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Distribution of *Culicoides sonorensis* (Diptera: Ceratopogonidae) in Nebraska, South Dakota, and North Dakota: Clarifying the Epidemiology of Bluetongue Disease in the Northern Great Plains Region of the United States

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Distribution of *Culicoides sonorensis* (Diptera: Ceratopogonidae) in Nebraska, South Dakota, and North Dakota: Clarifying the Epidemiology of Bluetongue Disease in the Northern Great Plains Region of the United States

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ABSTRACT The presence or absence of the biting midge *Culicoides sonorensis* Wirth & Jones (Diptera: Ceratopogonidae), a primary vector of bluetongue viruses (genus *Orbivirus*, family *Reoviridae*, BTV) in North America, was assessed on ranches and farms across the Northern Great Plains region of the United States, specifically Nebraska, South Dakota, and North Dakota, as part of a 2-yr regional study of BTV exposure among cattle. Blacklight/suction trap samples totaling 280 2-night intervals were taken at 140 aquatic sites (potential larval habitat for *C. sonorensis*) on 82 livestock operations (ranches and farms) that span a south-to-north gradient of expected decreasing risk for exposure to BTV. In Nebraska, *C. sonorensis* populations were common and widespread, present at 15 of 18 operations. Of 32 operations sampled in South Dakota, seven of which were sampled in successive years, 18 were positive for *C. sonorensis*; 13 of 14 operations located west of the Missouri River were positive, whereas 13 of 18 operations east of the river were negative. Of 32 operations sampled in North Dakota, seven of which were sampled both years, 12 were positive for *C. sonorensis*. Six of eight operations located west and south of the Missouri River in North Dakota were positive, whereas 18 of 24 operations east and north of the river were negative for *C. sonorensis*. These data illustrate a well-defined pattern of *C. sonorensis* spatial distribution, with populations consistently present across Nebraska, western South Dakota, and western North Dakota; western South Dakota, and North Dakota encompass the Northwestern Plains Ecoregion where soils are nonglaciated and evaporation exceeds precipitation. In contrast, *C. sonorensis* populations were largely absent east of the Missouri River in South Dakota and North Dakota; this area comprises the Northwestern Glaciated Plains Ecoregion and Northern Glaciated Plains Ecoregion where surface soils reflect Wisconsinan glaciation and precipitation exceeds evaporation. In defining a well-demarcated pattern of population presence or absence on a regional scale, the data suggest that biogeographic factors regulate the distribution of *C. sonorensis* and in turn BTV exposure. These factors, ostensibly climate and soil type as they affect the suitability of larval habitat, may explain the absence of *C. sonorensis*, hence limited risk for exposure to BTV, across the eastern Northern Plains, upper Midwest, and possibly Northeast, regions of the United States.

KEY WORDS *Culicoides*, bluetongue viruses, epidemiology, animal export, vector *Culicoides* spatial distribution

Historically, bluetongue (BT) disease has been an impediment for cattlemen in the United States who export cattle due to nontariff trade restrictions on international movements of animals and their germplasm (OIE 2005). Losses in export trade attributable to bluetongue virus (genus *Orbivirus,* family *Reoviridae,* BTV) worldwide have been estimated at >US$3 billion annually (Roberts et al. 1993) and may be more damaging to livestock industries of some countries than the disease itself (Oviedo et al. 1992). Annual losses to U.S. livestock industries attributable to restricted trade with BTV-free countries have been estimated at US$144 million (Hoar et al. 2003). Maclachlan et al. (2009) updated assessment of the impacts of BTV infection on the international movement and trade of ruminants. The sale or movement of livestock from defined BT-free regions or zones within BTV-positive countries (OIE 2005) is permitted by international regulations. Canada recently relaxed re-

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strictions on the import of U.S. livestock relative to BTV serotypes considered endemic to North America (Anonymous 2010).

In the United States, where exposure to BTV has been recognized since the mid-1960s (Walton 2004), the prevalence of exposure is defined by geographic regions that support populations of the biting midge *Culicoides sonorensis* Wirth & Jones (Diptera: Ceratopogonidae), a primary proven vector of BTV in North America (Tabachnick 1996). The epidemiology of recently discovered BTV serotypes from the Caribbean Basin in cattle in the southeastern United States (Johnson et al. 2007) is poorly understood (Wilson et al. 2009). The northeastern region of the United States historically has been considered to be free of BTV (Walton 2004) based on1) the absence of *C. sonorensis* (Wirth and Jones 1965, Schmidtmann et al. 1983, Holbrook et al. 1996), 2) the low seroprevalence (<2%) of BTV antibodies in cattle (Pearson et al. 1992, Ostlund et al. 2004), and 3) the low susceptibility of the eastern sister species *Culicoides variipennis* (Coquillett) to BTV infection (Tabachnick and Holbrook 1992). In contrast, BTV is endemic across much of the western United States (Pearson et al. 1992), where populations of *C. sonorensis* are widespread and abundant in proximity to livestock operations (Wirth and Morris 1985).

