VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 15, Number 11, 2015
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DOI: 10.1089/vbz.2015.1810

Management Factors Associated with Operation-Level Prevalence of Antibodies to Cache Valley Virus and Other Bunyamwera Serogroup Viruses in Sheep in the United States

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Abstract

A cross-sectional study was performed to identify operation-level risk factors associated with prevalence of antibody to Bunyamwera (BUN) serogroup viruses in sheep in the United States. Sera were obtained from 5150 sheep in 270 operations located in 22 states (three in the west, nine central states, and 10 in the east) and tested at a dilution of 1:20 by a plaque reduction neutralization test (PRNT) using Cache Valley virus (CVV). Antibodies that neutralized CVV were identified in 1455 (28%) sheep. Animal-level seroprevalence was higher in the east (49%) than the central (17%) and western (10%) states. A convenient subset ($n=509$) of sera with antibodies that neutralized CVV was titrated and further analyzed by PRNT using all six BUN serogroup viruses that occur in the United States: CVV, Lokern virus (LOKV), Main Drain virus (MDV), Northway virus (NORV), Potosi virus (POTV), and Tensaw virus (TENV). Antibodies to CVV and LOKV were identified in sheep in all three geographic regions; MDV and POTV activity was detected in the central and eastern states, NORV activity was restricted to the west, and antibodies to TENV were not detected in any sheep. Several management factors were significantly associated with the presence of antibodies to BUN serogroup viruses. For instance, sheep housed during the lambing season inside structures that contained four walls and a roof and a door closed most of the time were more likely to be seropositive than other sheep. In contrast, herded/open-range sheep were less likely to be seropositive than their counterparts. These data can be used by producers to implement strategies to reduce the likelihood of BUN serogroup virus infection and improve the health and management practices of sheep.

Key Words: Orthobunyavirus—Bunyamwera serogroup—Cache Valley virus—Sheep—Risk factors.

Introduction

All viruses in the genus *Orthobunyavirus* (family Bunyaviridae) are maintained and amplified in natural transmission cycles involving blood-feeding arthropods (*i.e.*, mosquitoes and midges) and vertebrate hosts (Schmaljohn and Nichol 2007). The genus is divided into 18 serogroups on the basis of antigenic relationships (International Committee on Taxonomy of Viruses 2012). One of the largest serogroups within the genus is the Bunyamwera (BUN) serogroup. Six viruses in this serogroup occur in the United States: Cache Valley virus (CVV), Lokern virus (LOKV), Main Drain virus (MDV), Northway virus (NORV), Potosi virus (POTV), and Tensaw virus (TENV) (Calisher et al. 1986, Francy et al. 1990).

CVV is the most widely distributed BUN serogroup virus in the United States. The virus was first isolated from

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mosquitoes in Utah in 1956 and has since been detected across most of the nation as well as parts of Canada and Mexico (Holden and Hess 1959, Calisher et al. 1986). Subtypes of this virus also occur in Central and South America (Calisher et al. 1983, Mangiafico et al. 1988). CVV is the most important BUN serogroup virus in the United States in terms of its impact on human and animal health. CVV infections in sheep are common and can result in embryonic and fetal death, stillbirths, and multiple congenital defects, such as arthrogryposis and hydranencephaly (McConnell et al. 1987, Edwards et al. 1989, Chung et al. 1990a, b, Hoffmann et al. 2012). CVV has also been responsible for three cases of severe human disease (Sexton et al. 1997, Campbell et al. 2006, Nguyen et al. 2013). LOKV, MDV, POTV, NORV, or TENV have not been associated with any cases of naturally occurring disease in sheep, although experimental infection studies have shown that MDV is a cause of severe musculoskeletal and nervous system malformations and death in ovine fetuses (Edwards et al.1997). Additionally, MDV has been isolated from brain tissue of a horse with encephalitis (Emmons et al. 1983), and TENV has been implicated in one case of human encephalitis (McGowan et al. 1973).

BUN serogroup viruses are similar antigenically, and therefore antibodies to one BUN serogroup virus often cross-react with other viruses in this serogroup (Hunt and Calisher 1979). To reduce the likelihood of serologic misdiagnosis, the plaque reduction neutralization test (PRNT), the gold-standard serologic technique for the identification of BUN serogroup virus infections, should be used during antibody surveys. It is also important that the PRNTs are performed using all BUN serogroup viruses known to occur in the geographic region in question and infect the vertebrate species under investigation. Unfortunately, most medical and veterinary diagnostic laboratories rarely use this approach when testing for antibodies to BUN serogroup viruses. Often only viruses known to cause human or animal disease (*i.e.*, CVV) are included in the PRNTs or less-specific assays are used. Furthermore, many diagnostic laboratories do not routinely test for BUN serogroup viruses, and therefore their seroprevalence and impact on public and veterinary animal health may be severely underestimated.

The overall objectives to this study are to determine the seroprevalence of BUN serogroup viruses in sheep in the United States and to identify operation-level risk factors associated with evidence of virus infection. To achieve these objectives, sera were collected from sheep in 22 of the nation's major sheep-producing states and analyzed by PRNT using all indigenous BUN serogroup viruses. The relationships between sheep with and without antibodies to BUN serogroup viruses were evaluated to identify farm management factors potentially associated with viral exposure. The investigation was performed using sera and management data collected during the US Department of Agriculture (USDA) National Animal Health Monitoring System (NAHMS) 2011 sheep study.

Materials and Methods

Data and sample collection

Data and blood samples were collected as part of the NAHMS sheep 2011 study, which was conducted to characterize the health and management of sheep operations in 22 states (US Department of Agriculture 2012, 2013). These states accounted for 70.1% of US farms with ewes and 85.5% of the US ewe inventory. Operation-level data and animal-level biologic results were categorized into three regions—west (California, Oregon, and Washington), central (Colorado, Idaho, Kansas, Montana, New Mexico, South Dakota, Texas, Utah, and Wyoming), and east (Iowa, Kentucky, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Virginia, and Wisconsin). All data are presented and discussed according to region to protect the confidentiality of study participants.

