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## FOOT-AND-MOUTH DISEASE IN A SMALL SAMPLE OF EXPERIMENTALLY INFECTED PRONGHORN (*ANTILOCAPRA AMERICANA*)

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**ABSTRACT:** There is limited information on the pathogenesis and epidemiology of foot-and-mouth disease (FMD) in North American wildlife and none concerning pronghorn (*Antilocapra americana*). In an experimental study of 13 pronghorn and six steers (*Bos taurus*), we compared the susceptibility of pronghorn to FMD virus (FMDV) strain O, with that of cattle (*Bos taurus*). We also determined the potential for intra- and interspecies transmission of FMDV strain O in pronghorn and cattle, assessed the application of conventional laboratory tests in their suitability to detect FMDV infection in pronghorn, and evaluated the potential role of pronghorn as efficient long-term carriers of FMDV. After acclimation to containment at Plum Island Animal Disease Center, two pronghorn and one steer were each infected by intraepithelial tongue inoculation with 10,000 bovine tongue infective doses of FMDV, strain O1 Manisa. Inoculated animals were housed with contact animals. When contact-exposed animals developed fever they were placed in rooms with previously unexposed animals. All inoculated and exposed cattle and pronghorn developed clinical disease typical of FMD. Pronghorn developed severe foot lesions and mild to moderate oral lesions, primarily on the tongue. Duration of clinical signs in both species was 2–3 wk with foot abnormalities evident to the end of the study (51 d postexposure). Other lesions included pancreatitis, myositis of the tongue, and secondary lesions including pleuritis, pneumonia, decubital ulcers, and tenosynovitis. Virus transmission occurred between pronghorn, from cattle to pronghorn, and from pronghorn to cattle. Conventional laboratory tests detected virus and antibodies against nonstructural and structural FMDV proteins in pronghorn and cattle. Virus was present in some animals for 1 wk but was not detectable by virus isolation or PCR at 3 wk postinfection or afterward.

**Key words:** *Antilocapra americana*, foot-and-mouth disease, FMD, pathology, PCR, pronghorn, ungulate, wildlife.

### INTRODUCTION

Foot-and-mouth disease (FMD) has never been diagnosed in wild pronghorn (*Antilocapra americana*) in North America or, to our knowledge, in captive zoologic collections. Wild artiodactyls are considered susceptible,

and the disease has been observed in several antelope and cervid species (Thomson et al. 2003; Arzt et al. 2011). Experimental work in wildlife has shown marked interspecies variability in susceptibility to infection and in the potential of the host to transmit FMD virus (FMDV) (Forman and Gibbs 1974; Gibbs et

al. 1975; Rhyan et al. 2008). The pronghorn is the only species in the family Antilocapridae and is not a true antelope. Pronghorn are widely distributed in interior western and central North America where they commonly come in contact with cattle on range (O'Gara and Yoakum 2004). In the case of an FMD outbreak in the western US, knowledge of the susceptibility of pronghorn to infection, transmission capability, clinical disease, laboratory findings, and possible carrier status of pronghorn would be valuable for disease management planning.

We experimentally infected pronghorn and cattle (*Bos taurus*) with FMD virus (FMDV) strain O. Our goals were to establish the susceptibility of pronghorn to the virus, characterize the clinical signs and lesions of the disease in pronghorn, determine the potential for intra- and interspecies transmission of the virus, evaluate conventional laboratory tests in their suitability to detect infection in pronghorn, and investigate the potential role of pronghorn as efficient long-term carriers of FMDV.

