Ivermectin and related avermectins are among the most commonly used antiparasitic drugs in both pets and livestock (Campbell 1989). Its toxicity to insects and arachnids involves the hyperpolarization of nerve and muscle cells and increases in the permeability of cell membranes to chloride ions, causing muscular paralysis and death (Turner and Schaffer 1989). Blood-feeding insects are exposed to ivermectin when they ingest blood from treated animals. Some Culicoides spp. are susceptible to ivermectin and suffer up to 99% mortality when fed on animals treated with 200 µg/kg of ivermectin (Standfast et al. 1984). Based on laboratory bioassays, Culicoides sonorensis Jones and Wirth (Diptera: Ceratopogonidae) is tolerant to ivermectin when it is mixed directly with blood in the laboratory and when fed on treated cattle (Holbrook 1994, Holbrook and Mullens 1994). However, animals treated with ivermectin can be less attractive to some Culicoides spp. than untreated animals (Sollai et al. 2007). Insecticidal treatments of animals with ivermectin have been proposed as an alternative to slaughter or quarantine when the vectors of disease are susceptible (Standfast et al. 1984). The bioavailability of ivermectin varies among formulations, route of entry, and animal species, and interactions between ivermectin and vector competence to arboviruses is poorly documented (Lo et al. 1985, Marriner et al. 1987).

In North America, C. sonorensis is a vector of bluetongue (BTV) and epizootic hemorrhagic disease (EHDV) viruses (Foster et al. 1977, 1963). It is a potential vector of exotic arboviruses such as Akabane and African horse sickness viruses (Jennings and Mellor 1989, Martin et al. 1997). The effects of ivermectin on vector competence to BTV and EHDV are unknown and the toxicity of ivermectin in blood of elk and ponies to C. sonorensis has not been assessed. In addition, Ivomec Plus (Merial, Duluth, GA), used to control parasites in cervids and cattle, contains clorsulon that kills Fasciola hepatica (Linnaeus) (Echinostomida: Fasciolidae) by inhibiting enzymes in the glycolytic pathway (Martin et al. 1997). The toxicity of ivermectin with clorsulon to C. sonorensis is unknown. Our goal was to address these questions.

All animals were verified to be antibody negative for BTV and EHDV prior to experimentation. Six Shetland ponies were treated with oral ivermectin paste at a dose of 200 µg/kg. Blood was drawn from two ponies prior to treatment, two at 24 h post-treatment, and two at four days post-treatment. A total of six sheep were treated with 400 µg/kg of injectable ivermectin. Blood was drawn from two sheep prior to treatment, two at 24 hours post treatment, and two at 11 days post treatment. Five elk were treated with an injectable subcutaneous dose of 200 µg/kg ivermectin and 2000 µg/kg clorsulon (Ivomec Plus). Blood was drawn from all five elk immediately prior to treatment and 24 h post-treatment. Blood from the ponies and sheep was citrated with sodium citrate (Becton Dickinson, Franklin Lakes, NJ). Blood from the elk was treated with sodium heparin (Becton Dickinson, Franklin Lakes, NJ).

Colonies of C. sonorensis were continuously maintained at the USDA-Arthropod-Borne Animal Diseases Research Laboratory as described by Hunt (1994). Teneral adult midges were held in a cage without a sugar meal for three days prior to feeding. Females of C. sonorensis were fed control or treated blood with membrane feeders described by Hunt and McKinnon (1990). Mortality was assessed three days post-feeding (dpf) for control and treatment flies. After blood feeding, midges were maintained on a 10% sugar water diet for 12 days at 24°C. Females of C. sonorensis fully digest their blood meal and oviposit in 3-4 dpf (Hunt 1994).