For the original study, a stratified random sample of 4920 operations with one or more ewes was selected from the National Agricultural Statistics Service (NASS) list frame. Stratified sampling was based on state and flock size. The size strata were based on the number of sheep and lambs for each operation on the list sampling frame at the time of survey selection and larger operations were selected with a higher probability. Producers on operations with 20 or more ewes were personally interviewed by NASS enumerators on site from January 1 to February 11, 2011, and interviewed a second time by federal or state veterinary medical officers or animal health technicians on site from March 14 to June 16, 2011. The surveys consisted of 164 questions in subsections related to: (1) General management, (2) last completed lamb crop, (3) lamb management during 2010, (4) disease control, illness, and death, (5) parasites and deworming, and (6) pasture management and feeding practices. The complete version of the study questionnaires is publically available at www.aphis.usda.gov/animal_health/nahms/sheep/downloads/sheep11ques/GSMQ.pdf

Operations that completed both questionnaires were eligible to have blood collected from up to 30 randomly selected adult ewes (lambed at least once) per operation. To reduce the likelihood of oversampling from small flocks, the number of samples from each flock varied by the number of ewes in the flock on the day of sampling—flocks with up to 49 ewes had 16 ewes sampled, 50–99 ewes had 22 ewes sampled, 100–199 ewes had 25 ewes sampled, and 200 or more ewes had 30 ewes sampled. In total, 13,252 ewe samples were collected from 563 operations. For this study, a subset of sera was selected using a convenient sampling method, and our final sample population consisted of sera from 5150 sheep in 270 operations (85–582 sheep per state).

Cell culture and viruses

African green monkey kidney (Vero) cells were cultured at 37°C with 5% CO_2 in Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. Six BUN serogroup viruses were used in this study: CVV (strain CVV-478), LOKV (strain FMS 4332), MDV (strain BFS 5015), NORV (strain 0234), POTV (strain BeAr7272), and TENV (strain A9-171b). CVV-478 was isolated from mosquitoes collected in Mexico (Farfan-Ale et al. 2010, Blitvich et al. 2012a). All other viruses were obtained from the World Arbovirus Reference Collection at the University of Texas Medical Branch in Galveston, Texas.

Plaque reduction neutralization tests

PRNTs were performed in six-well plates containing confluent monolayers of Vero cells following standard

methods (Beaty et al. 1995). Initially, all sera were screened at a single dilution of 1:20 using CVV. These assays are not CVV specific; BUN serogroup viruses are antigenically similar and, therefore, antibodies to other BUN serogroup viruses are also detected. A subset of sera that had CVV-neutralizing antibodies (selected based on convenience) were further diluted and tested by comparative PRNT to identify the BUN serogroup viruses responsible for these infections. Comparative PRNTs were performed using CVV, LOKV, MDV, NORV, POTV, and TENV. Titers were expressed as the reciprocal of highest serum dilutions yielding $\geq 90\%$ reduction in the number of plaques ($PRNT_{90}$). For etiologic diagnosis, the $PRNT_{90}$ antibody titer to the respective virus was required to be at least four-fold greater than that to the other viruses tested. The exception to this rule was when the $PRNT_{90}$ titers for two or more viruses were ≥ 1280. In such instances, the sheep was suspected to have had at least two BUN serogroup virus infections but was assigned the conservative diagnosis of ''seropositive to an undetermined BUN serogroup virus(es)'' to avoid potential misdiagnosis because the antibody responses in vertebrates sequentially infected with BUN serogroup viruses are not well understood.

Prevalence estimation

The primary outcome of interest was to estimate the individual-level and flock-level seroprevalence for BUN serogroup viruses. A positive flock was defined as any flock with at least one animal shown to possess CVV-neutralizing antibodies in the initial PRNT analysis. For each geographic region, the prevalence of positive flocks was summarized as the number of positive operations divided by the total number of operations with samples tested in our subset. A similar procedure was followed for estimates of animal-level seroprevalence. Results were reported for all regions and stratified by region (west, central, and east).

Associations with management practices

From the original NAHMS survey, eight items were selected on the basis of biological plausibility for assessment of association with serological responses. The eight survey items selected for assessment were region, breed (General Sheep Management Questionnaire [GSMQ] survey item section A-5, 10 possible responses), primary use (survey item section GSMQ A-6, six possible responses), flock type (survey item section GSMQ B-8, five possible responses), method used to house the majority of ewes in the winter, summer and lambing season (VS-A9, four possible responses for each season), occurrence of stillbirths in 2010 (VS-A19, four possible responses) (VS-C36, two possible responses), primary water source in the summer and winter (VS-F99, five possible responses each season), and insecticide and chemical usage (GSMQ survey item section K-1, two possible responses).

For each survey item, when survey responses were not mutually exclusive, the responses were dichotomized and associations tested. For example, survey item 6 requested information on the primary use of animals, and options consisted of meat production, wool production, show/competition/club/4-H, seed stock/breeding stock, milk production, and other. The owners were able to select multiple responses if more than one breed was present in the flock. These data were rearranged to create variables that indicated if a farm had at least one animal with meat production as a primary use, or at least one animal with wool production as a primary use, etc. Two-by-two classification tables were then created with flock CVV-neutralizing antibody status as positive or negative and the response as positive or negative combined over all regions. For example, for the breed survey item, Fisher's exact tests were applied to the six classification tables, as there were six possible responses for that survey item. For each test, the exact p value for the Fisher exact test is presented in most cases. We did not adjust for multiplicity. When analyses were not possible due to zero cells, this is indicated. The prevalence ratio and 95% confidence intervals were also calculated. In the subset tested very few missing data existed and these one to two examples are reported. The analyses were conducted using PROC FREQ SAS 9.3, and two-sided probabilities of being less than or equal to p is reported.

Results

Sera from 5150 sheep in 270 operations and 22 states in the United States were tested at a single dilution of 1:20 by PRNT using CVV. Antibodies that neutralized this virus were identified in 1455 (28.3%) sheep from 194 (71.9%) operations (Table 1). Animal-level seroprevalence was higher in the eastern states compared to the western and central states. In the east, 976 of 2005 (48.7%) sheep had antibodies that neutralized CVV. In contrast, 68 of 686 (9.9%) sheep in the west and 411 of 2459 (16.7%) sheep in the central states contained CVV-neutralizing antibodies. Analysis of the PRNT data at the operation level revealed that the seroprevalence in the east (96.4%) was significantly higher than the seroprevalence in the central (53.3%, $p < 0.00001$) and western (58.9%, $p < 0.00001$) states.

A subset of sera ($n = 509$) with antibodies that neutralized CVV was titrated and further analyzed by PRNT using CVV, LOKV, MDV, NORV, POTV, and TENV to determine the identities of the viruses responsible for the infections. Of the 169 sheep from the central United States that were tested, 53 were seropositive for CVV, two were seropositive for LOKV, one was seropositive for MDV, two were seropositive for POTV, and 111 were seropositive for an undetermined BUN serogroup virus. Of the 285 sheep from the east, 93 were seropositive for CVV, 11 were seropositive for LOKV, two were seropositive for MDV, seven were seropositive for POTV, and 172 were seropositive for an undetermined BUN serogroup virus. Of the 55 sheep from the west, nine were seropositive for CVV, one was seropositive for LOKV, four were seropositive for NORV, and 41 were seropositive for an undetermined BUN serogroup virus. Antibodies to TENV were not identified in any sheep. Of the 324 sheep seropositive for an undetermined BUN serogroup virus, the $PRNT_{90}$ titers were usually highest for CVV, and often these titers were two-fold higher than the corresponding LOKV $PRNT_{90}$ titers. Six sheep seropositive for an undetermined BUN serogroup virus had $PRNT_{90}$ titers that were ≥ 1280 for at least two viruses.

