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SHORT COMMUNICATIONS

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Serologic Survey for Selected Arboviruses and Other Potential Pathogens in Wildlife from Mexico

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ABSTRACT: During 1988 and 1989, a serologic survey of wildlife was conducted in northeastern Mexico to determine the presence, prevalence, and distribution of arboviruses and other selected disease agents. Eighty mammal specimens were tested. Antibodies to vesicular stomatitis-Indiana, Venezuelan equine encephalitis–Mena II, Rio Grande virus, and vesicular stomatitis-New Jersey were detected predominately in small mammals. Deer and mouflon (*Ovis musimon*) had antibodies to bluetongue and epizootic hemorrhagic disease. Two species had serologic evidence of recent exposure to *Francisella tularensis*. A white-tailed deer (*Odocoileus virginianus*) had antibodies to *Anaplasma marginale*. All specimens tested for antibodies against *Yersinia pestis* and *Brucella abortus* were negative. Sera from 315 birds were tested for antibody against five equine encephalitis viruses and six avian pathogens. During 1988, antibodies to Venezuelan equine encephalitis–Mena II, Venezuelan equine encephalitis-TC83, St. Louis encephalitis, eastern equine encephalitis, and western equine encephalitis were detected in birds of several species. Antibodies to *Pasteurella multocida* and Newcastle disease virus were also detected. Birds from five species presented antibodies to *Mycoplasma meleagridis*. Specimens tested for *M. gallisepticum*, *M. synoviae*, and *Chlamydia psittaci* were negative. To the best of our knowledge, this survey represents the first serologic evidence of bluetongue, Cache Valley virus, epizootic hemorrhagic disease, Jamestown Canyon virus, vesicular stomatitis–Indiana, vesicular stomatitis–New Jersey, Rio Grande virus, and tularemia reported among wildlife in Mexico.

Key words: Arboviruses, avian cholera, disease survey, Newcastle disease, tularemia, wildlife.

Much of the information known about diseases in wildlife has been aquired from serologic surveys. A number of factors, however, such as viremia titer and duration, antivector behavior, and longevity may bias results. Another limitation of serology includes the size and distribution of the samples. Samples from a single flock or herd may be insufficient. Also, the trapping technique may bias the composition of the collection, resulting in a non-representative sample of wildlife tested. Despite these limiting factors, serologic surveys are considered important in that they reveal evidence of the presence of disease when apparent infections and clinical cases have not been detected. Also, presence of selected disease agents may be determined when limited information is known about a specific disease in a geographic area.

The prevalence of arboviruses in wild vertebrates in Mexico has been previously documented (De Mucha-Macias, 1963; Campillo-Sainz, 1969; Scherer et al., 1971; Dickerman et al., 1972b). Other disease agents have been reported from wildlife in that country. For example, Varela and Vazquez (1954) isolated *Yersinia pestis* from spleens of Mexican prairie dogs (*Cynomys mexicanus*) trapped in the State of Coahuila, demonstrating for the first time that sylvatic plague was present in Mexico. However, we know of no serologic

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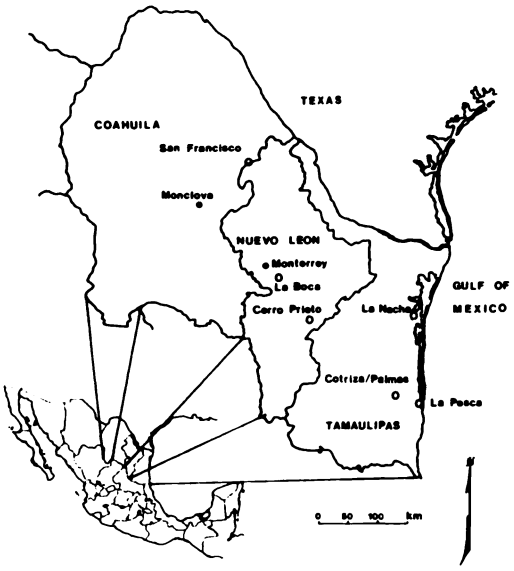


FIGURE 1. Study areas from which serum samples were collected from wildlife for serologic survey, Mexico 1988–89.

surveys performed in wildlife in Mexico since that time. The objective of this study was to determine, through a serologic survey, the presence and prevalence of arboviruses and other potential pathogens in resident wildlife species from northeastern Mexico.