This study documents the distribution of *C. sonorensis* in the North Central region of the United States as part of a Bluetongue Surveillance Pilot Project (BSPP) that evaluated the status of exposure to BTV in cattle and identified associated risk factors on a regional basis (Green et al. 2005). Based on BTV antibody seroprevalence in market cattle (Pearson et al. 1992), the BSPP study area spans a south-to-north gradient of epidemic to incursive risk for exposure to BTV (Gibbs and Greiner 1994). Previous geographic-scale investigations of the *C. variipennis* complex (Rowley 1967, O'Rourke et al. 1983, Schmidtmann et al. 1983, Holbrook and Tabachnick 1995, Holbrook et al. 1996, Schmidtmann et al. 1998), and the compilations of Wirth and Jones (1957) and Holbrook et al. (2000), have defined the general distribution of *C. sonorensis*, but little collection data exist for the Northern Great Plains region of the United States. Anderson and Holloway (1993) assessed the species of adult *Culicoides* associated with white-tailed deer, *Odocoileus virginianus* (Zimmermann), habitat and livestock operations across North Dakota, but they did not differentiate between *C. sonorensis* and *C. variipennis* due to the unavailability of diagnostic characters. A recent study in Canada characterized the seasonal abundance, parity, and survival of *C. sonorensis* at cattle facilities in southern Alberta, Canada (Lysyk 2007).

The BSPP was coordinated by the USDA–APHIS, Veterinary Services Centers for Epidemiology and Animal Health, Ft. Collins, CO, with collaboration from the Arthropod-Borne Animal Diseases Research Laboratory (ABADRL), USDA–ARS, Laramie, WY, and state and federal veterinarians, and with the cooperation of participating beef and dairy producers (Dargatz et al. 2004).

Materials and Methods

Study Experimental Design. Beef and dairy cattle operations were selected for participation in the BSPP to provide a broad geographic representation of both beef and dairy cattle populations in the Northern Great Plains region of the United States (Dargatz et al. 2004). Operations were identified to represent the distribution of beef and dairy cattle operations at the county level by respective State coordinators for Nebraska, South Dakota, and North Dakota. Of the 149 BSPP-enrolled operations in 2001, approximately every other operation ($n = 68$) was identified for *Culicoides* (vector) sampling. Additional vector sampling was conducted in 2002 in South Dakota and North Dakota at 12 and 16 randomly selected operations, respectively. Five and nine operations were new, respectively, and seven operations in each state were sampled in successive years. Light trap vector sampling was carried out between mid-July and mid-September, the primary seasonal period of transmission of BTV (Tabachnick 1996) and before onset of inclement weather and low temperatures.

Adult *Culicoides* Sampling. Black light/suction traps (model 1212, J. S. Hock, Gainesville, FL) were used to assess the presence or absence of *C. sonorensis*. The light traps were not supplemented with CO_2 because of logistical problems associated with acquisition and use of dry ice, and because black (UV) light alone attracts male, as well as nulliparous, parous, and gravid female *C. sonorensis* (Anderson and Linhares 1989). At each operation, a light trap was placed at one or two aquatic habitats (potential *C. sonorensis* larval habitats) for a 2-wk period. Traps were operated for two consecutive nights during the first week and for two consecutive nights during the subsequent week, totaling four trap night samples for each habitat. The trap fan and black light, set to activate at dusk and shut down at daylight, were powered by a 6-V dry cell battery (Energizer Battery Company, Inc., St. Louis, MO) that provided operation for a minimum of 2 nights. Traps were protected from livestock by installation of temporary enclosures constructed of T-stakes and hog-wire mesh. We partially filled 0.9-liter (1-quart) catch jars with ethylene glycol (nonpoisonous R.V. antifreeze, Triangle Manufacturers, Dinkle, IA) to entrap and preserve insects. Catch jars were harvested after 2 nights of sampling and labeled by date, site, and operation.

The contents of catch jars were examined for *Culicoides* by inspection under 10× magnification. Up to 25 females of either or both *C. sonorensis* and *C. variipennis*, identifiable by their distinctive wing patterns and large size, were removed and placed in 70% ethanol for identification, as were all other *Culicoides*. Negative catch jar samples were examined a second time to ensure that no *Culicoides* were present. Specimens of *C. sonorensis* and *C. variipennis* were identified by the condition of the third palpal segment, either expanded laterally with large sensory pit or narrow with small sensory pit, respectively (Holbrook et al. 2000). Other *Culicoides* were identified by respective wing patterns by using the photograph figures in Wirth et al. (1985). Voucher specimens were slide mounted for further inspection.

