Prairie dog (*Cynomys ludovicianus*) is not a host for porcine reproductive and respiratory syndrome virus

Rodney B. Baker, DVM, MS; Wanqin Yu; Martha Fuentes, DVM, PhD; Craig R. Johnson, PhD; LaRae Peterson; Kurt Rossow, DVM, PhD; C. Scanlon Daniels, DVM; Angela M. Daniels, DVM; Dale Polson, DVM, PhD; Michael P. Murtaugh, PhD

Summary

**Objective:** To determine if the prairie dog is a biological host for porcine reproductive and respiratory syndrome virus (PRRSV).

**Materials and methods:** Sixteen wild-caught prairie dogs were inoculated intraperitoneally with a high-titered mixture of wild-type local PRRSV strains, and seven animals were un inoculated controls. Serum and tissues were collected at 3- to 7-day intervals through 28 days for reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of viral RNA and determination of an anti-PRRSV immune response, using a novel ELISA to measure specific prairie dog IgG responses to nine viral polypeptides.

**Results:** A variety of tissues were negative for PRRSV RNA at all time points in both treated and control animals. Clinical signs were unremarkable and no histopathological lesions of PRRS were observed. Seroconversion was not observed in any animal over the 28-day study time course. Individual variation in background antibody levels and RT-PCR results were observed.

**Implications:** Prairie dogs do not support replication of PRRSV and are not a reservoir of the virus. Quantitative RT-PCR values for PRRSV in serum and tissue samples overlap with negative background values near the limits of detection, increasing the risk of false-positive interpretations. Methods, materials, and resources for diagnostic investigation of the prairie dog are now available to the veterinary community.

**Keywords:** swine, biosecurity, porcine reproductive and respiratory syndrome virus, polymerase chain reaction, wildlife

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Resumen – El perrito de la pradera (*Cynomys ludovicianus*) no es un huésped para el virus del síndrome reproductivo y respiratorio porcino

**Objetivo:** Determinar si el perrito de la pradera es un huésped biológico para el virus del síndrome reproductivo y respiratorio porcino (PRRSV por sus siglas en inglés).

**Materiales y métodos:** Se inocularon par enm aux las muestras de suero y de tejido se trasladaron a niveles de fondo de anticuerpos y resultados de RT-PCR.

**Resultados:** Se observó seroconversion en ningún animal durante el estudio de 28 días. Se observaron variaciones individuales en los niveles de fondo de anticuerpos y resultados de RT-PCR.

**Implicaciones:** Los perritos de la pradera no permiten la replicación del PRRSV y no son un reservorio del virus. Los valores cuantitativos de RT-PCR para PRRSV en las muestras de suero y de tejido se trastapan con valores de fondo negativos cerca de los límites de detección, aumentando el riesgo de interpretaciones falsas positivas. Los métodos, materiales, y recursos para la investigación diagnóstica del perrito de la pradera ya están disponibles para la comunidad veterinaria.

Résumé – Le chien de prairie (*Cynomys ludovicianus*) n’est pas un hôte pour le virus du syndrome reproducteur et respiratoire porcin

**Objectif:** Détecter si le chien de prairie est un hôte biologique pour le virus du syndrome reproducteur et respiratoire porcin (PRRSV).

**Matériels et méthodes:** Seize chiens de prairie capturés en nature ont été inoculés par voie intrapéritonéale avec un mélange
Transmission of porcine reproductive and respiratory syndrome virus (PRRSV) among farms may occur by a variety of means in spite of extensive biosecurity safeguards. The role of wildlife reservoirs in PRRSV transmission has not been demonstrated, outside of one observation of shedding in ducks that has not been replicated. Nevertheless, wild-life species are often reservoirs of animal disease, and they might provide a natural reservoir for PRRSV. Here, we report on an investigation of the prairie dog (Cynomys ludovicianus), a rodent species, as a possible reservoir of PRRSV. Prairie dogs are widely distributed in the central and southwestern United States, forming large colonies containing hundreds of individuals. We reasoned that rodent species such as the prairie dog are potential reservoirs of PRRSV, since the house mouse, Mus musculus, is the natural host for lactate dehydrogenase elevating virus, the arterivirus most closely related to PRRSV. The possibility that prairie dogs might circulate PRRSV in a local area was raised by a series of outbreaks in a breeding swine herd in the southwestern United States. Diagnostic ORF 5 sequencing has shown that PRRSV genotypes that most closely matched isolates from the past reappeared periodically over a 5-year period, yet no new viruses appeared to have been introduced from breeding stock replacements, semen, or area spread in this large and isolated production system.