The study areas, located in the states of Coahuila, Nuevo Leon, and Tamaulipas, Republic of Mexico (Fig. 1), have been previously described (Bennett et al., 1991). Mammals and birds were captured in all study areas from 18 May to 8 August 1988 and 10 June to 11 August 1989. Capture techniques and field procedures used in this study were similar to those described by Sudia et al. (1970). Sherman traps and leghold traps for mammals were operated for not more than 3 days in the same study area and were visited once a day early in the morning. An average of 41 Sherman trap-nights and five leg-hold trap-nights were used during a 65-day period over the two summers. Carnivores and other large mammals were chemically immobilized with ketamine (Ketaset, Bristol Laboratories, Syracuse, New York 66201, USA) at recommended dosages (Jessup, 1982). At the time of this survey, several wild-caught species were temporarily held in corrals and pens at Rancho San Francisco, Coahuila. For the purposes of this study, a wild-caught captive animal included any mammal or bird held in captivity for a week or longer. Wild birds were captured using mist nets at ground level for a total of 560 net hours (1 net hr = one 12-m mist net operated for 1 hr of daylight) (McLean et al., 1989). Nets were checked every 2 hr. Mist nets were set for 1 to 3 days on the study sites from 8:00 to 17:00 each day. Waterfowl and other birds species were manually captured in nesting boxes or while on ground nests (Markum and Baldassarre, 1989a, b). A number of nesting black-bellied whistling ducks (*Dendrocygna autumnalis*) were captured by hand at night using a spotlight.

Blood specimens (0.5–10 ml) were taken by venipuncture from the heart, radial, tarsal or jugular veins, allowed to clot and refrigerated overnight at 4 C. After blood specimens were centrifuged, sera were removed, placed in vials and frozen on dry ice. In the laboratory, serum specimens were heat-inactivated at 56 C for 30 min and tested by the plaque-reduction neutralization test (McLean et al., 1985, 1989) for antibody against the following arboviruses: St. Louis encephalitis (SLE) (TBH-28 strain), eastern equine encephalitis (EEE) (NJ-160 strain), Venezuelan equine encephalitis (VEE) (TC83, 1AB strain and Mena II, 1E strain), western equine encephalitis (WEE) (Fleming MI-2959B strain), vesicular stomatitis–Indiana (VSV–In) (lab strain), vesicular stomatitis–New Jersey (VSV–NJ) (Hazelhurst strain), epizootic hemorrhagic disease (EHD) (New Jersey strain), bluetongue (BT) [BT-13 (67-41B) and BT-17 strains], Cache Valley virus (CV) (M23355 strain), Jamestown Canyon virus (JC) (61V2235 strain), Nepuyo virus (NEP) (HBL7-2BG strain), and Rio Grande virus (RG) (TBM4-719 strain). Samples presenting a linear neutralization index of $\geq 10^{0.8}$ log of plaque counts compared with controls were considered pos-

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itive (McLean et al., 1985, 1989). The passive hemagglutination test and passive hemagglutination inhibition control test were used to detect antibodies against *Y. pestis* (Wolff and Hudson, 1974). Titers ≥16 were considered indicative of exposure. The micro-agglutination test was used to detect antibodies against *Francisella tularensis*. Titers ≥128 were considered to provide evidence of previous exposure (Stewart, 1988). Serum specimens from ungulate species were submitted to the U.S. Department of Agriculture Brucellosis Laboratory (Denver, Colorado 80211, USA) for the detection of antibodies against *Brucella abortus* and *Anaplasma marginale*. *Pasteurella multocida* and Newcastle disease virus antibodies were tested by using the ELISA Antibody Test Kit (Agritech Systems, Inc., Portland, Maine 04101, USA). Values for samples to known positive (S/P) ratio of ≤0.2 were considered as not providing evidence of previous exposure. Bird specimens obtained in 1988 were submitted to the National Animal Health Reference Laboratory (Ames, Iowa 50010, USA) to test for serologic evidence of exposure to *Mycoplasma meleagridis*, *M. gallisepticum*, *M. synoviae*, and *Chlamydia psittaci*. Serum samples obtained during summer 1989 were tested for *M. gallisepticum* and *M. synoviae* antibodies by the stained antigen rapid plate agglutination test (Intervet America Inc., Millsboro, Delaware 19966, USA).