Results of the potential risk factor analysis are presented in Tables 2–9. Given that the study was not designed to detect any particular difference, it is unclear if p value and significance testing are useful, and we would propose that examination of the magnitude of difference between antibody-positive and antibody-negative groups by potential risk factor is more meaningful. Some factors were statistically

Table 1. Individual- and Operation-Level Prevalence of Antibodies to Bunyamwera (BUN) Serogroup Viruses in Sheep in Different Regions of the United States

	Region			
	West	*Central*	*East*	*Overall*
No. sheep positive/tested[a] (%)	68/686 (9.9)	411/2,459 (16.7)	976/2,005 (48.7)	1,455/5,150 (28.3)
No. farms positive/tested (%)	23/39 (58.9)	64/120 (53.3)	107/111 (96.4)	194/270 (71.9)

[a]Sera were tested at a dilution of 1:20 by plaque reduction neutralization test using Cache Valley virus and those that reduced the number of plaques by ≥90% were considered positive.

significant with a $p < 0.05$. Because region was also strongly associated with infection rates, the findings are also presented with p values after taking into account regional variations. Not surprisingly, a positive association between age and seroprevalence was observed (Table 2). The average age of animals seropositive for BUN serogroup viruses was higher than the average age of negative animals (the mean difference in individual age was 0.7 years, 95% confidence intervals ([5% CI] 0.42–0.97, $p < 0.0001$; data not shown). At the operation level, seropositive flocks had a higher average age than negative flocks (the mean difference in flock mean age was 0.4 years, 95% CI –0.03–0.83, $p < 0.0001$; data not shown). A positive association also existed between flock size and seroprevalence. Animals in small (20–99) and medium-sized (100–499) flocks were significantly more likely that those in large flocks (500+) to have antibodies to BUN serogroup viruses ($p = 0.0006$ and 0.0002, respectively; data not shown). At the operation level, the length of time that the primary operator owned or managed sheep was not significantly associated with antibody status (the mean difference in age of operator was −1.8 years, 95% CI − 5.4–1.8, $p = 0.44$; data not shown).

Antibodies to BUN serogroup viruses were identified in sheep from every breed tested (Table 3). Fine wool white-faced sheep were significantly less likely those of other breeds to be seropositive (Table 3; prevalence ratio = 0.78 [0.65–0.93]), but when region was accounted for, this difference was not significant. An association between primary use and antibody status was also observed; sheep reared for wool production were less likely to have BUN serogroup virus-specific antibodies (prevalence ratio = 0.76 [0.59–0.97]), but this difference was not significant when accounting for geography (Table 4). Sheep raised in pastures were significantly more likely to be seropositive compared to those raised by other means (prevalence ratio = 1.41 [1.18–1.69]), but once again this difference was not significant after accounting for region (Table 5). In contrast, herded/open-range sheep were less likely to be seropositive than other sheep (prevalence ratio = 0.20 [0.09–0.40]), and this continued to be significant when accounting for regional prevalence variations (Table 5).

There was an association between the type of housing used during the lambing season and antibody status (Table 6). After accounting for geography, sheep housed inside structures with

Table 2. Descriptive Information of Individual- and Operation-Level Characteristics for Sheep Stratified by Region and Bunyamwera (BUN) Serogroup Virus Antibody Status

	Region							
	West		*Central*		*East*		*Overall*	
	Antibody status							
	+	−	+	−	+	−	+	−
Animal level ($n = 5150$)								
Mean age (standard error [SE])	5.8 (0.4)	3.9 (0.1)	4.3 (0.2)	4.0 (0.1)	4.6 (0.1)	3.8 (0.1)	4.6 (0.1)	3.9 (0.1)
Mean flock size (SE)	578 (245)	966 (294)	542 (143)	1160 (212)	205 (31)	279 (75)	318 (48)	886 (134)
Mean number of years primary operator owned or managed sheep (SE)	27.9 (4.3)	29.8 (2.6)	30.8 (3.4)	31.0 (1.7)	25.5 (1.5)	24.0 (1.7)	27.1 (1.4)	28.9 (1.2)
Operation level ($n = 270$)								
Mean age (SE)	4.4 (0.2)	4.0 (0.2)	4.2 (0.1)	3.9 (0.2)	4.3 (0.1)	3.4 (0.9)	4.3 (0.1)	3.9 (0.2)
Mean flock size (SE)	632 (253)	984 (426)	758 (160)	1047 (281)	201 (34)	136 (42)	436 (66)	986 (226)
Mean number of years primary operator owned or managed sheep (SE)	29.7 (3.2)	27.4 (3.6)	29.3 (2.2)	32.2 (2.1)	24.7 (1.3)	24.7 (9.2)	26.8 (1.1)	30.9 (1.8)
Flock size								
20–99	9/20	11/20	23/42	19/42	56/58	2/58	88/120	32/120
100–499	10/11	1/11	24/41	17/41	48/50	2/50	82/102	20/102
500+	4/8	4/8	17/37	20/37	3/3	0/3	24/48	24/48
Total	23/39	16/39	64/120	56/120	107/111	4/111	194/270	76/270

Table 3. Operation-Level Prevalence of Antibodies to Bunyamwera (BUN) Serogroup Viruses in Sheep According to Breed