The objective of this study was to determine if prairie dogs could be a wildlife reservoir of PRRSV. For a period of 28 days after inoculation of captive wild prairie dogs with PRRSV, serological testing for antibody formation, and sequential virological and histopathological examination of tissues for evidence of viral RNA and classical lesions of PRRS were performed.

Materials and methods

Animals and housing

A total of 23 male and female prairie dogs of unknown age were captured by live trapping for approximately 3 months from a wild colony in the midst of a swine operation in the southwestern United States, located more than 80 km from the nearest other swine operation. Animal capture and care procedures were approved by the Food and Drug Administration Center for Veterinary Medicine. Animals were housed by treatment group (controls and infected) in two rooms with separate ventilation systems. Animals were provided with timothy hay (Kaytree Products, Inc, Chilton, Wisconsin) and water ad libitum, and were limit-fed rodent chow (Bluebonnet Feeds, Brownwood, Texas). Controls were always handled first. Footwear, outerwear, and gloves were changed between groups.

Study design

Serum samples collected from all animals prior to the experiment tested negative for PRRSV by quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and for antibody by enzyme-linked immunosorbent assay (ELISA). Animals were randomly assigned to the control group (n = three females and four males) and the treatment group (n = seven females and nine males). On Day 0, animals in both groups were anesthetized with a reconstituted combination of tiletamine HCl and zolazepam HCl (Telazol; Fort Dodge Animal Health, Fort Dodge, Iowa) at 0.55 mL per kg body weight injected intramuscularly. Treatment animals were then inoculated intraperitoneally with a high-titered mixture of six wild-type PRRSV strains. Control animals were not inoculated.

As it was possible that PRRSV might reside in tissues from animals showing little or no viremia (compared to pigs), two inoculated animals and one control were sacrificed on each of Days 0, 3, 7, 10, 14, 21, and 28, and quantitative RT-PCR for PRRSV was performed on tissues from lung, heart, liver, kidney, spleen, thymus, tonsil, salivary gland, and pulmonary and mesenteric lymph nodes (ie, tissues from which PRRSV is commonly isolated in swine). Animals were sacrificed by inducing anesthesia as described, then exsanguinating via cardiac puncture. Tissues were also harvested from an inoculated prairie dog that died on Day 8 due to an anesthesia complication, and from an additional treatment-group prairie dog on Day 28. One hundred to 700 mg of tonsil, pulmonary lymph node, mesenteric lymph node, and lung tissue were preserved in 5 mL of RNAlater (Ambion, Austin, Texas) and stored at 4°C for quantitative RT-PCR. The remaining tissues were stored in formalin at 4°C.

Blood samples were collected by cardiac puncture from all animals remaining at each time point and frozen at -20°C for ELISA and quantitative RT-PCR. Blood collection was facilitated by Telazol anesthesia as described. The amount of serum obtained from blood samples varied between 500 and 1000 μL per animal.

Viruses

Six PRRSV isolates from infected pigs in the swine operation were grown on MA-104 cells or MARC 145 cells to the following titers (in median tissue culture infective doses per mL; TCID₅₀ per mL): 04-45228-31, 5 × 10⁵; 04-45229-33, 5 × 10⁴; SD PRRSV 04-100, 5 × 10³; SD PRRSV 04-101, 5 × 10⁵; SD PRRSV 04-102, 5 × 10³; and SD PRRSV 04-103, 5 × 10⁴. The first two strains were kindly provided by Dr Marie Gramer, University of Minnesota Veterinary Diagnostic Laboratory, St Paul, Minnesota, and the last four by Dr Jane Christopher-Hennings, Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings.
South Dakota. Equal aliquots were combined to make a 6-ml inoculum in cell culture medium that contained 1 mL of each viral isolate.

**Histopathology**

Samples of lung, pulmonary lymph node, spleen, and heart from control and inoculated animals sacrificed on Days 0, 7, and 21 were submitted in formalin to the Minnesota Veterinary Diagnostic Laboratory.