Aliquots of 7 log_{10} TCID_{50} of BTV-17 and EHDV-2 in cell culture media were mixed 1:1 with treated or untreated blood and fed to females of C. sonorensis (Ausman and AK colonies). After virus feeding, midges were maintained on a sugar water diet for 12 days at 24°C. Positive control midges (five to six per feeding) were fed virus as described above and then frozen immediately after the 2 h feeding. All insects were sorted on a chill table, decapitated, and the heads and bodies were macerated separately in homogenization buffer with tungsten beads (Spirit River, Roseburg, OR).
using a Tissue Lyser (Qiagen, Valencia, CA) with previously described protocols (Kato and Mayer 2007). A sterile scalpel was used for each decapitation to avoid contamination and all tools were sterilized with 4% bleach and 70% ethanol between each transfer. A non-template control was included for each extraction. RNA was extracted from the homogenate using an RNeasy kit (Qiagen, Valencia, CA). BTV was detected by infrared-RT-PCR using previously described protocols (Kato and Mayer 2007). EHDV was detected using the same reaction conditions with primers EHDv 63-F1 (5’-AACAGTTACTACGCACAAATCA-3’) and EHDv245-R1 (5’-AGCCATTTCCAGCCAATCT-3’) synthesized with a 58 IR-Dye 800 chromophore (LI-COR, Inc., Lincoln, NE). These primers were designed to detect EHDV-1 and EHDV-2.

Mortality of treated and control insects was analyzed with a chi square test. Treatments were statistically analyzed with a Randomized Complete Block Design of a Factorial Analysis (RCBD ANOVA) using SAS version 9.1.

There were no significant differences (P>0.05) in mortality between C. sonorensis fed on controls or treated blood. Negative controls performed as expected in both the BTV-17 and EHDV-2 assay. Both BTV-17 and EHDV-2 were detected in the bodies but not heads of positive control flies. This indicated that virions did not stick to the mouthparts or head of the fly after feeding. All heads that tested positive for BTV-17 or EHDV-2 were associated with bodies that also tested positive. There were no PCR positive heads without positive bodies.

Of the midges fed control blood, 40% of the bodies were positive for EHDV-2 and 38% of the treatment bodies were positive. Virus was detected in 14% and 12% of the control and treatment heads. There was no significant difference (P>0.05) in the infection rate between the midges fed on Ivomec Plus-treated elk blood mixed with EHDV-2 and control blood. In the BTV-17 treatment, 60% of the bodies of the midges fed control blood were positive for virus, while 18% of the bodies from midges fed treated blood were positive. Virus was detected in 43% of the heads from midges fed control blood, while 14% of the heads from treated blood fed midges were positive. There was a significant difference (P<0.05) in the infection rate of midges fed BTV-17 mixed with sheep blood treated with ivermectin and control blood. This significant difference held for dissemination of virus into the heads of the midges. Significantly fewer midges were infected with BTV-17 or had disseminated virus in the head. There was a greater than 40% reduction in the number of midges that were infected with virus between those fed control blood and those fed ivermectin treated blood. Of the infected midges, there was a more than 30% reduction in virus dissemination rate between midges fed on control blood and those fed on blood with ivermectin.

Ivermectin did not kill a significant number of blood-fed C. sonorensis. These results agree with previous studies using cattle and ivermectin (Holbrook 1994, Holbrook and Mullens 1994). The addition of clorsulon had no detectable effect on midge survival. These data support the previous studies that show a failure to control C. sonorensis with ivermectin and demonstrate that the failure to control was not related to factors in the blood of cattle.

Injectable ivermectin at a dose of 400 µg/kg significantly reduced the susceptibility of C. sonorensis to BTV-17. The reason for the reduced infection rate could include cellular inhibition or toxicity to midgut cells. While there was no significant mortality between treatment and control insects, there could be subclinical toxicity or physiological responses of the midgut cells that inhibited the virus or the infection process. Standfast et al. (1984) suggested that ivermectin could be used to control bluetongue because it could kill potential vectors and block transmission. Our data do not support this assertion, but they do suggest that ivermectin can inhibit the infection of C. sonorensis by BTV-17. The benefits of reduced vector susceptibility are unlikely to be financially significant to ranchers. However, the data support the use of ivermectin for other purposes, such as parasite control, during the transmission season of BTV-17, because it does not increase the susceptibility of C. sonorensis to BTV-17.

Our data do not support the use of ivermectin and clorsulon to control EHDV-2 in elk. There was no significant effect of the combined drugs. However, there was no indication that injectable ivermectin and clorsulon increased the susceptibility of C. sonorensis to EHDV-2. Thus, there is no contraindication to using these drugs during the transmission season.

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REFERENCES CITED


Holbrook, F.R. and B.A. Mullens. 1994. Effects of ivermectin on survival, fecundity, and egg fertility in *Culicoides*