Serum specimens from 80 mammals of 14 families and 21 species were tested for antibody against 13 selected arboviruses, *Y. pestis*, *F. tularensis*, *B. abortus*, and *A. marginale*. Results for serologic tests for arboviral antigens in mammals are listed in Table 1. During 1988, 12 species from San Francisco presented antibodies to VSV–NJ and seven species had antibody to VSV–In. Of these, six species presented antibodies to both viruses. In 1988, antibodies to enzootic strain VEE–Mena II were present in seven species predominated by small mammals. Antibody to VEE–TC83 strain was detected in three species including deer and two species of lagomorphs. From all the ruminants tested in 1988, white-tailed deer (*Odocoileus virginianus texanus*) was the only species with serologic evidence of exposure to both BT serotypes and EHD. During 1989, two of six mouflon sheep (*Ovis musimon*) had antibodies to BT. All other ungulate sera tested negative to hemorrhagic disease. Desert cottontail (*Sylvilagus auduboni*) was the only species with serologic evidence to NEP (1/6). Jackrabbits (*Lepus californicus*) (3/10) and a striped skunk (*Mephitis mephitis*) trapped during summer 1988, had diagnostically significant titers (128) to *F. tularensis*. A white-tailed deer serum had serologic evidence of exposure to *A. marginale*. There was no evidence of exposure to *Y. pestis* and *B. abortus* in the mammalian specimens tested.

Sera from 315 birds of 24 families and 44 species were tested for antibody to five arboviruses and six other selected avian pathogens (Table 2). During summer 1988, birds from 14 species had serologic evidence to the enzootic strain of VEE–Mena II. Antibodies to *P. multocida* were detected in five bird species in 1988 and two bird species in 1989. Newcastle disease virus antibodies were also identified in two species for both years. A scaled quail (*Callipepla squamata*) had a positive titer of 160 to *M. meleagridis*. No serologic evidence to selected arboviral antigens was found in 27 species of birds. Birds from 18 species had no antibodies to avian cholera and Newcastle disease. No serologic evidence of exposure to *M. gallisepticum*, *M. synoviae* and *C. psittaci* was identified in the bird specimens tested.

The present study indicates that transmission of at least 12 arboviruses occurred prior to the summers of 1988 and 1989 in the mammal species tested in northeastern Mexico. Previous evidence of exposure to equine encephalitis viruses in wild and domestic mammals has been reported in Mexico (Scherer and Dickerman, 1972; Gonzalez-Cortes et al., 1975). An endemic strain of VEE virus was isolated from opos-

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TABLE 1. Serologic results of wild mammals tested for selected arboviruses by the plaque reduction neutralization[a] test in Mexico, 1988–89.

Species[b]	VSV-NJ[c]	VSV-In[c]	VEE-TC83[c]	VEE-Mena II[c]	WEE[c]	SLE[c]	CV[c]	JC[c]	RG[c]
1988									
Rancho San Francisco									
Urocyon cinereoargenteus	1/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1	1/1
Ursus americanus[d]	1/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1	1/1
Procyon lotor	2/2	0/2	0/2	1/2	0/2	0/2	0/2	0/2	2/2
Mephitis mephitis	1/1	1/1	0/1	1/1	0/1	0/1	0/1	—	—
Lynx rufus[d]	0/1	1/1	0/1	0/1	0/1	0/1	0/1	—	—
Tayassu tajacu[d]	3/5	1/5	0/5	0/5	0/5	0/5	0/5	—	—
Odocoileus virginianus	3/5	5/5	1/5	1/5	0/5	0/5	2/5	1/5	—
Dipodomys merriami	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/1
Peromyscus leucopus	3/4	1/4	0/4	2/4	0/4	0/4	0/4	0/4	2/4
Baiomys taylori	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Neotoma mexicana	3/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	2/5
Lepus californicus	8/10	3/10	4/10	1/10	1/10	1/10	2/10	5/10	0/10
Sylvilagus audubonii	1/6	0/6	1/6	2/6	1/6	0/6	0/6	0/6	0/6
Presa Cerro Prieto									
Procyon lotor	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Sciurus griseus	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	1/2
Laguna La Nacha									
Myocastor coypu	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
1989									
Rancho San Francisco									
Antilope cervicapra[d]	4/7	0/7	0/7	0/7	0/7	0/7	0/7	0/7	0/7
Ovis musimon[d]	0/6	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6
Dipodomys merriami	0/1	0/1	1/1	0/1	0/1	0/1	0/1	0/1	1/1
Lepus californicus	7/14	1/14	0/14	0/14	0/14	0/14	1/14	0/14	0/14

[a] ≥80% plaque reduction in Vero-cell culture.