**RNA isolation and quantitative RT-PCR**

Isolation of RNA was performed for all samples using a Nucleospin II RNA Isolation kit (BD Biosciences, San Jose, California). A titrated PRRSV strain, MN30100, was aliquoted into a series of eight 10-fold dilutions that were used to generate a positive-control RT-PCR standard curve.\(^4\) RNA was extracted from 100 µL of each dilution, eluted in 50 µL RNase-free water provided in the kit, and stored at -80°C. For prairie dog serum samples, RNA was extracted from 100 µL of serum, eluted in 50 µL of RNase-free water, and stored at -80°C. Tissue samples were homogenized in five volumes of lysis buffer using a Polytron PT 3100 (Brinkman, Westbury, New York). Two hundred µL of homogenate was extracted, and purified RNA was eluted in 50 µL RNase-free water for storage at -80°C. For real-time RT-PCR assays, samples were dried in a Savant SpeedVac (Thermo Electron Corp, Waltham, Massachusetts) and dialyzed against 20 mM Tris HCl (pH 8.0) and 150 mM NaCl. Protein concentration was determined by Bradford assay (BioRad, Hercules, California). Purified protein solutions were stored at -80°C before coating on ELISA plates.

**Prairie dog IgG-specific antiserum**

As seroconversion in prairie dogs might be a more sensitive indicator of PRRSV infection at low levels or in tissues not tested by RT-PCR, an ELISA was tested to detect antigen-specific humoral immune responses to PRRSV. Nine recombinant PRRSV proteins from strain VR2332 were expressed from the plasmid pET 24b as fusion proteins containing an amino terminal myc-tag and a carboxyl terminal 6x histidine tag in BL 21(DE3)-RP cells (Stratagene, La Jolla, California) (Table 1). For purification, denatured proteins were dialyzed overnight in 0.1 M Tris HCl (pH 8.0), 6 M guanidine HCl, and 2 mM EDTA at 4°C, then adjusted to a concentration of 3 mg per mL. Dithiothreitol was added to a final concentration of 300 mM and the solution was filtered through a 0.45-µm membrane. Reduced protein was added into refolding buffer (100 mM Tris HCl (pH 8.0); 0.5 M L-arginine; 8 mM oxidized glutathione; 2 mM EDTA; 10 µM peptatin A; 10 µM leupeptin; and 1 mM phenylmethylsulfonylfluoride), filtered (0.22 µm), and stirred overnight at 4°C.\(^5\) The purified protein was concentrated by tangential flow filtration (Pellicon XL Ultrace 5 k; Millipore, Billerica, Massachusetts) and dialyzed against 20 mM Tris HCl (pH 8.0) and 150 mM NaCl. Protein concentration was determined by Bradford assay (BioRad, Hercules, California). Purified protein solutions were stored at -80°C before coating on ELISA plates.

**Table 1:** Porcine reproductive and respiratory syndrome virus (PRRSV) proteins and polypeptides used in an ELISA for testing serum of wild-caught prairie dogs inoculated intraperitoneally with six strains of PRRSV

<table>
<thead>
<tr>
<th>Protein or polypeptide</th>
<th>Description</th>
<th>Nucleotide sequence range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleocapsid (N)</td>
<td>Complete 123 amino acid sequence of N</td>
<td>14889-15257</td>
</tr>
<tr>
<td>Nonstructural protein (nsp) 1</td>
<td>Complete predicted 383 amino acid sequence of nsp1</td>
<td>193-1341</td>
</tr>
<tr>
<td>Nsp2p</td>
<td>Amino terminal 719 amino acid fragment of nsp2</td>
<td>1339-3495</td>
</tr>
<tr>
<td>Envelope glycoprotein 5 (GP5) 5’</td>
<td>Amino terminal 35 amino acid fragment of mature GP5</td>
<td>13875-13979</td>
</tr>
<tr>
<td>GP5 3’</td>
<td>Carboxy terminal 72 amino acid fragment of mature GP5</td>
<td>14172-14387</td>
</tr>
<tr>
<td>GP5-matrix protein ectodomain (GP5-M)</td>
<td>87 amino acid synthetic peptide with predicted ectodomain regions linked with a neutral peptapeptide (GP5 &amp; M)</td>
<td>13875-13979</td>
</tr>
<tr>
<td>GP5 5’total</td>
<td>52 amino acid synthetic peptide external to the envelope GP5</td>
<td>14085-14114</td>
</tr>
<tr>
<td>M 5’total</td>
<td>32 amino acid synthetic peptide external to the envelope M</td>
<td>14375-14431</td>
</tr>
<tr>
<td>M 3’</td>
<td>Carboxy terminal 88 amino acid fragment of M</td>
<td>14633-14896</td>
</tr>
</tbody>
</table>

