. J. Vector Ecol. 41: 292–294.

Chistyakov, A. F. 1968. Skin lesions in people due to bite of Lipoptena cervi. Vestn. Dermatol. Venerol. 42: 59–62.

Dade, H. A. 1962. Anatomy and dissection of the honeybee. International Bee Research Association, Cardiff, United Kingdom.

Dehio, C., U. Sauder, and R. Hiestand. 2004. Isolation of Bartonella schoenbuchensis from Lipoptena cervi, a blood-sucking arthropod causing deer ked dermatitis. J. Clin. Microbiol. 42: 5320–5323.

Härkönen, S., M. Laine, M. Vornanen, and T. Reunala. 2009. Deer ked (Lipoptena cervi) dermatitis in humans – an increasing nuisance in Finland. Alces. 45: 73–79.

Heine, K. B., P. J. DeVries, and C. M. Penz. 2017. Parasitism and grooming behavior of a natural white-tailed deer population in Alabama. Ethology Ecol. Evol. 29: 292–303.

Hornok, S., J. de la Fuente, N. Biró, I. G. Fernández de Mera, M. L. Meli, V. Elek, E. Gönczi, T. Meili, B. Tánczos, R. Farkas, et al. 2011. First molecular evidence of Anaplasma ovis and Ricetettia spp. in keds (Diptera: Hippoboscidae) of sheep and wild ruminants. Vector Borne Zoonotic Dis. 11: 1319–1321.

Izenour, K., S. Zikeli, A. Kalalah, S. D. Ditchkoff, L. A. Starkey, C. Wang, and S. Zohdy. 2020. Diverse Bartonella spp. detected in white-tailed deer (Odocoileus virginianus) and associated keds (Lipoptena mazamae) in the southeastern United States. J. Wildl. Dis. 56: 1–7.

Kellogg, F. E., T. P. Kistner, R. K. Strickland, and R. R. Gerrish. 1971. Arthropod parasites collected from white-tailed deer. J. Med. Ent. 8: 495–498.

Klei, T. R., and D. L. De Giusti. 1973. Ultrastructural changes in salivary glands of Pseudolynchia canariensis (Diptera: Hippoboscidae) infested with sporozoites of Haemoproteus columbae. J. Invert. Pathol. 22: 321–328.

Kynkäänniemi, S. M., M. Kettu, R. Kortet, L. Härkönen, A. Kaitala, T. Paakkonen, A. M. Mustonen, P. Nieminen, S. Härkönen, H. Ylönen, et al. 2014. Acute impacts of the deer ked (Lipoptena cervi) infestation on reindeer (Rangifer tarandus tarandus) behaviour. Parasitol. Res. 113: 1489–1497.

Laukkanen, A., P. Ruooppi, and S. Mäkinen-Kiljunen. 2005. Deer ked-induced occupational allergic rhinoconjunctivitis. Ann. Allergy. Asthma Immunol. 94: 604–608.

Lejal, E., S. Moutailler, L. Šimo, M. Vaysier-Tausat, and T. Pollet. 2019. Tick-borne pathogen detection in midgut and salivary glands of adult Ixodes ricinus. Parasites Vectors 12: 152. doi:10.1186/s13071-019-3418-7

Madslien, K., B. Ytrehus, T. Vikøren, J. Malmsten, K. Isaksen, H. O. Hygen, and E. J. Solberg. 2011. Hair-loss epizoonic in moose (Alces alces) associated with massive deer ked (Lipoptena cervi) infestation. J. Wildl. Dis. 47: 893–906.
Skvarla, M. J., and E. T. Machtinger. 2019. Deer Keds (Diptera: Hippoboscidae: Lipoptena and Neolipoptena) in the United States and Canada: new state and county records, pathogen records, and an illustrated key to species. J. Med. Entomol. 56: 744–760.

Smith, R. H. 1923. Technique in studying by dissection the internal anatomy of small insects. Ann. Entomol. Soc. Am. 16: 277–278.

Tarshis, I. B. 1955. Transmission of Haemoproteus lophortyx O’Roke of the California quail by hippoboscid flies of the species Stilbometopa impressa (Bigot) and Lynchia hirsuta Ferris. Exp. Parasitol. 4: 464–492.

Tarshis, I. B. 1957. The diverticulum of Stilbometopa impressa (Bigot) as a functioning organ (Diptera: Hippoboscidae). Ann. Entomol. Soc. Am. 50: 519–521.

Theodor, O. 1928. Ueber ein nicht pathogenes Trypanosoma aus der Ziege und seine Übertragung durch Lipoptena caprina Austen. Zeitschr. F. Parasitenk. 1: 283–330.

Ueti, M. W., J. O. Reagan, Jr, D. P. Knowles, Jr, G. A. Scoles, V. Shkap, and G. H. Palmer. 2007. Identification of midgut and salivary glands as specific and distinct barriers to efficient tick-borne transmission of Anaplasma marginale. Infect. Immun. 75: 2959–2964.