Breed	No. operations positive/tested (%) West	Central	East	Overall	Operations without breed of interest	Prevalence ratio (95% confidence interval), p value[a]	p value when accounting for region[b]
Black face	16/26 (61.5)	37/66 (56.1)	54/57 (94.7)	107/149 (71.8)	87/121 (71.9)	0.99 (0.85–1.16), 1.00	0.64
Fine wool white-faced	5/9 (55.6)	31/63 (49.2)	19/19 (100.0)	55/91 (60.4)	139/179 (77.7)	0.78 (0.65–0.93), 0.0041	0.48
Medium wool white-faced	12/16 (75.0)	19/45 (42.2)	49/50 (98.0)	80/111 (72.1)	114/159 (71.7)	1.00 (0.86–1.16), 1.00	0.61
Long wool	4/7 (57.1)	3/4 (75.0)	4/4 (100.0)	11/15 (73.3)	183/255 (71.8)	1.02 (0.75–1.39), 1.00	0.60
Mottle-faced, brockle or speckle-faced crossbred	7/11 (63.6)	13/20 (65.0)	23/24 (95.8)	43/55 (78.2)	151/215 (70.2)	1.10 (0.99–1.31), 0.31	0.29
Colored wool	3/3 (100.0)	2/4 (50.0)	9/10 (90.0)	14/17 (82.4)	180/253 (71.1)	1.15 (0.92–1.46), 0.41	0.74
Hair sheep	3/5 (60.0)	9/11 (81.8)	17/18 (94.4)	29/34 (85.3)	165/236 (69.9)	1.20 (1.03–1.43), 0.07	0.15
Milk sheep	1/1 (100.0)	1/2 (50.0)	3/3 (100.0)	5/6 (83.3)	189/264 (71.6)	1.16 (0.80–1.67), 1.00	0.61
Other ($n=269$)[c]	1/2 (50.0)	4/6 (66.7)	13/13 (100.0)	18/21 (85.7)	175/248 (70.6)	1.21 (1.00–1.47), 0.21	0.46
Unknown	0	1/2 (50.0)	3/3 (100.0)	4/5 (80.0)	190/265 (71.7)	1.12 (0.71–1.74), 1.00	0.96
Total[d]	23/39 (59.0)	64/120 (53.3)	107/111 (96.4)	194/270 (71.9)	—	—	—

[a]Two-sided (unadjusted) *p* value.
[b]*p* value for significance of prevalence between operations that had any of this breed and those that did not when accounting for geography.
[c]Information on ''other breeds'' is not available for one of the 270 operations.
[d]Some operations contain more than one breed of sheep.

Table 4. Operation-Level Prevalence of Antibodies to Bunyamwera (BUN) Serogroup Viruses in Sheep According to their Primary Use

Primary purpose	No. operations positive/tested (%) West	Central	East	Overall	Operations without sheep type of interest	Prevalence ratio (95% confidence interval), p value[a]	p value when accounting for region[b]
Meat production	16/30 (53.3)	56/100 (56.0)	87/91 (95.6)	159/221 (72.0)	33/47 (70.2)	1.02 (0.83–1.25), 0.86	0.90
Wool production	3/5 (60.0)	18/39 (46.2)	10/10 (100)	31/54 (57.4)	161/214 (75.2)	0.76 (0.59–0.97), 0.01	0.40
Show, competition, 4-H or club lambs	3/5 (60.0)	4/10 (40.0)	13/13 (100)	20/28 (71.4)	172/240 (71.7)	0.99 (0.78–1.28), 1.00	0.70
Seedstock/Breedingstock	9/14 (64.3)	16/35 (45.7)	27/28 (96.4)	52/77 (67.5)	140/191 (73.3)	0.92 (0.77–1.09), 0.37	0.53
Milk production	1/1 (100)	0	3/3 (100)	4/4 (100)	188/264 (71.2)	N/A	—
Other	0	2/2 (100)	3/3 (100)	5/6 (83.3)	187/262 (71.4)	1.17 (0.81–1.68), 1.00	0.61
Total[c]	23/39 (59.0)	64/120 (53.3)	107/111 (96.4)	194/270 (71.9)	—	—	—

[a]Two-sided (unadjusted) *p* value.
[b]*p* value for significance of prevalence between operations that had this primary purpose and those that did not when accounting for geography.
[c]Some operations rear sheep for more than one purpose.
N/A, not applicable.

Table 5. Operation-Level Prevalence of Antibodies to Bunyamwera (BUN) Serogroup Viruses in Sheep According to Primary Flock Type

Primary flock type	No. operations positive/tested (%) West	Central	East	Overall	Operations without flock type of interest	Prevalence ratio (95% confidence interval), p value[a]	p value when accounting for region[b]
Herded/open-range	0/4 (0)	5/27 (18.5)	0	5/31 (16.1)	189/239 (79.1)	0.20 (0.09–0.4), <0.0001	Ref.[c]
Fenced-range	4/6 (66.7)	24/35 (68.6)	8/8 (100.0)	36/49 (73.5)	158/221 (71.5)	1.02 (0.88–1.24), 0.86	<0.001
Pasture	17/26 (65.4)	29/48 (60.4)	87/91 (95.6)	133/165 (80.6)	61/105 (58.1)	1.41 (1.18–1.69), <0.0001	0.0001
Dry lot or feedlot	2/3 (66.7)	6/10 (60.0)	12/12 (100.0)	20/25 (80.0)	174/245 (71.0)	1.13 (0.91–1.39), 0.48	0.002
Total	23/39 (59.0)	64/120 (53.3)	107/111 (96.4)	194/270 (71.9)			

[a]Two sided (unadjusted) *p* value.
[b]*p* value for significance of prevalence between operations that had any of this flock type and those that did not when accounting for geography.
[c]Reference value to which other data were compared.

four walls and a roof with a door closed most of the time were significantly more likely to have antibodies to BUN serogroup viruses than other sheep. Summer and winter housing were not significantly associated with antibody status after accounting for region. Likewise, the summer water source was also not significantly associated with antibody status after accounting for region (Table 7). There was no association between seroprevalence and the percentage of lambs that were stillborn in 2010 (Table 8). BUN serogroup virus activity was detected on 69 of 104 (66.4%) operations that treated sheep with insecticides or similar products and on nine of 11 (81.8%) operations that did not but this difference was not significant (Table 9).

Discussion

This report describes the largest survey for antibodies to BUN serogroup viruses in sheep. BUN serogroup virus activity was detected in all three geographic regions investigated (*e.g.*, the west, central, and eastern United States), and the overall seroprevalence was 28%. This is presumably a slight underestimate and additional seropositive animals most likely would have been identified if the initial PRNTs were not restricted to CVV. Another limitation of our study is that a random sampling strategy was not used to select the sera. Although the sera collected in the NAHMS study were from ewes that were representative of the ewe population on the operations, the subset of sera selected for our PRNTs was a convenient sample. Convenient sampling limits our understanding of how the findings of the study population relate to the source or target population. Nevertheless, we provide compelling evidence that BUN serogroup viruses commonly infect sheep in the United States. Other serological investigations have also estimated the seroprevalence of BUN serogroup viruses in North American sheep, although these studies were performed with much smaller sample populations (Buescher et al. 1970, Crandell et al., 1989, Chung et al. 1991, Blitvich et al. 2012b).