* Nucleotide sequence ranges are from Genbank accession U87392.
Concentration of IgG was determined by spectrophotometry (Nanodrop ND-100; Nanodrop Technologies, Wilmington, Delaware) using an extinction coefficient $E_{1\%260} = 13.7$. Purity was determined by 10% SDS-PAGE analysis (Figure 1A). Purified material was used to immunize goats to produce anti-prairie dog IgG antisera (Bethyl Laboratories, Montgomery, Texas). Goat IgG containing antibodies to prairie dog IgG was purified by triple 40% saturated ammonium sulfate precipitation, dialysis in phosphate buffered saline (PBS), and adjustment to the original serum volume (Figure 1B).

ELISA for PRRSV-specific antibodies was performed by coating microtiter plates with 300 ng of each of the nine recombinant PRRSV proteins in carbonate buffer (pH 9.6), blocking with 5% nonfat dry milk in PBS containing 0.05% Tween 20, and reacting with serum samples diluted 1:100 in PBS containing 5% nonfat dry milk and 0.05% Tween-20. Wells were washed and replicate samples were incubated with 100 µL of pre-immune or immune goat anti-prairie dog IgG diluted 1:100. After 1 hour, the plates were washed and developed with horseradish peroxidase-conjugated rabbit anti-goat IgG in a dilution of 1:5000 for 1 hour. Plates were then washed and color was developed with tetramethyl blue tetrazolium substrate (KPL, Gaithersburg, Maryland). Reactions were stopped with 1 M phosphoric acid and plates were read at 450 nm.

ELISA for prairie dog IgG was performed by coating microtiter plates with 0 to 320 ng of prairie dog IgG per well as described above. Blocking and washing steps were performed as described above. Samples were incubated with 100 µL of pre-immune or immune IgG in 10-fold dilutions from 1:100 to 1:10$^7$ for 1 hour. Color development was performed as described above, using horseradish peroxidase-conjugated rabbit anti-goat IgG to detect the goat anti-prairie dog IgG bound to prairie dog IgG.

Since the ELISA involves three binding reactions, we were concerned that negative results might indicate a failure of the assay to detect prairie dog antibody binding to plate-bound antigen. To address this possibility, we determined the ability of prairie dog natural antibodies to react with bacterial antigens instead of PRRSV antigens on an ELISA plate. ELISA for prairie dog natural antibodies was performed by preparing an overnight culture of DH5α Escherichia coli, treating with lysozyme for 30 minutes,

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**Figure 1:** Prairie dog (PD) IgG purification and anti-IgG production in goat. Figure 1A: prairie dog IgG was purified by multiple rounds of ammonium sulfate precipitation and visualized by SDS-PAGE. Lane 1: 10 µg purified IgG; Lane 2: lysozyme (Lys); Lane 3: molecular weight (MW) standards. Figure 1B: goat anti-PD IgG purified by ammonium sulfate precipitation before (lane PI) and after (lane I) immunization with purified PD IgG. Each lane contains 5 µg of total protein. Lane 3 contains MW markers.
heating at 90˚C for 5 minutes, and pellet-
ing at 10,000 g for 10 minutes. The pellet
was resuspended in one-tenth volume of
PBS, sonicated, and coated undiluted and
at ten-fold dilutions in 200 μL total vol-
ume at pH 9.4, as described above. *Esche-
richia coli* antigens were detected with three
antibody reactions, consisting of prairie
dog IgG followed by goat anti-prairie dog
IgG and horseradish peroxidase-conjugated
rabbit anti-goat IgG. Prairie dog sera from
one control and two PRRSV-inoculated
animals at Day 28 were diluted 1:100 and
applied in duplicate to the wells. Block-
ing and washing steps were performed as
described above. All wells were then treated
with a 1:100 dilution of goat pre-immune
IgG or immune anti-prairie dog IgG.
Immune complexes of prairie dog IgG and
goat anti-prairie dog IgG were detected
with horseradish peroxidase-conjugated
rabbit anti-goat IgG as described above.