## MATERIALS AND METHODS

### Animal sources and pre-experimental preparation

Nine neonatal pronghorn fawns were captured at the Hart Mountain Antelope Refuge (42°30'N, 119°36'W) in south-central Oregon over a 2-d period. Fawns were immediately transported to the Colorado State University, Animal Population Health Institute wildlife research pens in Fort Collins, Colorado. Three weeks later, eight 2–3-wk-old fawns were obtained from rehabilitators in Colorado and the Colorado Division of Wildlife Foothills Wildlife Research Facility. Fawns were hand raised in groups of 4–6 in 4.3×13.4 m, plywood-sided runs. They received extensive human contact and training consisting of handling, blindfolding, examining, manipulation of feet, and phlebotomy. They were trained to a crate for handling (Phillips et al. 1998), trained to load and travel in a stock trailer, and acclimated to dried apples as a reward.

Six 1-mo-old, male, Jersey calves were purchased from a local dairy, castrated, and raised in proximity to the pronghorn. Calves were periodically placed in pens with pronghorn for short intervals to familiarize the two species. At 10 mo of age, male pronghorn were castrated by

banding due to development of aggressive behavior.

Temperature transmitters (Advanced Telemetry Systems, Incorporated, Isanti, Minnesota, USA), each having a unique radiofrequency, were installed in the peritoneal cavities of calves and all but one pronghorn. The procedure was, in brief: following tranquilization with tiletamine HCl and zolazepam HCl (Telazol; 6–12 mg/kg IV; Zoetis, Florham Park, New Jersey, USA), surgical preparation of the left paralumbar fossa and local infiltration of subcutis and musculature with lidocaine (2%), the skin was incised, underlying musculature bluntly dissected in a grid approach, and a 5 cm incision made in the peritoneum. The sterile transmitter was then inserted into the peritoneal cavity, and the incision closed with surgical glue (Vetbond®, 3M Company, St. Paul, Minnesota, USA) and surgical staples. All animals were given one dose of postsurgical antibiotics (Nuflo; florfenicol, Merck Animal Health, Summit, New Jersey, USA; 40 mg/kg by subcutaneous injection).

After 11 mo in confinement, nine male and four female pronghorn and the six Jersey steers were transported in a darkened stock trailer to the Plum Island Animal Disease Center (PIADC), Orient, New York. Prior to transport and once during shipment, pronghorn were administered the long-acting neuroleptic zuclopenthixol acetate (Clopixol Acuphase; Abbott Renuka Pharmacy, Vancouver, BC, Canada; 1 mg/kg by intramuscular [IM] injection) to control excitability. On arrival, animals were placed in 40-m<sup>2</sup> biocontainment rooms. Four pronghorn and three steers were placed in one room and separated by a padded partition. Remaining pronghorn and steers were housed separately in two rooms. Of the 17 original pronghorn, 10 were included in the study; seven died or were euthanized due to illnesses and injuries during hand-raising, transport, and introduction to biocontainment. After 1 wk of acclimation, animals were moved to new rooms such that each room housed 3–5 pronghorn and 2–3 steers (Fig. 1). Animal rooms had 23–30 air exchanges per hour and temperatures of 25–28 C. Animals were fed alfalfa hay cubes and a mixed grain and molasses feed. Pronghorn and cattle were commingled during the study without separating partitions. The two species generally segregated themselves, occupying different corners of the rooms. Body temperatures were monitored remotely every 30 min for the first 3 wk using the intraperitoneal temperature transmitters and a receiver/datalogger (model R4500S, Advanced Telemetry Systems) located outside the containment building. Animals were cared for in accordance with the institutional animal care and use guidelines of Colorado State University and the PIADC.