Aquatic Sediment Samples. Surface sediments (top 2–4 cm) of ≈0.3-liter volume were taken just below the mud–water interface from most aquatic sites nearest to a light trap sample site. A sample typically consisted of a several small aliquots of sediment. Typical habitats were shoreline margins of stock pond dams, ponds or small lakes, soils saturated by water from overflowing stock tanks, and backwaters of small streams. Samples were placed in a labeled Ziploc lock bag and held temporarily on crushed ice in an insulated container before shipment by express service to the ABADRL. Sediment samples were processed for chemical analysis by the Soils Testing Laboratory of the University of Wyoming following procedures in Schmidtmann et al. (2000).

Values for each of the soil chemistry analytes were compared for *C. sonorensis*-positive and -negative sample sites by using the Genmod procedure in SAS (SAS Institute, Cary, NC). Samples were designated as clustered by operation. To further explore the data and look for explanatory power among the soil chemistry values, a discriminant analysis was performed using SAS (SAS Institute). This multivariate technique accounts for the lack of independence between soil chemistry measurements. The analysis sought to determine the best function to correctly classify sites as positive or negative for *C. sonorensis.*

Geographic Analysis. Operation habitats that were sampled for adult *C. sonorensis* were identified by latitude and longitude coordinates by using a Garmin 12SL global positioning system (Garmin International, Olathe, KS). Site coordinates were geo-coded in ArcView (Environmental Systems Research Institute, Redlands, CA). A Level II ecoregion shapefile was imported into ArcView for visualization and spatial analysis. The "measure tool" of ArcView was used to determine distances between selected operations and the Missouri River.

Results

Adult *Culicoides* Sampling. Table 1 lists the livestock operations by county in Nebraska, South Dakota, and North Dakota that were investigated, as well as the types of aquatic habitats that were sampled, and the presence or absence of *C. sonorensis* and the sibling species *C. variipennis.* Overall, 68 and 28 operations were investigated in 2001 and 2002, respectively.

As listed in Table 2, a progressive decrease in abundance of *C. sonorensis* was observed from Nebraska northward through South Dakota and North Dakota. This trend, observed both years of study, was collectively expressed in the number of operations where *C. sonorensis* was detected, the number of positive sample sites, and the number of positive light trap samples. Of the 14 operations that were sampled both years of study, 12 persisted as either positive or negative for *C. sonorensis.*

Geographic Distribution. A consistent pattern of operations positive for female *C. sonorensis* was observed across Nebraska (Fig. 1), with the possible exception of the easternmost region, where several operations were negative. In South Dakota, *C. sonorensis* was detected at 18 operations, of which 13 of 14 operations located west of the Missouri River were positive, and 13 of 18 operations east of the Missouri River were negative. A similar geographic distribution was observed in North Dakota, where six of eight operations located west and south of the Missouri River were positive. In contrast, 20 of 24 operations located north and east of the Missouri River were negative. The eight positive operations east of the Missouri River in South Dakota and North Dakota are, with one exception, geographically contiguous with positive operations west of the Missouri River, all of which lie within the Northern Great Plains Ecoregion (Fig. 1). The eight positive operations average a distance of 30.4 km east of the Missouri River.

Other *Culicoides*. Several species of *Culicoides* other than *C. sonorensis* were captured in light trap samples. The second most prevalent species was *C. variipennis,* a sibling species of *C. sonorensis* that was geographically widespread at scattered locations across Nebraska, South Dakota, and North Dakota (Fig. 1). Other *Culicoides* captured were *Culicoides crepuscularis* Malloch, *Culicoides cockerellii* (Coquillett), *Culicoides stellifer* (Coquillett), *Culicoides haematopotus* Malloch, *Culicoides* (*Selfia*) Khalaf sp., and *Culicoides gigas* Root & Hoffman. With the exception of *C.* (*Monoculicoides*) *gigas*, these *Culicoides* were captured sporadically and in small numbers. Numerous *C. gigas*, a species closely related to the *C. variipennis* complex, were captured from several locations in eastern South Dakota and North Dakota.

Soil Chemistry of Larval Habitats. Soil chemistry data were available for 72 aquatic sites (sediment samples) that were collected on 52 operations. From one to three sediment samples were analyzed per operation. Table 3 lists the mean, standard error, and maximum and minimum values of soil chemistry parameters for habitats where adult *C. sonorensis* was captured (positive sites) and not captured (negative sites). Forty positive sample sites were available, with the exception that 28 sites were tested for phosphate. In total, 32 negative site samples were tested, with the exception that 24 sites were tested for phosphate.

Comparisons between positive and negative samples showed no differences between soil chemistry values, with P values <0.05 for each parameter. Mean calcium values for negative sites, although nearly double those of positive sites, did not differ significantly, $P = 0.25$. Mean electrical conductivity values, a measure of collective dissolved salts, were 3.63 mg/kg for positive sites and 2.32 mg/kg for negative sites, and they did not differ ($P = 0.17$). In addition, discriminant analysis did not find a combination of soil chemistry factors that discriminated between positive and negative sites.