Animal-level seroprevalence for BUN serogroup viruses was approximately three- to five-fold higher in the east compared to the central and western states, and the most common BUN serogroup virus was CVV. These findings could indicate that there is a higher abundance of competent arthropod vectors or reservoir hosts for CVV and possibly other BUN serogroup viruses in the eastern United States. Currently, the principal vectors and reservoir hosts of the viruses examined in this study are not known, although likely candidates have been identified for some of these viruses. CVV has been isolated from many different mosquito species but most frequently from *Anopheles* (*An.*) *quadrimaculatus*, *Culiseta inornata*, and *Coquillettidia* (*Cq.*) *perturbans* (Calisher et al. 1986). *An. quadrimaculatus* and to a lesser extent *Cq. perturbans* are competent vectors of CVV in the laboratory (Blackmore et al. 1998), whereas the vector competence of *Cs. inornata* has not been evaluated. *An. quadrimaculatus* is common in the eastern half of the United States and does not occur in the western United States (Carpenter 1955, Levine et al. 2004).

White-tailed deer have been implicated as the principal reservoir hosts of CVV (Blackmore and Grimstad 1998) and are more abundant in eastern half of the United States (Paddock and Yabsley 2007). White-tailed deer are also the preferred source of blood for *An. quadrimaculatus* in the eastern United States (Molaei et al. 2009). Therefore, the higher seroprevalence of BUN serogroup viruses in the east

Table 6. Operation-Level Prevalence of Antibodies to Bunyamwera (BUN) Serogroup Viruses in Sheep According to Housing Method

Housing according to season[a]	No. operations positive/tested (%) — Region: West	Central	East	Overall	Operations without housing of interest	Prevalence ratio (95% confidence interval), p value[b]	p value when accounting for region[c]
Winter housing ($n=265$)							
Structure with four walls and a roof with door closed most of the time	1/1 (100)	2/6 (33.3)	20/22 (90.9)	23/29 (79.3)	167/236 (70.8)	1.21 (0.91–1.36), 0.38	0.30
Structure with four walls and a roof with door open most of the time	3/4 (75.0)	12/20 (60.0)	49/50 (98.0)	64/74 (86.5)	126/191 (66.0)	1.33 (1.40–1.50), 0.0007	0.34
Structure with a roof and three or fewer walls (*e.g.*, loafing shed)	8/16 (50.0)	18/32 (56.2)	19/20 (95.0)	45/68 (66.2)	145/197 (73.6)	0.89 (0.74–1.08), 0.27	1.00
No structure	10/17 (58.8)	29/58 (50.0)	19/19 (100.0)	58/94 (61.7)	132/171 (77.2)	0.79 (0.67–0.95), 0.011	Ref.[d]
Summer structure ($n=264$)							
Structure with four walls and a roof with door closed most of the time	0	0	0	0	189/264 (71.6)	N/A	N/A
Structure with four walls and a roof with door open most of the time	2/3 (66.7)	4/8 (50.0)	29/32 (90.6)	35/43 (81.4)	154/221 (69.7)	1.16 (0.98–1.38), 0.14	0.37
Structure with a roof and three or fewer walls (*e.g.*, loafing shed)	5/10 (50.0)	15/25 (60.0)	25/26 (96.2)	45/61 (73.8)	144/203 (70.9)	1.04 (0.87–1.23), 0.74	0.81
No structure	15/25 (60.0)	41/82 (50.0)	53/53 (100.0)	109/160 (68.1)	80/104 (76.9)	0.88 (0.76–1.02), 0.12	Ref.[d]
Lambing season ($n=266$)							
Structure with four walls and a roof with door closed most of the time	3/4 (75.0)	21/25 (84.0)	46/47 (97.9)	70/76 (92.1)	121/190 (63.7)	1.44 (1.27–1.64), 0.38	0.05
Structure with four walls and a roof with door open most of the time	8/14 (57.1)	16/36 (44.4)	36/38 (94.7)	60/88 (68.2)	131/178 (73.6)	0.93 (0.78–1.09), 0.38	0.30
Structure with a roof and three or fewer walls (*e.g.*, loafing shed)	8/16 (50.0)	12/32 (37.5)	13/14 (92.9)	33/62 (53.2)	158/204 (77.5)	0.69 (0.54–0.87), 0.001	0.10
No structure	4/5 (80.0)	12/23 (52.2)	12/12 (100.0)	28/40 (70.0)	163/226 (72.1)	0.91 (0.43–1.8), 0.84	Ref.[d]

[a]Information on housing is not available for several operations.
[b]Two-sided (unadjusted) *p* value.
[c]*p* value for significance of prevalence between operations that had any of this housing type and those that did not when accounting for geography.
[d]Reference value to which other data were compared.
N/A, not applicable.

Table 7. Operation-Level Prevalence of Antibodies to Bunyamwera (BUN) Serogroup Viruses in Sheep According to Summer Water Source

Primary summer water source (n = 266)[a]	No. operations positive/tested (%) — Region: West	Central	East	Overall	Operations without water source of interest	Prevalence ratio (95% confidence interval), p value[b]	p value when accounting for region[c]
Pond/lake/reservoir	0/1 (0.0)	11/21 (52.4)	6/6 (100)	17/28 (60.7)	174/239 (72.8)	0.83 (0.61–1.12), 0.18	0.75
Stream	2/4 (50.0)	8/25 (32.0)	13/13 (100)	23/42 (54.8)	168/224 (75.0)	0.73 (0.54–0.97), 0.01	0.19
Bucket, trough or waterer	20/32 (62.5)	30/48 (62.5)	70/73 (95.9)	120/153 (78.4)	71/113 (62.8)	1.24 (1.06–1.47), 0.006	0.67
Other water source	0	2/5 (40.0)	1/1 (100)	3/6 (50.0)	188/260 (72.3)	0.69 (0.31–1.54), 0.35	0.55
Multiple water sources	1/2 (50.0)	10/17 (58.8)	17/18 (94.4)	28/37 (75.7)	163/229 (71.2)	1.06 (0.87–1.29), 0.695	Ref.[d]

[a]Information on summer water source is not available for four of the 270 operations.
[b]Two-sided (unadjusted) *p* value.
[c]*p* value for significance of prevalence between operations that had this primary water source and those that did not when accounting for geography.
[d]Reference value to which other data were compared.