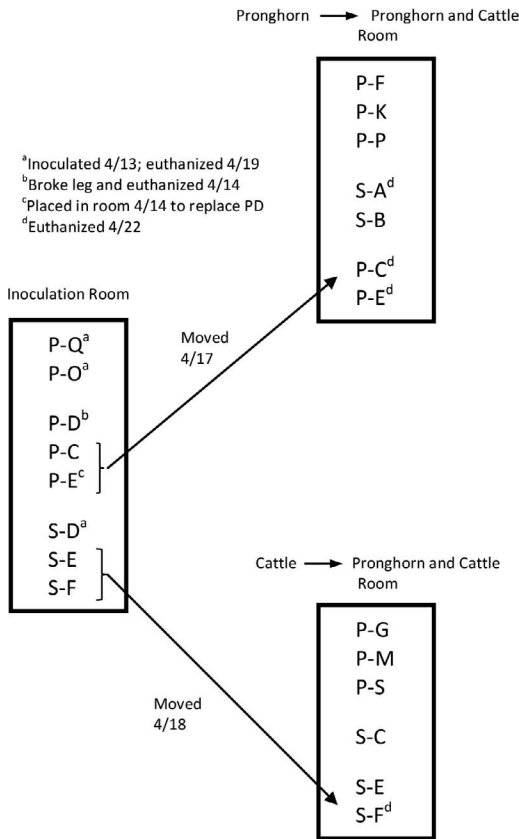


FIGURE 1. Flow diagram depicting housing of pronghorn (*Antilocapri americana*) and cattle (*Bos taurus*; animal ID beginning with P=pronghorn, S=cattle) with movements of inoculated and exposed animals in pronghorn and cattle transmission studies.

#### Virus strain, inoculations, and contact exposure

Two pronghorn and one steer were each inoculated with 10,000 50% bovine tongue infective doses (BTID<sub>50</sub>) of FMDV, strain O1 Manisa in 0.8 mL minimum essential medium. O1 Manisa is a well-characterized FMDV strain often used in experimental infections. Ten thousand BTID<sub>50</sub> of FMDV consistently produces disease in cattle and bison (*Bison bison*) but only minimal disease in elk (*Cervus elaphus*; Rhyan et al. 2008). Pronghorn were chemically restrained using 2.0 mL medetomidine (0.5 mL intravenously [IV], 1.5 mL IM) and 0.5 mL Telazol IV. Medetomidine was antagonized with 2.0 mL (0.5 mL IV and 1.5 mL IM) atipamezole-HCl (5 mg/mL; Antisedan, Zoetis). The steer was tranquilized using 1.0 mL xylazine (100 mg/mL; AnaSed, Lloyd Laboratories, Shenandoah, Iowa, USA) IV; xylazine was antagonized with 4.5 mL tolazoline (100 mg/mL; Tolazine, Lloyd Laboratories) given by slow IV

injection. Animals were inoculated with FMDV by intraepithelial injection on the dorsal surface of the tongue using a 25 gauge 15.9 mm needle and 1.0 mL syringe. Each tongue was dried and injected at six sites in titrated doses with the most concentrated inoculum given at the back and a decreasing concentration of the virus at each site moving anterior. Each injection in the tongue consisted of 100  $\mu$ L. Remaining inoculum was given by IM injection in the hip.

Inoculated animals were initially housed with two pronghorn and two steers to serve as contacts (Fig. 1). Animals in the inoculation room were examined daily. Steers were manually restrained in a head catch. Pronghorn were routinely caught and sedated with 1.0 mL medetomidine and 0.5 mL butorphanol (10 mg/mL; Tobugesic, Zoetis) delivered by IV injection. Medetomidine was antagonized with 1 mL atipamezole by IV injection. Contact animals in the inoculation room developed fever (>39.5 C) within 5 d postexposure (dpe) and were moved to the rooms containing unexposed animals (Fig. 1).

#### Clinical monitoring and sampling

Animals were examined and the following samples collected daily for the first week following inoculation or exposure to infected animals and weekly thereafter: whole blood, heparinized blood, ethylenediaminetetraacetic acid blood, nasal swab, and oral swab. Oropharyngeal secretions were collected from three pronghorn using a probang (Sutmoller and Gaggero 1965) 21, 32, 48, and 56 dpe. Following the development of clinical signs, pain was mitigated with flunixin meglumine (1.1–2.2 mg/kg IM) and butorphanol (0.1 mg/kg IM or IV) as needed. Animals were euthanized and necropsied at predetermined times or due to animal welfare concerns (Table 1). Lung, liver, kidney spleen, heart (atria, ventricles, and interventricular septum), pancreas, testis, prepuce, seminal vesicles, rumen, reticulum, abomasum, tongue, buccal tissue, dental pad, palatine tonsil, and lymph nodes (mandibular, parotid, retropharyngeal, tracheobronchial, mediastinal, mesenteric, ileocecolic, hepatic, and superficial cervical) were collected for microbiologic and histopathologic examinations.