[b] Negative mammals (number tested) were *Sus scrofa* (1), *Spermophilus mexicanus* (2), *Peromyscus eremicus* (1), *Neotoma lepida* (1).

[c] Vesicular stomatitis–New Jersey; Vesicular Stomatitis–Indiana, Venezuelan equine encephalitis–TC83; Venezuelan equine encephalitis–Mena II; western equine encephalitis; St. Louis encephalitis; Cache Valley virus; Jamestown Canyon virus; Rio Grande virus.

[d] Captive wildlife.

sums (*Didelphis marsupialis*) and bats. Antibodies were detected in seven of 10 species of wild terrestrial mammals in two endemic areas of Veracruz, Mexico (Scherer et al., 1971). Our survey identified antibodies to VEE in a cottontail rabbit, a white-footed mouse (*Peromyscus leucopus*), and a raccoon (*Procyon lotor*), species with historical serologic or virologic evidence to VEE in Mexico. This survey identified five new species with serologic evidence to VEE in Mexico including striped skunk, white-tailed deer, kangaroo rat (*Dipodomys merriami*), woodrat (*Neotoma mexicana*), and jackrabbit. It is unknown, however, if all of these species act as effective reservoir hosts. Nepuyo virus was isolated for the first time in Mexico in 1966 (Dickerman et al., 1971). There is evidence that this virus is widely distributed and can cause epidemics in humans similar to dengue. The present study provides the only serologic evidence of this arbovirus in cottontails in Mexico since that year.

Vesicular stomatitis and hemorrhagic disease are enzootic in domestic cattle in Mexico (Mason and Gutierrez, 1984; Stott et al., 1989). The role of wildlife in the transmission of these arboviruses is still un-

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TABLE 2. Serologic results of wild birds tested for selected pathogens in Mexico, 1988–89.

Species[a]	VEE–TC83[b]	VEE–Mena II[b]	WEE[b]	SLE[b]	EEE[b]	*Mycoplasma meleagridis*	*Pasteurella multocida*	Newcastle disease virus
1988								
Rancho San Francisco								
Charadrius vociferus	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Cathartes aura	1/1	1/1	0/1	0/1	0/1	0/1	1/1	0/1
Callipepla squamata	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6
Meleagris gallopavo[c]	0/4	1/4	1/4	4/4	0/4	0/4	2/4	0/4
Molothrus ater	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
Molothrus aeneus	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Presa La Boca								
Dendrocygna autumnalis	0/10	2/10	0/10	0/10	0/10	0/10	0/10	0/10
Presa Cerro Prieto								
Colinus virginianus[c]	0/6	1/6	0/6	0/6	0/6	0/6	1/6	0/6
Fulica americana	0/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1
Columbina inca	0/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1
Crotophaga sulcirostris	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
Psilorhinus morio	0/5	1/5	0/5	0/5	1/5	0/5	3/5	0/5
Laguna La Nacha								
Bubulcus ibis	1/6	1/6	0/6	0/6	0/6	0/6	0/6	1/6
Egretta tricolor	0/2	0/2	0/2	1/2	0/2	0/2	0/2	0/2
Dendrocygna autumnalis	1/17	4/17	0/17	0/17	0/17	0/17	1/17	0/17
Dendrocygna bicolor	0/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1
Tyto alba	1/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
Quiscalus mexicanus	0/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3
La Pesca								
Zenaida asiatica	0/11	0/11	0/11	2/11	0/11	0/11	0/11	0/11
Rancho Cotriza and Rancho Palmas								
Bubulcus ibis	0/8	1/8	1/8	0/8	1/8	0/8	0/8	0/8
1989								
Rancho San Francisco								
Anas diazi	0/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4
Presa La Boca								
Dendrocygna autumnalis	0/16	3/16	0/16	0/16	0/16	0/16	1/16	0/16
Anas diazi	0/22	0/22	0/22	0/22	2/22	0/22	0/22	2/22
Presa Cerro Prieto								
Icteria virens	0/2	0/2	0/2	0/2	1/2	0/2	0/2	0/2
Laguna La Nacha								
Bubulcus ibis	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
Dendrocygna autumnalis	0/110	5/110	0/110	1/110	4/110	0/110	3/110	3/110