Table 8. Operation-Level Prevalence of Antibodies to Bunyamwera (BUN) Serogroup Viruses in Sheep According to the Incidence of Stillbirths

% of new lambs stillborn per ewe bred in 2010 (n = 244)	No. operations positive/tested (%)[a] — Region: West	Central	East	Overall	Operations without % stillborns of interest	Prevalence ratio (95% confidence interval), p value[b]	p value when accounting for region[c]
0%	3/5 (60.0)	10/16 (62.5)	19/19 (100.0)	32/40 (80.0)	146/204 (71.6)	1.12 (0.93–1.33), 0.33	0.06
>0% but <5%	6/10 (60.0)	21/42 (50.0)	32/32 (100.0)	59/84 (70.2)	119/160 (74.4)	0.94 (0.79–1.15), 0.54	0.12
5 to <20%	11/17 (64.7)	18/36 (50.0)	46/49 (93.9)	75/102 (73.5)	102/142 (71.8)	1.02 (0.87–1.19), 0.88	0.15
≥20%	0/3	2/4 (50.0)	10/11 (90.9)	12/18 (66.7)	166/226 (73.5)	0.91(0.65–1.27), 0.58	Ref.[d]
Total	20/35 (57.1)	51/98 (52.0)	107/111 (96.4)	178/244 (73.0)			

[a]Information is not available for 26 of the 270 operations.
[b]Two-sided (unadjusted) *p* value.
[c]*p* value for significance of prevalence between operations that had this stillborn percentage category and those that did not when accounting for geography.
[d]Reference value to which other data were compared.

Table 9. Association Between Operation-Level Seroprevalence and Insect Control

Treatment	No. (%) operations with BUN serogroup virus-specific antibodies				p value when accounting for region
	Region				
	West	Central	East	Overall	
Insecticides or other chemical products were applied to sheep to control insects	11/20 (55.0)	31/55 (56.4)	27/29 (93.1)	69/104 (66.4)	0.35
Insecticides or other chemical products were not applied to sheep to control insects	0	5/7 (71.4)	4/4 (100.0)	9/11 (81.8)	
Total[a]	11/20 (55.0)	36/62 (58.1)	31/33 (94.0)	78/115 (67.8)	—

[a]Information is not available for 155 operations.

could be due to the greater abundance of white-tailed deer and *An. quadrimaculatus* in this region. Other mosquito species implicated as vectors of CVV include *An. punctipennis*, *Ochlerotatus* (*Oc.*) *sollicitans*, *Oc. taeniorhynchus*, and *Oc. trivittatus* (Ngo et al. 2006, Farfan-Ale et al. 2010, Andreadis et al. 2014). *An. punctipennis* occurs throughout most of the United States (Carpenter, 1955) and has been incriminated as the most likely mosquito vector of CVV in the northeastern United States on the basis of yearly isolation frequencies and spatial distribution patterns (Andreadis et al. 2014). Furthermore, although the preponderance of blood meals identified from this mosquito species in the northeast have been from white-tailed deer, Molaei and colleagues identified sheep-derived blood in 6% of *An. punctipennis* collected in New Jersey (Molaei et al. 2009). *An. punctipennis* is also a competent laboratory vector of CVV (Saliba et al. 1973). *Oc. sollicitans* and *Oc. taeniorhynchus* are common along the eastern seaboard (Carpenter 1955) and are also competent vectors of CVV (Yuill and Thompson 1970).

Several management factors were shown to be significantly associated with the presence of antibodies to BUN serogroup viruses once regional prevalence variations were accounted for. Sheep housed during the lambing season inside structures containing four walls and a roof with a door closed most of the time were more likely to have antibodies to BUN serogroup viruses than other sheep. Four-walled structures that have a roof and a door closed most of the time could facilitate increased animal–mosquito contact. Indeed, these structures could provide a suitable environment for overwintering mosquitoes, and the sheep inside these structures could provide a readily available blood source for the mosquitoes once they emerge in the spring. Sheep classified as herded/open-range were less likely than other sheep to have antibodies to BUN serogroup viruses. Herded/open-range sheep roam freely on unfenced lands (usually prairies) that are typically covered with perennial grass but have few, if any, trees and man-made structures. Thus, one explanation why herded/open-range sheep are less likely to be seropositive than other sheep is because they are raised on land that has less shade and therefore potentially fewer mosquito resting sites than other areas. Land used for herded/open-range sheep often has higher wind activity than other areas due to the sparse amount of tall vegetation and made-made structures and this could also potentially reduce the amount of mosquito activity.

There was no association between seroprevalence and the percentage of new lambs that were stillborn in the previous lambing season. These data could indicate that BUN serogroup virus infections are frequently asymptomatic or that limited BUN serogroup virus activity occurred in 2010. Alternatively, these data could indicate that most BUN serogroup virus infections often occurred outside of the pregnancy period. In this regard, the natural lambing season in the United States usually occurs from February to May, whereas the large majority of CVV isolations have been made from mosquitoes collected during the late summer and early fall (Calisher et al. 1986, Ngo et al. 2006, Andreadis et al. 2014). For instance, in Connecticut, CVV activity peaked in August to mid-September (Andreadis et al. 2014). Another explanation for our findings is that most stillbirths were either due to other pathogens or noninfectious factors. One limitation of our study is that the questionnaire was limited to general stillbirths rather than those characterized by arthrogryposis, which is the most common musculoskeletal defect observed in CVV-infected sheep (Edwards et al. 1989, Chung et al. 1990a, b). The inclusion of all stillbirths could have contributed to the lack of association, and a positive correlation may have been observed if our analysis was restricted to stillbirths characterized by arthrogryposis.

BUN serogroup virus activity was detected on 69 of 104 (66.4%) operations that applied insecticides or other chemical products to sheep and on nine of 11 (81.8%) operations that did not use these products. This difference is not significant, and, therefore, our study provides no evidence that insecticides reduce the likelihood of virus exposure. Because insecticide use is a practice adopted on most sheep operations, the number of insecticide-free operations was restricted to 11. This small sample size would have reduced the power of our analysis. Therefore, additional work is needed to assess more accurately the relationship between insecticide use and BUN serogroup virus exposure in sheep.

Fourteen sheep were seropositive for LOKV (11 in the eastern United States, two in the central United States, and one in the west). Prior to this study, LOKV activity had only been detected in the western and central United States (Crane et al. 1983, Calisher et al. 1986, Kramer et al. 1990, Johnson et al. 2014). Thus, these data indicate that the geographic distribution of LOKV is wider than previously recognized. Three sheep were seropositive for MDV (two in the east and one in the central United States). Previously, MDV was not known to occur east of Texas (Calisher et al. 1986), and, therefore, these data indicate that the geographic distribution of MDV is also wider than previously reported. Four sheep were seropositive for NORV, and all were from operations in the west. These

findings support previous studies that have shown that NORV activity is restricted to the western states (Calisher et al. 1974, Campbell et al. 1991). Antibodies to POTV were detected in nine sheep. Seven sheep were from operations in the east and two were from operations in the central United States, consistent with known geographic distribution of POTV (Francy et al. 1990, Harrison et al. 1995, Mitchell et al. 1996, 1998, Wozniak et al. 2001, Armstrong et al. 2005, Ngo et al. 2006). This is the first study to provide evidence that sheep are susceptible to POTV infection. Antibodies to TENV were not detected in any sheep. One explanation for this finding is that sheep are not a preferred source of blood for the principal vectors of TENV. Alternatively, TENV may replicate poorly, if at all, in sheep. Another, perhaps more likely, explanation is because most sheep sampled in this study were from states outside of the known geographic range of TENV. This virus has been reported in seven states in the southeast: Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, and Texas (Calisher et al. 1986, Wozniak et al. 2001). However, almost all isolates have come from Alabama, Florida, and Georgia, three states not represented in our study.