Table 1. Light/suction trap data for adults of the *C. variipennis* complex captured onfarms and ranches in Nebraska, North Dakota, and South Dakota

Yr/state	County	Operation no./ light trap site	Type of habitat	*C. sonorensis* +/−	*C. variipennis* +/−
2001					
Nebraska	Cheyenne	1/1	Natural water course <3 m wide	+	−
		1/2	Natural water course <3 m wide	+	−
	Cherry	2/1	Overflow from stock tank	+	−
		2/2	Stock dam pond	+	−
	Dawes	3/1	Stock dam pond	+	−
		3/2	Stock dam pond	+	−
	Garden	4/1	Overflow from stock tank or water trough	+	−
		4/2	Pond/lake	+	−
	Dundy	5/1	Wetlands	+	−
		5/2	Overflow from stock tank or water trough	+	−
	Grant	6/1	Overflow from stock tank or water trough	+	+
		6/2	Overflow from stock tank or water trough	+	−
	Keith	7/1	Overflow from stock tank or water trough	+	−
		7/2	Overflow from stock tank or water trough	+	−
	Custer	8/1	Natural water course <3 m wide	+	+
		8/2	Pond/lake	−	−
	Dawson	9/1	Rainfall collection	+	+
		9/2	Stock dam pond	+	−
	Garfield	10/1	Overflow from stock tank	+	−
		10/2	Overflow from stock tank	+	−
	Sherman	11/1	Stock dam pond	+	+
		11/2	Stock dam pond	+	+
	Antelope	12/1	Stock dam pond	+	+
		12/2	Stock dam pond	−	+
	Butler	13/1	Stock dam	−	−
	Gage	14/1	Dairy lagoon	+	−
		14/2	Dam on drainage ditch	+	−
	Otoe	15/1	Unlined waste water lagoon	−	+
		15/2	Pond/lake	−	+
	Nance	16/1	Pond/lake	−	−
		16/2	Pond/lake	+	−
	Hall	17/1	Pasture slough	−	−
		17/2	Pasture slough	−	−
	Clay	18/1	Pond/lake	+	−
		18/2	Natural water course <3 m wide	+	+
South Dakota	Harding	1/1	Stock dam	+	−
		1/2	Wetlands	+	−
	Meade	2/1	Stock dam pond	+	−
	Lawrence	3/1	Stock dam pond	+, +*	+
	Pennington	4/1	Stock dam pond	+	+
	Haakon	5/1	Stock dam pond	−	−
		5/2	Stock dam pond	+	−
	Hughes	6/1	Pond/lake	+	−
	Jones	7/1	Pond/lake	+	−
		7/2	Pond/lake	+, +	−
	Lyman	8/1	Overflow from stock tank or water trough	−, +	−
	Buffalo	9/1	Overflow from stock tank or water trough	+	−
	Aurora	10/1	Stock dam pond	−	−
	Sanborn	11/1	Overflow from stock tank or water trough	−	−
	Davison	12/1	Natural water course <3 m wide	−	−
	Custer	13/1	Stock dam pond	−	−
	Fall River	14/1	Stock dam pond	+	−
		14/2	Stock dam pond	+	−
	Bennett	15/1	Stock dam pond	+, +	+
	Tripp	16/1	Pond/lake	+, +	−
	Turner	17/1	Natural water course <3 m wide	−	+
	Brookings	18/1	Natural water course <3 m wide	−	−
	Codington	19/1	Stock dam pond	−	−
	Marshall	20/1	Pond/lake	−	−
	Hand	21/1	Overflow stock tank or water trough	+, +	−
	Hyde	22/1	Stock dam pond	−, −	−
	Lake	23/1	Stock dam pond	−	−
	Moody	24/1	Pond/lake	−	+
	Potter	25/1	Stock dam pond	+	+
		25/2	Stock dam pond	+	+
	Roberts	26/1	Natural water course <3 m wide	−	−
	Sully	27/1	Stock dam pond	−	−

