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# Species Identification of Ovine-Feeding Mosquitoes in Southwestern Montana

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## Abstract

In order to contribute to research on Cache Valley Virus, an ovine virus that leads to fetus malformations and abortion, this study investigated the specific species of mosquitoes that blood feed on domestic sheep. Nine sheep were separated into three groups and were contained in a constructed trap one night per week for seven weeks (July-August 2011) in a rural area in southwestern Montana. Mosquitoes that entered the sheep-baited trap during the evening were then collected for species identification and bloodfed status the following morning. This study also examined the effectiveness of two different insecticide treatments. The three groups of sheep consisted of a control group, a group with Python insecticide ear tags, and a group with a permethrin pour-on. Bloodfed mosquitoes were found in each of the 16 mosquito species collected from the sheep-baited traps. The species collected from the sheep-baited traps were then compared to species collected from a CDC light trap located near the study site. The data collected from this study showed that in the sheep-baited traps *Ochlerotatus increpitus, Ochlerotatus idahoensis, and Aedes vexans* were the three most prevalent species; *Ochlerotatus dorsalis, Aedes vexans,* and *Ochlerotatus idahoensis* were the three most prevalent species in the light trap collections. Furthermore, *Cs. inornata, Oc. intrudens, Oc. sierrensis, Oc. implicatus* are potential reservoirs of CVV because they were present only in the sheep-baited traps. These results help to direct further investigations of potential vectors for Cache Valley Virus in Montana.

## Introduction

Little research has been conducted on mosquito species that feed on ovines (domestic sheep) and the economical and epidemiological effects of insect bloodfeeding. However, research investigating bloodfeeding mosquitoes on other domestic livestock has been conducted. Various species of mosquitoes have been known to feed on other domestic livestock, such as *Oc. dorsalis* and *Ochlerotatus melanimon* on ponies and Hereford cattle (Schmidtmann et al., 2001).

If specific species of mosquitoes that feed on ovines are known, it may be possible to identify those species that carry viruses, such as Cache Valley Virus (CVV) that cause fetal malformations in sheep. If a specific carrier of a virus is known, improved methods for mosquito control and prevention can be developed. The objective of this study was to identify which species of mosquitoes feed on domestic sheep in southwestern Montana in order to contribute to research conducted on the effects of bloodfeeding mosquitoes that have potential to infect ovines with CVV.

First detected in Cache Valley, Utah in 1956, Cache Valley Virus has since threatened ovine agriculture due to its ongoing spread throughout North America (Holden and Hess, 1959; Chung et al., 1990). Insect hosts of CVV have been detected in a wide range of climates including Canada, (Iversen et al., 1979) Northern Mexico, Jamaica, and Argentina (Tauro et al., 2009). Along with the diverse locations CVV has been identified, 26 mosquito species have been detected as potential CVV vectors in North America. Although CVV has not been detected in Montana, CVV has been detected in mosquitoes in states near Montana, including Oregon, Colorado,

Utah, and South Dakota (Calisher et al., 1986). Three mosquito vector species have also been detected in North Dakota (G. Johnson, pers. com., 2011).

Characteristic signs of CVV are birth defects and malformations in lambs. Birth defects include stillbirths, mummified fetuses, central nervous system defects and musculoskeletal problems (Chung et al., 1990). Infection in mammals has included: humans, sheep, cattle, horses, goats, swine, and wildlife such as white-tailed deer that may serve as amplifying reservoirs (Blackmore & Grimstad, 1998). Although rare, two cases of Cache Valley Virus have been detected in humans (Campbell et al., 2006).

A 1987 study conducted in San Angelo, TX, concluded that, of the ewes studied, 100% of those who gave birth to defected lambs infected with arthrogryposis hydranencephaly (AGH) tested positive for the CVV antibody and 62% of the ewes that gave birth to normal lambs also tested positive for CVV antibodies (Chung et al., 1990). This same study detected CVV antibodies in pre-colostrum lambs. This indicates that, because ewes only pass antibodies onto the lamb after birth through colostrum feeding, the virus is capable of directly infecting the fetus without having been passed on from the ewe (Chung et al., 1990). As Chung states, the infection rate of CVV is time sensitive. Such is the case in a similar virus, the Akabane virus, in which the majority of infections occur within the first 30-35 days of gestation, leading researchers to make a similar conclusion about the time sensitivity of CVV (Chung et al., 1990).