#### Serology

Serologic tests comprised the 3ABC enzyme-linked immunosorbent assay (ELISA), a commercially available test that reacts with nonstructural proteins that indicate FMDV replication and infection, the 3D ELISA, an in-house assay that also reacts with nonstructural proteins, and virus neutralization, which reacts with FMDV structur-

TABLE 1. Clinical signs and necropsy results of pronghorn (*Antilocapra americana*) and cattle (*Bos taurus*) following inoculation or exposure to O 1 Manisa strain of foot-and-mouth disease virus, grouped by species and necropsy day.

Animal ID <sup>a</sup>	Source of exposure	Onset of clinical signs (dpi/dpe) <sup>b</sup>	Clinical signs <sup>c</sup>	Day of necropsy (dpi/dpe) <sup>b</sup>	Postmortem findings <sup>c</sup>
PO, PQ	Inoculated	1	Fever (40.4 C, 41.2 C), shifting leg lameness, foot shaking, depressed, tongue and foot v/e/u	6	Tongue, foot, and rumen v/e/u; iliac and popliteal LNs enlarged; focal pneumonia (both); tongue myositis (PQ)
PC, PE	PQ, PO, SD	4, 3	Fever (40.1 C, 40.3 C), lameness, foot shaking, depressed, foot and tongue v/e/u	9	Tongue, foot, and rumen v/e/u (both); pancreatitis (PE)
PS, PP, PM	PS, PM: SE, SF; PP: PC, PE	4, 3, 4	Fever (40.8–40.9 C), lameness, foot shaking, depressed, foot v/e/u (no oral lesions observed)	24, 28, 32	Severe foot v/e/u; decubital ulcers (all); pleuritis and pneumonia (PP, PM); tenosynovitis (PS)
PG, PF, PK	PG: SE, SF; PF, PK: SE, SF	4, 3, 6	Fever (40.0–40.7 C), lameness, foot shaking, depressed, oral v/e/u in PG, foot v/e/u in all	50, 51, 51	Hoof deformity with regrowth (all); rumen scars (PG, PF); decubital ulcer (PK)
SD	Inoculated	1	Fever (41.1 C), foot and oral v/e/u, lameness, pyalism, depressed	6	Oral (tongue, palate) and foot v/e/u; myocarditis
SA	PC, PE	3	Fever (41.0 C), foot and oral v/e/u, lameness, depressed, pyalism	5	Oral (tongue, dental pad, palate) v/e/u; tongue myositis
SF, SE	SD	3, 5	Fever (39.9, 40.2 C), foot and oral v/e/u, depressed, lameness, pyalism	9, 13	Oral (tongue, palate, labia, gingiva), foot and rumen v/e/u (both)
SC, SB	SE, SF: SC PC, PE: SB	2,3	Fever (40.4, 40.7 C), foot, oral and nares v/e/u, depressed, lameness	50, 51	Hoof deformity with regrowth (both); rumen scars (SC); mild focal myocarditis (SB)

<sup>a</sup> Animal ID beginning with P = pronghorn, S = cattle (steers).

<sup>b</sup> dpi/dpe = days postinoculation/days postexposure.

<sup>c</sup> Fever = highest temperature recorded for each animal; v/e/u = vesicles/erosions/ulcers; LN = lymph node.

al proteins indicating exposure, infection, or vaccination.