Continued on following page

Table 1. Continued

Yr/state	County	Operation no./ light trap site	Type of habitat	*C. sonorensis* +/−	*C. variipennis* +/−
2002					
South Dakota	Meade	28/1	Stock dam pond	+	−
	Butte	29/1	Stock dam pond	+	−
	Shannon	30/1	Stock dam pond	+	−
	Campbell	31/1	Stock dam pond	−	−
	Potter	32/1	Natural water course <3 m wide	+	−
2001					
North Dakota	McLean	1/1	Pond/lake	−, +	−
		1/2	Pond/lake	−	−
	Morton	2/1	Pond/lake	−	−
		2/2	Pond/lake	−	−
	Ransom	3/1	Stock dam pond	−	−
		3/2	Stock dam pond	−	−
	McIntosh	4/1	Pond/lake	−	−
		4/2	Wetlands	−	−
	Kidder	5/1	Stock dam pond	−	−
		5/2	Pond/lake	−	−
	Wells	6/1	Wetlands	−	−
		6/2	Wetlands	−	−
	McLean	7/1	Pond/lake	−	+
		7/2	Pond/lake	−	−
	Cavalier	8/1	Natural water course 3 m wide	−	−
		8/2	Wetlands	−	−
	Pembina	9/1	Pond/lake	−, −	+
	Benson	10/1	Natural water course >3 m wide	−	−
		10/2	Natural water course >3 m wide	−	−
	Billings	11/1	Stock dam pond	+	−
		11/2	Pond/lake	+	−
	Rolette	12/1	Stock dam	−	−
		12/2	Pond/lake	−	−
	Walsh	13/1	Wetlands	−	−
		13/2	Wetlands	−	−
	Dickey	14/1	Pond/lake	−	−
		14/2	Pond/lake	−, −	−
	Logan	15/1	Pond/lake	−	−
		15/2	Pond/lake	−	−
	Burleigh	16/1	Pond/lake	+, +	+
		16/2	Pond/lake	+	−
	Bottineau	17/1	Stock dam pond	−	−
		17/2	Pond/lake	−	−
	Cavalier	18/1	Pond/lake	−, −	−
		18/2	Pond/lake	−	−
	Benson	19/1	Pond/lake	−	−
		19/2	Pond/lake	−, −	−
	Towner	20/1	Pond/lake	−	−
		20/2	Pond/lake	−	−
	Benson	21/1	Natural water course >3 m wide	−	−
		21/2	Natural water course >3 m wide	−, −	−
	Golden Valley	22/1	Pond/lake	+	−
		22/2	Pond/lake	+	−
		23/1	Pond/lake	+	−
		23/2	Stock dam pond	+	−
2002					
North Dakota	McLean	1/1	Pond/lake	+	−
	Pembina	9/1	Pond/lake	−	−
	Dickey	14/1	Pond/lake	−, −	−
	Burleigh	16/1	Pond/lake	+	−
	Cavalier	18/1	Stock dam pond	−	−
	Benson	19/1	Pond/lake	−	−
		21/1	Natural water course >3 m wide	−	−
	Divide	24/1	Pond/lake	−	−
	Foster	25/1	Stock pond	−	−
	Ward	26/1	Pond/lake	−	−
		27/1	Dugout	−	−
	Emmons	28/1	Stock dam pond	+	−
	Ransom	29/1	Artesian well/stock tank	+	+
	Morton	30/1	Stock dam pond	+	−
	Hettinger	31/1	Dairy waste water	+	−
	Grant	32/1	Natural water course >3 m wide	+	−

* Presence of absence or *C. sonorensis* in 2002.

Table 2. Light/suction trap catch data for *C. sonorensis* on ranches and farms in Nebraska, South Dakota, and North Dakota

State	2001	2002	Total
No. positive operations/no. operations (%)			
Nebraska	15/18 (83)		15/18 (83)
South Dakota	14/27 (52)	10/12 (83)*(4/5 new)	24/39 (62)*18/32 (56)
North Dakota	5/23 (22)	7/16 (44)*(5/9 new)	12/39 (31)*10/32 (31)
No. positive sample sites (habitats)/no. sites (%)			
Nebraska	27/35 (77)		27/35 (77)
South Dakota	17/32 (53)	10/12 (83)	27/44 (61)
North Dakota	8/45 (18)	7/16 (44)	15/61 (25)
No. positive light trap catch samples/no. samples (%)			
Nebraska	41/70 (59)		41/70 (59)
South Dakota	34/64 (53)	20/24 (83)	54/88 (61)
North Dakota	16/90 (18)	14/32 (44)	30/122 (25)

* Data include duplicate samples taken the second year of study on seven operations in South Dakota and seven operations in North Dakota.

Discussion

Understanding the distribution of the biting midge species comprising the *C. variipennis* complex—*C. variipennis*, *C. sonorensis*, and *C. occidentalis*—is important because *C. sonorensis* is a primary vector of BTV among ruminant livestock and wildlife in North America (Tabachnick 1996). Accordingly, risk for exposure to BTV is defined geographically by the presence of *C. sonorensis* populations, which span western, south central, mid-Atlantic, and southeastern States (Holbrook et al. 2000). The sister species, *C. variipennis,* is associated with livestock operations across northeastern, mid-Atlantic, and southeastern regions (Schmidtmann et al. 1983, 1998; Holbrook et al. 1996), but is an inefficient vector of BTV (Tabachnick and Holbrook 1992). The third species, *C. occidentalis,* is associated with highly saline and isolated aquatic habitats of the far west and is of minor concern as a vector of BTV because populations are sympatric with *C. sonorensis* (Tabachnick 1996). Previous geographic-scale investigations of the *C. variipennis* complex (Rowley 1967; O'Rourke et al. 1983; Schmidtmann et al. 1983, 1998; Holbrook and Tabachnick 1995; Holbrook et al. 1996) and the compilations of Wirth and Jones (1957) and Holbrook et al. (2000) have established the general distributions of respective species, but they are incomplete relative to the Northern Great Plains region of the United States. Anderson and Holloway (1993) assessed the adult *Culicoides* associated with white-tailed deer habitats and livestock operations across North Dakota, but due to the unavailability of diagnostic characters at the time they were unable to differentiate between *C. sonorensis* and *C. variipennis.* A more recent study in Canada (Lysyk 2007) characterized the seasonal abundance, parity, and survival of *C. sonorensis* in southern Alberta).