A previous study conducted near Laramie, WY, used CDC light traps and sheep-baited traps and found that, when mosquito populations are high, there is a

tendency for mosquitoes to feed on available sheep and cattle (Jones and Lloyd, 1985). In the area of that particular study there were nine species of mosquitoes feeding during July of 1978. Of the nine species collected, six were collected from both the sheep-baited traps and the CDC light trap: *Cs. inornata, Oc. melanimon, Oc. dorsalis, Aedes campestris, Oc. idahoensis, Aedes fitchii,* and *Culex tarsalis* (Jones and Lloyd, 1985).

The current study was conducted July 7th through August 17th, 2011 in Toston, MT. Without proper knowledge of the mosquito species that feed on sheep, the carriers of CVV cannot be determined. Therefore, the goal of this study was to fill this information gap in ovine epidemiology in order to contribute to further studies on CVV and other ovine viruses.

## Materials and Methods

Domestic sheep were contained in traps overnight as mosquito bait. Thereafter, the mosquitoes remaining in the traps in the morning were collected and studied. Captured mosquitoes were sorted into various subgroups to be further examined for bloodfed status and species identification. Along with the nine sheep-baited traps, a CDC light trap was placed approximately 200 yards away from the sheep trap lot. Dry ice was used with the CDC light trap to attract mosquitoes. Mosquitoes collected from the CDC trap were compared to those collected from the sheep-baited traps.

The mosquito collection process was carried out once every seven days during the seven-week trial. Sheep traps were set up on a ranch approximately five miles east of Toston, MT, (N 46°09.993', W111°34.156', elev. 1,243 m) just off of

Montana State Highway 437. Beef cattle ranches and wheat farms are the principal industries of this rural area. The area around the study is mostly flat with few rolling hills towards the base of the distant mountains. An unused lot near the ranch house was used as the study site. No cattle or horses were present around the study area in the summer because they are released to the mountains to avoid the dense mosquito populations around the site. A small stream sparsely lined with trees is located on the south side of the lot. Due to the heavy June rains in 2011, the stream, which normally dries up in July, trickled throughout the summer. To the east and west of the lot are cattle corrals and machine shops, respectively. To the north of the lot is the gravel driveway separating the lot and an alfalfa field. Smooth brome grass, foxtail barley, western wheat grass, and timothy grass predominately covered the lot, as well as small patches of fan weed and leafy spurge.

Nine sheep traps were constructed to house one sheep per trap. The traps consisted of one large, screened trap (outside trap) and one smaller, wire trap (inside trap) placed within the large trap. Both traps were designed by Dr. Greg Johnson, Montana State University entomologist, and constructed by undergraduate research students, Justin Nagy and Reagan Grabbe. The nine outside traps had wooden frames with sides covered with silver-grey insect screen with 17 holes per inch. The dimensions of the outside trap were 1.52 m. H X 1.83 m. L X 1.22 m. W. Each trap had seven gable louver vents with three 30.5 cm. X 30.5 cm. vents on each six-foot side and one 30.5 cm. X 45.7 cm. vent on one four-foot side. Each vent had a piece of screen covering the outside that could be rolled up or pinned over the surface of the vent as desired. The inside trap was a five-sided cube shape with

dimensions of 0.91 m. H X 0.91 m. L X 0.61 m. W. The nine inside traps were made out of welded livestock wire with 15.24 cm. holes and covered in 2.54 cm. poultry wire on the outside of the trap (Fig. 1).


Figure 1. Sheep-baited trap at study site with sheep inside.

The inside traps were placed near the vented end within the outside traps. This placement within the outside trap made the collection process easier. The inside traps were held in position by placing the open end on the ground and staking the livestock wire into the ground. The traps were arranged in a three by three set up in the lot with 10 meters between each trap in a row and 30 meters between each trap in a column. The grass within and around each trap was mowed and the base of each outside trap was sealed with dirt so as to avoid any mosquitoes escaping (Fig. 2).