The majority (64%) of sera analyzed by comparative PRNT had antibodies to an undetermined BUN serogroup virus(es). A likely explanation for these findings is that most of the sheep from which these sera were collected had been exposed to two or more BUN serogroup viruses. If this is true, we consider it most likely that the sheep had been exposed to CVV and either LOKV or POTV because our data indicate that these viruses are more widespread and frequently associated with ovine infections than the other viruses included in this study. Another explanation is that an unrecognized BUN serogroup virus occurs in the United States. Similar to our observations, a high proportion of sheep and horses in the Yucatan Peninsula of Mexico in 2007–2008 were shown to have antibodies to an undetermined BUN serogroup virus (Blitvich et al. 2012b). Blackmore and Grimstad (1998) have provided some insight into the antibody responses in vertebrates challenged sequentially with different BUN serogroup viruses. In these studies, white-tailed deer were inoculated with POTV or CVV and, 5–7 months later, inoculated with the alternate virus. Seven days after the secondary inoculation, the mean reciprocal antibody titers in deer sequentially inoculated with POTV then CVV were 206 (±60 standard error [SE]) and 96 (±16 SE), respectively. It is important to note the approximate two-fold difference in mean antibody titers in these deer because many of the sheep in our study with antibodies to an undetermined BUN serogroup virus also exhibited a two-fold difference between their highest and second highest PRNT titer.

Conclusions

We provide evidence that BUN serogroup viruses commonly infect sheep in the United States and have identified a number of factors positively correlated to antibody status. The data generated in this study could assist producers in the implementation of strategies that reduce the likelihood of BUN serogroup virus infection and improve the health and management practices of sheep.

Acknowledgments

This study was supported by a grant from the Iowa Livestock Health Advisory Committee. The authors wish to thank the State and Federal personnel who visited sheep operations and collected the data and blood samples, and the sheep producers themselves who voluntarily participated in the study. The authors also thank Robert B. Tesh from the University of Texas Medical Branch in Galveston, Texas, for providing isolates of LOKV, MDV, NORV, POTV, and TENV.

Author Disclosure Statement

No competing financial interests exist.

References

Andreadis TG, Armstrong PM, Anderson JF, Main AJ. Spatial-temporal analysis of cache valley virus (Bunyaviridae: Orthobunyavirus) infection in anopheline and culicine mosquitoes (Diptera: Culicidae) in the northeastern United States, 1997–2012. Vector Borne Zoonotic Dis 2014; 14:763–773.

Armstrong PM, Andreadis TG, Anderson JF, Main AJ. Isolations of Potosi virus from mosquitoes (Diptera: Culicidae) collected in Connecticut. J Med Entomol 2005; 42:875–881.

Beaty BJ, Calisher CH, Shope RE. Arboviruses, In: Lennette E, ed. *Diagnostic Procedures for Viral and Rickettsial Diseases*. Washington DC: American Public Health Association, 1995: 189–212.

Blackmore CG, Grimstad PR. Cache Valley and Potosi viruses (Bunyaviridae) in white-tailed deer (*Odocoileus virginianus*): Experimental infections and antibody prevalence in natural populations. Am J Trop Med Hyg 1998; 59:704–709.

Blackmore CG, Blackmore MS, Grimstad PR. Role of *Anopheles quadrimaculatus* and *Coquillettidia perturbans* (Diptera: Culicidae) in the transmission cycle of Cache Valley virus (Bunyaviridae: Bunyavirus) in the midwest, USA. J Med Entomol 1998; 35:660–664.

Blitvich BJ, Lorono-Pino MA, Garcia-Rejon JE, Farfan-Ale JA. Nucleotide sequencing and serologic analysis of Cache Valley virus isolates from the Yucatan Peninsula of Mexico. Virus Genes 2012a; 45:176–180.

Blitvich BJ, Saiyasombat R, Travassos da Rosa A, Tesh RB, et al. Orthobunyaviruses, a common cause of infection of livestock in the Yucatan peninsula of Mexico. Am J Trop Med Hyg 2012b; 87:1132–1139.

Buescher EL, Byrne RJ, Clarke GC, Gould DJ, et al. Cache Valley virus in the Del Mar Va Peninsula. I. Virologic and serologic evidence of infection. Am J Trop Med Hyg 1970; 19:493–502.

Calisher CH, Lindsey HS, Ritter DG, Sommerman KM. Northway virus: A new Bunyamwera group arbovirus from Alaska. Can J Microbiol 1974; 20:219–223.

Calisher CH, Gutierrez E, Francy DB, Alava A, et al. Identification of hitherto unrecognized arboviruses from Ecuador: Members of serogroups B, C, Bunyamwera, Patois, and Minatitlan. Am J Trop Med Hyg 1983; 32:877–885.

Calisher CH, Francy DB, Smith GC, Muth DJ, et al. Distribution of Bunyamwera serogroup viruses in North America, 1956–1984. Am J Trop Med Hyg 1986; 35:429–443.

Campbell GL, Hardy JL, Eldridge BF, Reeves WC. Isolation of Northway serotype and other Bunyamwera serogroup bunyaviruses from California and Oregon mosquitoes, 1969–1985. Am J Trop Med Hyg 1991; 44:581–588.

Campbell GL, Mataczynski JD, Reisdorf ES, Powell JW, et al. Second human case of Cache Valley virus disease. Emerg Infect Dis 2006; 12:854–856.

Carpenter SJ, LaCasse WJ. *Mosquitoes of North America (North of Mexico).* Berkeley, CA: University of California Press, 1955.

Chung SI, Livingston CW Jr, Edwards JF, Crandell RW, et al. Evidence that Cache Valley virus induces congenital malformations in sheep. Vet Microbiol 1990a; 21:297–307.

Chung SI, Livingston CW Jr, Edwards JF, Gauer BB, et al. Congenital malformations in sheep resulting from in utero inoculation of Cache Valley virus. Am J Vet Res 1990b; 51:1645–1648.

Chung SI, Livingston CW Jr, Jones CW, Collisson EW. Cache Valley virus infection in Texas sheep flocks. J Am Vet Med Assoc 1991; 199:337–340.

Crandell RA, Livingston CW Jr, Shelton MJ. Laboratory investigation of a naturally occurring outbreak of arthrogryposis-hydranencephaly in Texas sheep. J Vet Diagn Invest 1989; 1:62–65.