Given the south-to-north decreasing prevalence of BTV antibodies in cattle within the BSPP study area (Pearson et al. 1992, Dargatz et al. 2004, Ostlund et al. 2004), the south-to-north decrease in operations positive for *C. sonorensis*—15 of 18 in Nebraska, 11 of 18 in North Dakota, and seven of 21 in North Dakota—is not surprising. Nevertheless, inspection of the data shows clearly that the decreasing prevalence of *C. sonorensis* in South Dakota and North Dakota is based not only along a south-to-north cline but also on a well demarcated pattern of population presence or absence west and east of the Missouri River. For example, *C. sonorensis* populations in Nebraska were widespread and common across the state, with the exception of the southeastern region. The absence of *C. sonorensis* at a cattle and hog farm located just west of the Missouri River may reflect an eastern limit in the central United States. Females of the sister species *C. variipennis* were captured at this operation, which shows that light/suction traps were operational and weather conditions were suitable for flight on sample nights. Immature populations of *C. sonorensis* occur at the latitude of Nebraska east of the Missouri River in Missouri, Kentucky, and Ohio, but they seem to be restricted to isolated saline springs (Schmidtmann et al. 2000).

An eastern limit to populations of *C. sonorensis* was more clearly defined in South Dakota, where 13 of 14 operations located west of the Missouri River were positive, whereas 13 of 18 operations east of the Missouri River were negative. The single negative operation in western South Dakota was located in the far western Black Hills, where elevation and intrusive substrate soils differ from the balance of the state (Rich 1985). A similar well defined pattern of *C. sonorensis* presence or absence was observed in North Dakota, where six of eight operations west and south of the Missouri River were positive, in contrast to the negative status of 20 of 24 operations east and north of the river. Moreover, the seven positive operations east of the Missouri River in South Dakota and North Dakota were, with one exception, located an average distance of only 30.4 km east of the river along a south-to-north transition zone of mixed population presence or absence. The correspondence between operations in South Dakota and North Dakota that were either positive or negative in successive years of study (12 of 14) indicates that blacklight/suction trap sampling provided a good assessment of *C. sonorensis* presence or absence, irrespective of the vagaries of weather, seasonal population dynamics, and logistics of large-scale sampling at often remote rural locations. In defining an eastern boundary for *C. sonorensis* that generally parallels the Missouri River in central and