Figure 2. 3 X 3 Trap Arrangement

Lambs were purchased from a ranch approximately 20 miles northeast of Geyser, MT. Nine 50-60 lb lambs were selected for the study. The lambs were separated into three designated groups for the study. Treatment group one (T1) was the control group. The T2 group had 9.5g. Python insecticide ear tag (zetacypermethrin) applied 14 days prior to the first trapping day. The T3 group was treated with 12 mL. of GardStar, a permethrin pour-on, on the first trapping day (July 7th) and again on July 13th, day seven of the trial. The first application of pour-on was applied to the entire body of the lambs and the second treatment was applied mainly to the face and ears. In the days between collection dates, the lambs were kept in three different pens at the Fort Ellis Research Farm, a Montana State

University Department of Animal and Range Sciences Center, where they had access to plant forage and water within each pen. This study was conducted under MSU AACU Protocol # 2011-AA03.

The sheep were transported approximately 80.5 kilometers to the rural study area each week of the trial. The sheep were randomized into pens with a T1, T2, and T3 sheep in each row. Each week after week one, the sheep were simply rotated one trap to the north. Therefore, every treatment stayed overnight in every pen at least once. Each trapping evening around 1700 hr MDT the sheep were put in their respective traps with water and alfalfa pellets and left overnight. When the sheep were put into their traps, the screen over each vent was rolled up, allowing full access for mosquitoes to enter. Each collection morning around 0730 hr MDT, before the aspiration process began, the screen over each of the vents was pulled down in order to prevent mosquitoes from escaping. Then, mosquitoes were aspirated from inside the larger trap before the sheep were removed from the small, inside trap. As soon as each trap was fully aspirated the sheep were released and transported back to the Fort Ellis Research Farm.

After aspiration, the mosquitoes were put into a cooler with dry ice and taken back to the lab to be sorted to species and bloodfed status. The mosquitoes caught using the CDC light trap were also collected each morning and taken back to the lab for study. Every mosquito caught in either type of trap was identified based on species characteristics and bloodfed status. The species and number of mosquitoes trapped in the sheep-baited traps were then compared to those caught in the CDC light trap.

## Results

The numbers of mosquitoes collected from the sheep-baited traps are represented in Table 1. The control group had the highest populations of both bloodfed and non-engorged mosquitoes. The group with spray treatment and the group with ear tags were similar in numbers, but the spray treatment group had higher populations of bloodfed and lower populations of non-bloodfed (non-engorged). This indicates that the zetacypermethrin in the ear tags was more effective in preventing mosquito feeding than the permethrin pour-on applied to group T3. Further information on the effectiveness of the spray versus the ear tag can be obtained from Dr. Greg Johnson at Montana State University Animal Science Department.

| | **CONTROL** | **EAR TAG** | **SPRAY** | **TOTAL** |
|---|---|---|---|---|
| **NON ENGORGED** | 3,334 | 3,099 | 2,952 | 9,385 |
| **BLOODFED** | 6,418 | 936 | 1,603 | 8,957 |
| **TOTAL** | 9,752 | 4,035 | 4,555 | 18,342 |

Table 1. Number and feeding status of mosquitoes collected by treatment.

Table 2 displays the species that were found in the sheep-baited traps and the percent of each species within the total population collected from the sheep-baited traps. In total, 16 different species were identified from the sheep-baited traps. *Oc. increpitus* was present in significantly higher numbers than the other species trapped. Eight different species account for less than one percent of the total number of mosquitoes collected from the sheep-baited traps. All 16 species collected from the sheep-baited traps had bloodfed individuals.

| SPECIES | % POPULATION |
|---|---|
| *Ochlerotatus increpitus* | 48 |
| *Ochlerotatus idahoensis* | 20 |
| *Aedes vexans* | 12 |
| *Ochlerotatus dorsalis* | 7 |
| *Culiseta inornata* | 4 |
| *Ochlerotatus spencerii* | 3 |
| *Culex tarsalis* | 2 |
| *Ochlerotatus melanimon* | 2 |
| *Oc. trivitattus, Ae. cinereus, Oc. canadensis, Oc. intrudens, Oc. sierrensis, Cs. incidens, An. earlei, Oc. implicatus* | < 1 |

Table 2. Percent population of identified species out of total population collected from sheep-baited traps.