[a] Negative birds (number tested) were *Phalacrocorax olivaceous* (1), *Butorides striatus* (5), *Dendrocygna bicolor* (1), *Cairina moschata* (15), *Ortalis vetula* (1), *Phasianus colchicus* (2), *Gallinula chloropus* (1), *Limnodromus scolopaceus* (1), *Himantopus mexicanus* (1), *Rhynchops niger* (1), *Zenaida macroura* (2), *Zenaida asiatica* (6), *Rhynchopsitta pachyrhyncha* (4), *Ara militaris* (3), *Speotyto cunicularia* (1), *Chordeiles minor* (1), *Melanerpes aurifrons* (2), *Myiarchus cinerascens* (3), *Camptostoma imberbe* (3), *Sporophila torqueola* (1), *Cardinalis sinatus* (1), *Passerina iris* (3), *Amphispiza bilineata* (1), *Parus bicolor* (1), *Agelaius phoeniceus* (1), *Corvus brachyrhynchos* (2).

[b] Venezuelan equine encephalitis–TC83; Venezuelan equine encephalitis–Mena II; western equine encephalitis; St. Louis encephalitis; eastern equine encephalitis.

[c] Captive wildlife.

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determined. During the present survey, three of five blackbuck (*Antilope cervicapra*) obtained from Texas were seropositive to VSV–NJ. These animals were sampled the same day they arrived at Rancho San Francisco, Coahuila. These results may indicate activity of VSV in Texas. The implications of disease movement caused by wildlife translocations should be considered. Although virus isolation was not attempted, the introduction of VSV into Mexico is a possibility. VSV is indigenous to the western hemisphere and epizootics have occurred in southeastern United States almost every year (Karstad, 1981). There is extensive serologic and virologic evidence of BT virus infection in domestic livestock from Mexico (Stott et al., 1989). This study confirms exposure of white-tailed deer to BT-13 and BT-17, strains previously isolated in Mexico. To the best of our knowledge this is the first confirmed report of serologic evidence to EHD, VS–NJ, VS–In, CV, JC, and RG in wildlife in Mexico. These arboviruses are enzootic in areas of the United States bordering Mexico (Karabatsos, 1985).

Tularemia titers obtained in jackrabbits and a skunk indicate that the disease may be active in Mexico. Implications to human health are important since it is known that jackrabbits are a source of food to people in Mexico. To the best of our knowledge, this is the first serologic evidence of tularemia reported in wild mammals from Mexico.

The present study indicates that the bird species sampled were exposed recently to four encephalitis viruses, of which three have been reported previously in Mexican birds. Campillo-Sainz (1969) found herons and egrets from five species with antibodies to SLE and WEE. SLE was isolated from water birds in Veracruz, Mexico (Dickerman et al., 1972a). The present survey of waterbirds identified similar numbers of reactors to the ones previously reported for VEE in resident birds collected in southeastern Mexico (Dickerman et al., 1972b).

It is known that some mammals and birds may develop persistent viremias and act as amplifying hosts of these diseases in epizootic situations (Dickerman et al., 1972b; McLean and Bowen, 1980; McLean et al., 1985). At least three of the equine encephalitis viruses (EEE, SLE, and VEE) have caused epidemics in humans and epizootics in horses in Mexico. The role of wildlife in any of these outbreaks is unknown. Birds appear to be particularly important in the ecology of arboviral diseases. Mammals play an incidental role in the natural cycle, but this fact does not exclude the possibility of infection during epizootic situations. SLE and other flaviviruses have been present in Mexico for many years (Sosa-Martinez, 1963). To the best of our knowledge this is the first laboratory confirmed serologic report of EEE in wild birds in Mexico. Further studies in the prevalence, transmission, and ecology of selected diseases in wildlife populations in Mexico are required. Increasing data on disease agents in a greater number of species and scattered locations raise questions regarding the possibilities of disease introduction and exchange between geographical areas. There is supported evidence of annual reintroduction of arboviruses from areas south of the United States by migratory birds (Stamm and Newman, 1963; Calisher et al., 1971; Dickerman et al., 1980).

Surveillance for currently known diseases and isolation of new etiologic agents can be the initial attempt to establish the laboratory confirmed status of selected diseases in Mexico. The systematic collection of samples to establish the presence of disease is essential in the continental management of migratory species as well as other wildlife in North America.

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