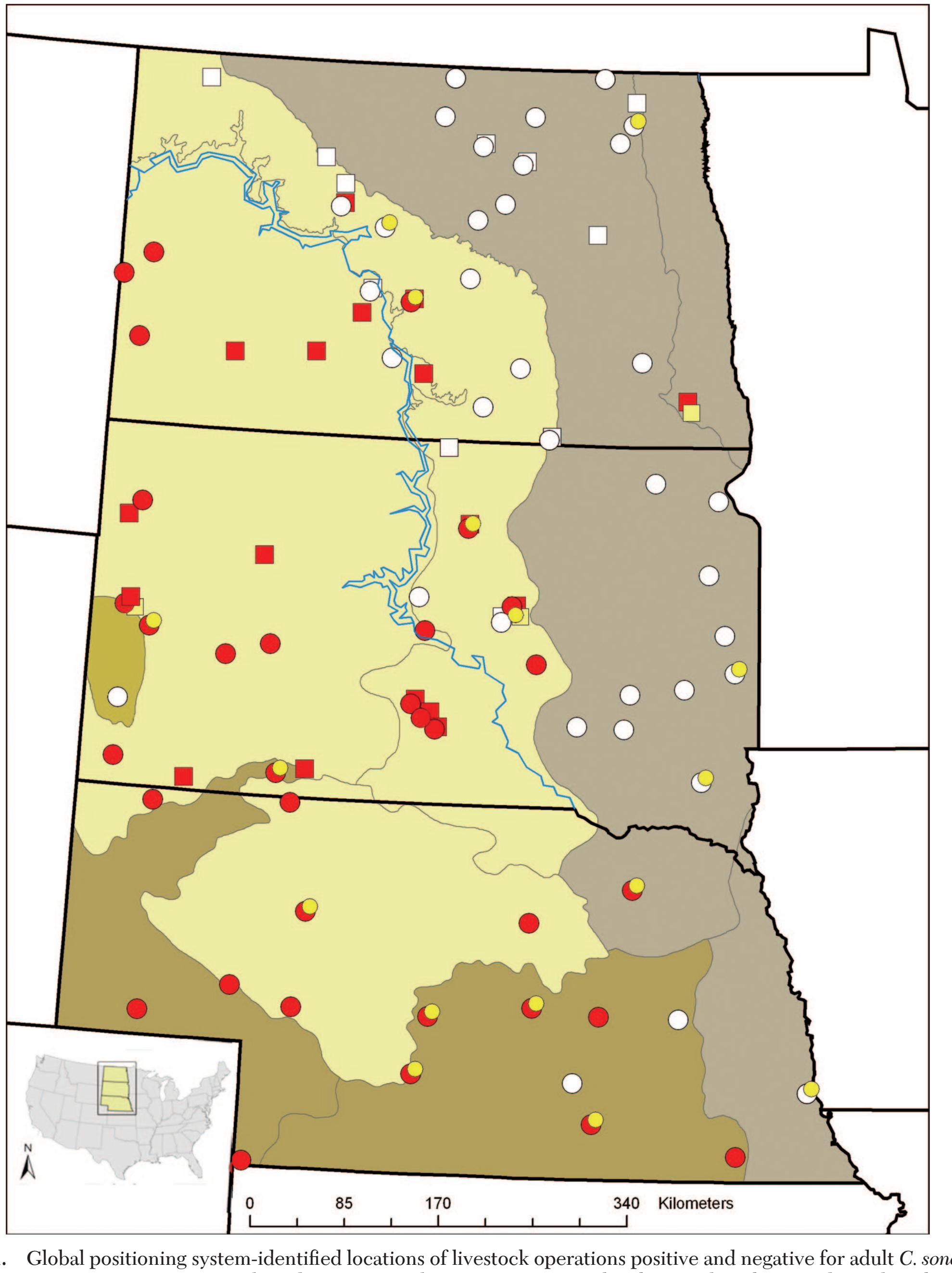

Fig. 1. Global positioning system-identified locations of livestock operations positive and negative for adult *C. sonorensis* and its sister species *C. variipennis* plotted against Level II ecoregions in Nebraska, South Dakota, and North Dakota. Red circles and squares represent operations positive for *C. sonorensis* in 2001 and 2002, respectively. White circles and squares represent operations negative for *C. sonorensis* in 2001 and 2002, respectively. Yellow circles and squares represent operations positive for *C. variipennis* in 2001 and 2002, respectively. The yellow-shaded landmass area represents the West Central semi-arid prairies ecoregion, and the gray-shaded landmass area represents the temperate prairies ecoregion.

northern regions of the United States, these results provide an entomologic basis for rating the eastern Northern Plains and Upper Midwest regions a minor risk for BTV due to the absence of *C. sonorensis.* This interpretation is in agreement with the low seroprevalence (<2%) of BTV antibodies in cattle from eastern North Dakota, Minnesota, Wisconsin, and Michigan (Ostlund et al. 2004).

Regardless of the well-defined pattern of *C. sonorensis* populations within the BSPP study area, "vector (*C. sonorensis*) presence" was not a significant risk factor for BTV seroconversion in cattle on the 61

Table 3. Soil chemistry values for aquatic habitats associated with light trap sample sites positive and negative for *C. sonorensis*

	Habitat status	
	C. sonorensis present (*n* = 40)	*C. sonorensis* absent (*n* = 32)
pH	7.36 (±0.10), 6.4–9.4	7.38 (±0.86), 6.7–9.0
Electrical conductivity (DS/m)	3.63 (±1.77), 0.10–43.5	2.32 (±0.20), 0.49–5.8
Magnesium (mg/kg)	532.10 (±90.34), 90.0–2,810.0	614.5 (±94.51), 70–2,280.0
Calcium (mg/kg)	4,382.90 (±888.50), 105.0–36,490.0	7,270.0 (±1,351.60), 960.0–32,980.0
Sodium (mg/kg)	524.30 (±162.50), 15.60–5,230.0	357.8 (±102.80), 27.0–3,180.0
Boron (mg/kg)	1.10 (±0.23), 0.1–7.7	0.95 (±0.16), 0.08–4.4
Phosphate (mg/kg)	23.29 (±6.44), 0–130.0	13.88 (±3.10), 0–60.0

operations where cattle were tested and light trap sampling was conducted (Green et al. 2005). A plausible explanation for the exclusion of vector presence as a BTV risk factor was the presence of *C. sonorensis* at three operations in northwestern North Dakota where cattle did not have BTV antibodies and, conversely, the absence of *C. sonorensis* at one operation in northeastern North Dakota where cattle were BTV positive. The latter operation was one of 11 farms under study in northeastern North Dakota, 10 of which were both negative for *C. sonorensis* and cattle with BTV antibodies. Follow-up questioning at the positive farm indicated that the few animals with BTV antibodies had been out of state and possibly exposed to BTV elsewhere (D.A.D., personal observation). The presence of *C. sonorensis* at three operations in northwestern North Dakota where cattle were negative for BTV antibodies may reflect environmental factors (e.g., temperature, population density, flight conditions) that negatively affect vectorial capacity and are expressed at the northern margin of the species' range (Mullens et al. 2004).