Table 3 displays the species that were found in the CDC light traps and the percent of each species within the total population collected from the CDC light traps. In total, 15 different species were identified. *Oc. dorsalis* was present in significantly higher numbers than the other species trapped. Seven different species account for less than one percent of the total number of mosquitoes collected from the CDC light trap. Figure 3 represents a comparison between the percent populations of species in the sheep-baited traps versus the CDC light trap. The listed species are those that represent 1% or more of the population from their respective trap.

| SPECIES | % POPULATION |
|---|---|
| *Oc. dorsalis* | 50 |
| *Ae. vexans* | 20 |
| *Oc. idahoensis* | 15 |
| *Oc. melanimon* | 6 |
| *Oc. increpitus* | 4 |
| *Oc. spencerii* | 2 |
| *Oc. trivitattus* | 1 |
| *Cx. tarsalis* | 1 |
| *Ae. v. nipponi, Ae. cinereus, Oc. canadensis, Oc. excrucians, An. earlei, Cs. incidens, Oc. nigromaculis* | < 1 |

Table 3. Percent population of identified species out of total population collected from the CDC light trap.

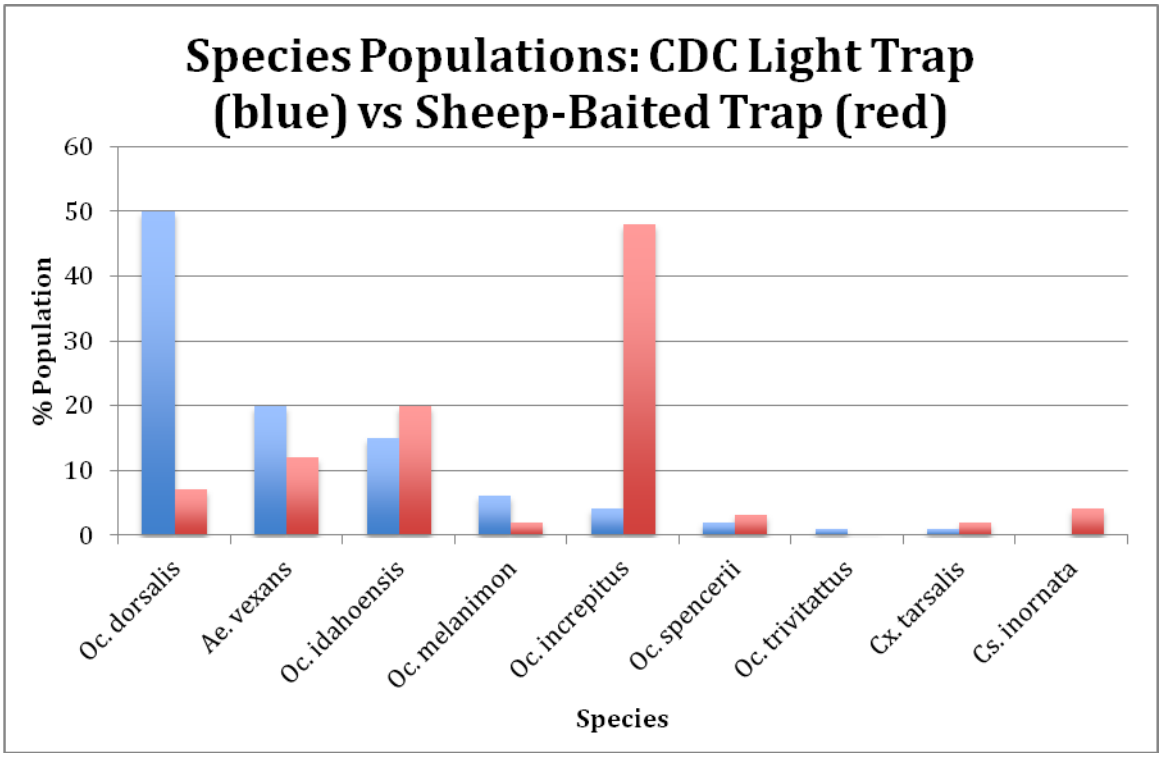


Figure 3. Species population of light trap versus sheep-baited traps.

Over the course of seven weeks, a total of 16 mosquito species were identified from sheep-baited traps and 15 species from CDC light traps. From the two traps, 19 different species were identified. Of these 19 species, there were 11 common species, five specific to the sheep-baited trap, and three specific to the CDC light trap.

| Sheep-Baited Trap Only | CDC Light Trap Only | Found In Both Traps |
|---|---|---|
| *Cs. inornata, Oc. intrudens, Oc. sierrensis, Oc. implicatus* | *Ae. v. nipponi, Oc. excrucians, Oc. nigromaculis* | *An. earlei, Oc. dorsalis, Ae. vexans, Oc. increpitus, Oc. idahoensis, Oc. melanimon, Oc. trivitattus, Ae. cinereus, Oc. canadensis, Cx. tarsalis, Oc. spencerii, Cs. incidens* |

Table 4. Species identified from each trap type.