**Results**

**Histopathology**

In prairie dogs sacrificed on Day 0 (before
inoculation with PRRSV), heart, lung,
spleen, and pulmonary lymph node were
devoid of lesions that could be attributable
to PRRSV infection. One animal had evi-
dence of myocardial inflammation which
had been resolved by fibrosis. Partial cuff-
ing by small lymphoid nodules was noted
in lungs of two animals and local replace-
ment of myocardial tissue with fibrous con-
nective tissue was noted in one animal. The
same observations were made on Day 7,
with additional observation of mild cardiac
thickening due to subacute inflammation
or infiltration by lymphocytes and mac-
rophages. Histopathology was unchanged
in control and inoculated animals at Day
21 except for clear cytoplasmic vacuoles in
a few alveolar macrophages of the control
animal, and focal inflammation centered
around inhaled plant material in one inoc-
ulated animal. Overall, no signs of PRRS,
including microscopic interstitial pneumo-
nia and cardiac arteritis, were noted in any
animals.

**RT-PCR screening for PRRSV**

Background real-time RT-PCR threshold
cycle (Ct) values for prairie dog serum,
determined on samples from all animals
before inoculation, were in the range of
36 to 45 (Figure 2). Sample Ct values
remained within this range at all sampling
day.
times after inoculation through Day 28. There was no difference in values in serum from inoculated and control prairie dogs (Figure 2).

The overall Ct range was 29 to 45 in tonsil, pulmonary lymph node, mesenteric lymph node, and lung samples collected on Days 0, 3, 7, 10, 14, 21, and 28 (Figure 2). Moreover, Ct values for tissues from control animals were frequently lower than for tissues from inoculated prairie dogs, further indicating that PRRSV RNA was not present, even though there was a wide range of results (Figure 2).

**Anti-PRRSV antibody responses**

Prairie dog serum contained approximately 12.6 ± 6.1 mg IgG per mL (mean ± SE). A checkerboard titration (ie, varying amounts of prairie dog IgG coated on the plates and varying amounts of pre-immune or immune goat antisera tested) was performed to determine the conditions for assessing the amount of anti-PRRSV antibody response. Prairie dog IgG-specific, dose-dependent immunoreactivity was readily detected (Figure 3). No PRRSV antigen-specific reactivity was observed against any of the nine PRRSV proteins or protein fragments in the 23 animals on Day 0 (ie, they had no pre-existing immunity to PRRSV). At 28 days post infection, sera were similarly negative for all PRRSV antigens, albeit with sporadic high absorbance values (Figure 4). Because only three infected animals remained at 28 days, serum samples were evaluated further for serological response to N and M-3’ antigens at all time points following infection. As shown in Figures 5A and 5B, individual absorbance values were the same in control and test animals, indicating the absence of anti-PRRSV antibodies.

Results of the *E coli* ELISA for prairie dog natural antibodies are shown in Figure 5C. Specific reactivity was observed only in wells containing control and inoculated prairie dog IgG and goat anti-prairie dog IgG. Pre-immune goat IgG values were the same as the negative control that included no antigen (data not shown). This result shows that the method involving three antibody-binding reactions detected prairie dog antibodies and supports the conclusion that prairie dogs did not produce an antibody response to PRRSV inoculation.

![Figure 3: Specific IgG antibody response of a goat immunized with prairie dog IgG. Reactivity of pre-immune (Figure 3A) and immune (Figure 3B) goat IgG is shown against prairie dog IgG coated in varying amounts (5-320 ng per well) onto microtiter plates. Uncoated wells gave absorbance values of ≤ 0.06.](image)
Discussion

Starting in 1998, PRRSV ORF 5 sequence information was collected in the isolated swine production system where chronic PRRS re-infection problems raised the original questions about prairie dogs as a potential zoonotic reservoir of PRRSV. After the year 2000, the data, consisting of > 600 sequences, indicated that no new viruses had entered the system from external, lateral introductions. Instead, viral isolates from periodic episodes of abortion and nursery mortality spikes appeared more similar to historical isolates than to contemporaneously occurring forms in the system. Many of these sequences differed by as much as 4.5% from circulating strains, but differed from older isolates by less than 2% on sequence analysis.

An explanation for this phenomenon could be that the large multi-site complex acted as a geographic continuous-flow system, and the less dominant older virus types remained present at low levels in the growing-pig units. For this to be the case, the virus would have to be efficiently spread by aerosol, infected-pig movement, or an unknown mechanical vector. Aerosol spread appears unlikely, since the re-appearance of older isolates followed no apparent pattern. Pig movement could not be linked to re-breaks. Biosafety at the time of the study was far more stringent than in other commercial systems in the United States. The system is in an isolated area more than 80 km from other pig production units. Transport vehicles were pressure-washed with heated water and dried for 24 hours. Personnel were dedicated to specific parts of the system. 48- to 96-hour downtime rules were enforced, and the site was fenced and patrolled continuously by security guards. The boar stud remained PRRSV-negative during the study period. Outside breeding stock (boars and gilts) were from known negative sources and remained negative until exposure after arrival.