Crane GT, Elbel RE, Francy DB, Calisher CH. Arboviruses from western Utah, USA, 1967–1976. J Med Entomol 1983; 20:294–300.

Edwards JF, Livingston CW, Chung SI, Collisson EC. Ovine arthrogryposis and central nervous system malformations associated with in utero Cache Valley virus infection: Spontaneous disease. Vet Pathol 1989; 26:33–39.

Edwards JF, Karabatsos N, Collisson EW, de la Concha Bermejillo A. Ovine fetal malformations induced by in utero inoculation with Main Drain, San Angelo, and LaCrosse viruses. Am J Trop Med Hyg 1997; 56:171–176.

Emmons RW, Woodie JD, Laub RL, Oshiro LS. Main Drain virus as a cause of equine encephalomyelitis. J Am Vet Med Assoc 1983; 183:555–558.

Farfan-Ale JA, Lorono-Pino MA, Garcia-Rejon JE, Soto V, et al. Detection of flaviviruses and orthobunyaviruses in mosquitoes in the Yucatan Peninsula of Mexico in 2008. Vector Borne Zoonotic Dis 2010; 10:777–783.

Francy DB, Karabatsos N, Wesson DM, Moore CG Jr, et al. A new arbovirus from *Aedes albopictus,* an Asian mosquito established in the United States. Science 1990; 250:1738–1740.

Harrison BA, Mitchell CJ, Apperson CS, Smith GC, et al. Isolation of potosi virus from *Aedes* albopictus in North Carolina. J Am Mosq Control Assoc 1995; 11:225–229.

Hoffmann AR, Welsh CJ, Varner PW, de la Concha-Bermejillo A, et al. Identification of the target cells and sequence of infection during experimental infection of ovine fetuses with Cache Valley virus. J Virol 2012; 86:4793–4800.

Holden P, Hess AD. Cache Valley virus, a previously undescribed mosquito-borne agent. Science 1959; 130:1187–1188.

Hunt AR, Calisher CH. Relationships of bunyamwera group viruses by neutralization. Am J Trop Med Hyg 1979; 28: 740–749.

International Committee on Taxonomy of Viruses (ICTV). *Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses*. San Diego: Elsevier Academic Press, 2012.

Johnson GD, Bahnson CS, Ishii P, Cochrane ZN, et al. Monitoring sheep and Culicoides midges in Montana for evidence of Bunyamwera serogroup virus infection. Vet Rec Open 2014; 1:e000071 doi:10.1136/vetreco-2014-000071.

Kramer WL, Jones RH, Holbrook FR, Walton TE, et al. Isolation of arboviruses from Culicoides midges (Diptera: Ceratopogonidae) in Colorado during an epizootic of vesicular stomatitis New Jersey. J Med Entomol 1990; 27:487–493.

Levine RS, Peterson AT, Benedict MQ. Distribution of members of *Anopheles quadrimaculatus* say s.l. (Diptera: Culicidae) and implications for their roles in malaria transmission in the United States. J Med Entomol 2004; 41:607–613.

Mangiafico JA, Sanchez JL, Figueiredo LT, LeDuc JW, et al. Isolation of a newly recognized Bunyamwera serogroup virus from a febrile human in Panama. Am J Trop Med Hyg 1988; 39:593–596.

McConnell S, Livingston C Jr, Calisher CH, Crandell RA. Isolations of Cache Valley virus in Texas, 1981. Vet Microbiol 1987; 13:11–18.

McGowan JE Jr, Bryan JA, Gregg MB. Surveillance of arboviral encephalitis in the United States, 1955–1971. Am J Epidemiol 1973; 97:199–207.

Mitchell CJ, Smith GC, Karabatsos N, Moore CG, et al. Isolations of Potosi virus from mosquitoes collected in the United States, 1989–94. J Am Mosq Control Assoc 1996; 12:1–7.

Mitchell CJ, Haramis LD, Karabatsos N, Smith GC, et al. Isolation of La Crosse, Cache Valley, and Potosi viruses from Aedes mosquitoes (Diptera: Culicidae) collected at used-tire sites in Illinois during 1994–1995. J Med Entomol 1998; 35:573–577.

Molaei G, Farajollahi A, Armstrong PM, Oliver J, et al. Identification of bloodmeals in *Anopheles quadrimaculatus* and *Anopheles punctipennis* from eastern equine encephalitis virus foci in northeastern U.S.A. Med Vet Entomol 2009; 23:350–356.

Ngo KA, Maffei JG, Dupuis AP 2nd, Kauffman EB, et al. Isolation of Bunyamwera serogroup viruses (Bunyaviridae, Orthobunyavirus) in New York state. J Med Entomol 2006; 43:1004–1009.

Nguyen NL, Zhao G, Hull R, Shelly MA, et al. Cache valley virus in a patient diagnosed with aseptic meningitis. J Clin Microbiol 2013; 51:1966–1969.

Paddock CD, Yabsley MJ. Ecological havoc, the rise of white-tailed deer, and the emergence of *Amblyomma americanum*-associated zoonoses in the United States. Curr Top Microbiol Immunol 2007; 315:289–324.

Saliba EK, DeFoliart GR, Yuill TM, Hanson RP. Laboratory transmission of Wisconsin isolates of a Cache Valley-like virus by mosquitoes. J Med Entomol 1973; 10:470–476.

Schmaljohn CS, Nichol ST. Bunyaviridae., In: Knipe DM, ed. *Fields Virology. Fifth Edition.* Philadelphia: Lippincott Williams and Wilkins, 2007:1741–1789.

Sexton DJ, Rollin PE, Breitschwerdt EB, Corey GR, et al. Life-threatening Cache Valley virus infection. N Engl J Med 1997; 336:547–549.

US Department of Agriculture. Sheep 2011, Part I: Reference of Sheep Management Practices in the United States, 2011. 2012. www.aphis.usda.gov/animal_health/nahms/sheep/downloads/sheep11/Sheep11_dr_PartI.pdf

US Department of Agriculture. Sheep 2011, Part III: Health and Management Practices on U.S. Sheep Operations, 2011. 2013. www.aphis.usda.gov/animal_health/nahms/sheep/downloads/sheep11/Sheep11_dr_PartIII.pdf

Wozniak A, Dowda HE, Tolson MW, Karabatsos N, et al. Arbovirus surveillance in South Carolina, 1996–98. J Am Mosq Control Assoc 2001; 17:73–78.

Yuill TM, Thompson PH. Cache Valley virus in the Del Mar Va Peninsula. IV. Biological transmission of the virus by *Aedes sollicitans* and *Aedes taeniorhynchus*. Am J Trop Med Hyg 1970; 19:513–519.

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