## Discussion

The data from this study were compared to that of the study conducted by Jones and Lloyd (1985) study near Laramie, WY because the studies were carried out in similar intermountain meadow regions in the northwestern United States. They also compared species of mosquitoes found in sheep-baited traps with those collected in a CDC light trap. The Jones and Lloyd study collected on two separate occasions. They collected from only sheep-baited traps for 12 consecutive nights and collected from both a CDC light trap and sheep-baited traps simultaneously for five consecutive nights (Jones and Lloyd, 1985). The results from the five-day simultaneous trapping will be used for comparison because the methods are most similar to that of the Toston study. In the Jones and Lloyd study nine species of mosquitoes were collected from the sheep-baited traps and the CDC light trap, with six of those collected in both traps. In both types of traps, Jones and Lloyd (1985)

found similar species compared to the Toston study. The Wyoming study found *Cs. inornata* to have the highest populations in both types of traps, and the Toston study found *Cs. inornata* to have the highest population only in the sheep-baited trap. Also, similar to the Toston study, Jones and Lloyd (1985) found that in the sheep-baited traps *Oc. melanimon, Oc. dorsalis,* and *Oc. idahoensis* were found in second, third, and fourth highest populations, respectively. The most significant difference in species type between the two studies is that the sheep-baited trap in the Toston study showed that 48% of that population was *Oc. increpitus*. However, this species can be dismissed as a possible vector for Cache Valley Virus because it is solely a nuisance bitter and not capable of transmitting the virus (G. Johnson, pers. com., 2011).

Like the Toston study, the study conducted by Jones and Lloyd (1985) showed a small difference between the species that were caught in the sheep-baited traps compared to the CDC light traps. Of the nine species collected in the Wyoming study, three were not found in both types of traps. *Cs. incidens* and *Ae. flavescens* were found only in the CDC light trap, and *Ae. fitchii* was found only in the sheep-baited trap. However, each of these was found in less than one percent of the total population of mosquitoes trapped (Jones and Lloyd, 1985).

During both the 12-day and the 5-day study, two flocks of sheep and two herds of cattle were in the area of the Jones and Lloyd study so the mosquitoes had the opportunity to feed on either of these insecticide-free livestock species. Of the total 614 captured mosquitoes, there were only 34 bloodfed mosquitoes. Of these 34 mosquitoes, 17 had fed on sheep, 9 on cattle, and 8 on unidentified sources (Jones and Lloyd, 1985). In comparison, the Toston study observed 8,957 bloodfed

mosquitoes out of the total 18,342 captured. Since these bloodfed mosquitoes were captured inside a sheep-baited trap, it can be assumed that they fed on the caged sheep.

There were only a total of 245 mosquitoes trapped in the five-day Wyoming study (Jones and Lloyd, 1985), compared to the Toston study with 18,342 collected from the sheep-baited trap and 24,219 collected from the CDC light trap for a total of 42,561 mosquitoes trapped during the one-night-per-week, seven week period. Therefore, the Toston study provides a larger sample size, and, perhaps, a larger number of potential disease vectors. It may be worth testing whether *Cs. inornata, Oc. intrudens, Oc. sierrensis, Oc. implicatus* are potential reservoirs of CVV because they were present in the sheep-baited traps, but not the CDC light trap. This could be worthwhile because there are other genera from which the CVV has been isolated, including: *Aedes, Psorophora, Anopheles, Coquillettidia, Culex* and *Culiseta* (Calisher et al., 1986, Corner et al., 1979, Iversen et al., 1979).

The results from this study help to direct further investigation of potential vectors of CVV. Further investigations should test for CVV in *Cs. inornata, Oc. intrudens, Oc. sierrensis, Oc. implicatus.* These species are candidates for testing because this study found that they fed on the available ovines and were not detected in the CDC light trap. This study is simply one step in the investigation of CVV and further research should consider other possible vectors, such as *Culicoides sonorensis* (biting midges) (G. Johnson, pers. com., 2011). Research on CVV is in its infancy, but through the discovery of what species of mosquitoes feed on ovines, science is one step closer to eradicating this virus.

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