The strict biosecurity measures and results of viral sequencing analysis seemed to preclude external sources of new infections. Thus, it was possible that older PRRSV isolates were being maintained in a nonporcine rodent reservoir such as the indigenous prairie dog. However, the immunological, virological, histopathological, and clinical findings presented here rule out the prairie dog as a host species for PRRSV. The source of PRRSV before its discovery as a viral pathogen of swine in the 1980s remains to be determined.8,9 Mice were determined previously not to be a host species for PRRSV,10 and a report of PRRSV growth and shedding in ducks could not be reproduced.3 Until the host range possibilities of PRRSV are more fully investigated, the evolutionary trajectory and origins of PRRSV, including the hypothesis of a non-swine reservoir, are speculative.11,12 The findings help to focus attention on mechanisms of viral transmission by excluding the prairie dog as a possible reservoir and vector.

Screening of serum and tissues by RT-PCR for viral RNA revealed a wide range of C<sub>t</sub> values for negative samples. Real-time PCR is a powerful method for detection and quantification of nucleic acids, but its application to diagnostic specimens gives results that may show overlap between weakly positive samples and negative samples with high backgrounds. Here, serum samples from negative animals gave C<sub>t</sub> values as low as 36 cycles, and tissue samples gave C<sub>t</sub> values as low as 29 cycles, which is well within the range of true-positive results.13 These findings emphasize the reality that tissue samples, in particular, are subject to high backgrounds and spurious false-positive results, and that RT-PCR should not be relied on exclusively to provide evidence of PRRSV infection, especially when additional test methods, such as serology, are available, or when the PCR results are near the limit of assay sensitivity. New information was also gained about humoral IgG of the prairie dog. The concentration of IgG in serum of wild-caught adults, 12.6 ± 6.1 mg per mL, is less than the serum IgG concentration in swine (20 to 30 mg per mL, unpublished data, W. Yu and M. P. Murtaugh, 2005), but is similar to that in humans (normal range = 8 to 16 mg per mL).14,15 While the prairie dog is
Figure 5: Absence of specific anti-PRRSV antibody response in prairie dogs. Prairie dog serum samples were obtained from 0 to 28 days after animals were inoculated with PRRSV (Treated) or not inoculated (Control). Serum was applied to microtiter plates coated with membrane protein (M-3'; Figure 5A) or nucleocapsid (N; Figure 5B), reacted with goat anti-prairie dog IgG, and detected with horseradish peroxidase-conjugated rabbit anti-goat IgG. Figure 5C: Detection of Control (n = 1) and Treated (n = 2) serum reactivity to bacterial lysate antigens. Plates were coated with various dilutions of *Escherichia coli* lysate and reacted with Day 28 prairie dog IgG. Absorbance was determined following incubation with immune goat anti-prairie dog IgG, horseradish peroxidase-conjugated rabbit anti-goat IgG, and color substrate.

![Absorbance over time for Control and Treated samples](image-url)

not a reservoir for PRRSV, it is a reservoir of human disease.16-18 Thus, it might play a role in the spread of other swine diseases. The methods and reagents presented here would then be useful for development of appropriate diagnostics.

**Implications**
- The prairie dog can be excluded as a PRRS reservoir in unexplained PRRS outbreaks in the geographic home range of the animal.
- Methods, materials, and resources for diagnostic investigation of the prairie dog are now publicly available to the swine veterinary community.
- More detailed information about the sensitivity and specificity of real-time RT-PCR assays for PRRSV RNA in serum and tissue samples would help in diagnostic interpretations.

**Acknowledgements**
We thank Dr Richard Mock at the Texas Veterinary Medical Diagnostic Laboratory, Amarillo, Texas, for assistance in identifying resources familiar with capture, housing, and care of prairie dogs, and Dr Marie Gramer at the Texas Animal Disease Research and Diagnostic Laboratory, Brookings, for providing virus and related viruses. In: Fields BN, Knipe DM, Howley PM, eds. *Fields Virology*. 3rd edition. Philadelphia: Lippincott-Raven; 1997:55;329–336.