The classification of one cattle operation in southeastern North Dakota as positive for *C. sonorensis* was based on the capture of a single female. Follow-up light trap sampling at this operation and three adjacent farms over 2 wk in September 2003 failed to detect *C. sonorensis* (E.T.S., unpublished data). Females of *C. variipennis* were captured, however, indicating suitable climatic conditions for flight and operational light traps. The three next-nearest populations of *C. sonorensis* in this study averaged 245 km to the west and southwest, and the levels of dissolved salts at the positive site, an artesian spring-fed stock pond, were not consistent with the highly saline springs where *C. sonorensis* occurs east of the Missouri River.

Sediment samples taken from aquatic sites where adult *C. sonorensis* were present or absent in light trap samples did not differ significantly in terms of soil chemistry factors. Levels of dissolved salts as measured by electrical conductivity were generally consistent with the moderate levels of dissolved salts that characterize aquatic habitats with immature *C. sonorensis* in other regions of the United States (Schmidtmann et al. 2000, and greater than the levels of dissolved salts in habitats with the sister species *C. variipennis* (Schmidtmann 2006). Given that most positive operations in South Dakota and North Dakota were located west of the Missouri River, where evaporation exceeds precipitation and dissolved salts are concentrated in surface soils, higher levels of dissolved salts might have been expected at *C. sonorenesis*-positive sites. The lack of difference in soil chemistry between positive and negative habitats may be due to the assessment of presence or absence being based on capture of adults, which in some instances may have been attracted to light traps from a distance, thus confounding possible differences in habitat soil chemistry. It is also possible that the soil chemistry profiles of aquatic habitats tested in this study did not differ between positive and negative habitats, irrespective of differences in soil chemistry among habitats occupied by differing species-level larval populations of the *C. variipennis* complex (Schmidtmann et al. 2000).

The second most prevalent biting midge captured in this study was *C. variipennis*, with populations present in a scattered pattern across Nebraska and the Dakotas; these states represent the westernmost range of this eastern species (Holbrook et al. 2000). *C. variipennis* is an inefficient vector of BTV (Tabachnick and Holbrook 1992) and, given the scattered distribution of populations across the BSPP study area, of little concern relative to transmission of BTV. Other species of biting midges captured were *C. crepuscularis*, *C. cockerellii*, *C. stellifer*, *C. haematopotus*, *C.* (*Selfia*) sp., and *C. gigas*. With the exception of *C. gigas*, these species were captured sporadically and in small numbers. Female *C. gigas*, known previously from British Columbia and Alberta, Canada, and Nebraska (Wirth et al. 1985, Lysyk 2006), were prevalent on operations in eastern South Dakota and eastern North Dakota. The possible role of *C. gigas* as a vector of BTV is unknown, but based on the limited distribution of populations, as well as sympatry with *C. sonorensis*, there is little reason to suspect an association with BTV seropositive cattle in this study.

As a last point of discussion, the widespread occurrence of *C. sonorensis* across western and central South Dakota and North Dakota suggests a biogeographically based spatial dependence with the Northwestern Great Plains Level III ecoregion, an ecologically distinct area where evaporation exceeds precipitation and soils were not glaciated during the Pleistocene (Bryce et al. 1998). This interpretation is in agreement with the finding that "elevation," which increases west of the Missouri River (Rich 1985), was a significant risk factor for exposure to BTVs in the BSPP study (Green et al. 2005). Furthermore, similar conditions of elevation and aridity occur across much of the greater western range of *C. sonorensis,* where larval populations typically occur in aquatic sediments with moderate levels of dissolved salts that derive from evaporation and contamination with cattle manure (Schmidtmann et al. 2000).

In contrast, the lesser prevalence and absence of *C. sonorensis* east and north of the Missouri River in South Dakota and North Dakota suggest a spatial dependence with the Northwestern Glaciated Plains and Northern Glaciated Plains Level III ecoregions. These ecoregions mark the westernmost extent of Pleistocene continental glaciation, where surface soils consist of Wisconsinan glacial till over Cretaceous Pierre shale and precipitation exceeds evaporation (Bryce et al.1998). Thus biogeographic factors at the ecoregion level, possibly climate and soil type or condition as they influence larval habitat suitability, may regulate the distribution of *C. sonorensis* in the upper midwestern United States. Clarification of the role of these or other factors will be meaningful as further evidence that *C. sonorensis* does not occur consistently in the temperate prairies and Upper Midwest regions, thus explaining the limited risk for exposure to BTV in the Upper Midwest, and possibly Northeast, regions of the United States.

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