Detection of anti-3ABC antibody on Prionics CHECK<sup>®</sup> FMDV NS (Prionics Lelystad B.V., Lelystad, the Netherlands) was based on the manufacturer's protocol (also see Sørensen et al. 2005). Six washes with 1× wash buffer were done after each incubation step. Eighty microliters of ELISA buffer were added to wells of a dried assay plate followed by 20 µL of test serum, negative control, and standards. Plates were incubated overnight at room temperature, and 100 µL/well of antibody-horseradish peroxidase conjugate was added followed by 1 h incubation at room temperature. Next horseradish peroxidase substrate solution was added to plates (100 µL/well). Chromogenic reaction was developed for 20 min and terminated by the addition of Stop Solution (100 µL/well). Optical density (OD) at 450 nm of the reaction product in each well was determined on an ELISA reader (VersaMax, Molecular Devices, Sunnyvale, California, USA). Antibody level of each sample is expressed as the percentage inhibition (PI):  $PI = 100 - [100 \times (OD_{\text{sample}} / OD_{\text{max}})]$ . Samples were considered positive when  $PI > 50\%$ . The 3D ELISA was performed as described by Sørensen et al. (1998).

Virus neutralization assays were done using a swine kidney cell line, IBRS-2. Sera were thawed, heat inactivated at 56 C for 30 min, and serially diluted in 96-well plates with supplement medium. To this was added titered O1 Manisa FMDV (approx. 100 50% tissue culture infective doses [TCID<sub>50</sub>]/well), and plates were incubated in a CO<sub>2</sub> incubator at 37 C for 1 h. We added  $4 \times 10^5$  cells in 100 µL of minimum essential medium containing 4% fetal bovine serum to each well and shook the plate to distribute cells evenly. Plates were incubated up to 4 d and monitored daily for cytopathic effect. Plates were then stained with crystal violet and a final reading done. Neutralization titers were computed using the Spearman-Kärber Method (Cottral 1978) and reported as the reciprocal of the final serum dilution that neutralized 100 TCID<sub>50</sub> of O1 Manisa FMDV in 50% of the wells.

#### Detection of FMDV

Virus was detected by real-time reverse-transcriptase-PCR (rtRT-PCR), and virus isolation (VI). Extraction of RNA was done using the Qiagen Viral RNA Kit (Qiagen, Valencia, California, USA); we extracted 140 µL of supernatant from oral and nasal swab samples, tissue extracts, and cell culture supernatants that exhibited cytopathic effect. The final volume of the extracted RNA was 50 µL. Of this 10 µL was reverse transcribed using random hexamers as a primer source. Using TRIzol<sup>®</sup> Reagent (Gibco

BRL, Life Technologies, Grand Island, New York, USA), 125 µL of ethylenediaminetetraacetic acid blood was diluted with 125 µL of RNA-grade water and extracted as described by the manufacturer. Viral RNA was coprecipitated with GlycoBlue<sup>™</sup> (Ambion, Foster City, California, USA), and the briefly dried pellet was dissolved in 50 µL of RNA grade water. Dissolved RNA was stored at -70 C until assayed by PCR. The rtRT-PCR was done using primers and probes described by Callahan et al. (2002). The primers target a conserved region of the 3D RNA polymerase gene. The forward primer was 5' ACT-GGG-TTT-TAC-AAA-CCT-GTG-A 3'; the reverse primer was 5' GCG-AGT-CCT-GCC-ACG-GA 3'; the probe was 5' TCC-TTT-GCA-CGC-CGT-GGG-AC 3' and was labeled with a 5'-reporter dye, 6-carboxyfluorescein and a 3' quencher tetramethylrhodamine. Reactions were conducted in a 25 µL reaction volume using 2.5 µL of RNA template. Concentrations of reagents for the reaction were Mn(OAc)<sub>2</sub> (5 mM), primers (0.3 mM), probe (0.3 mM), dATP/CTP/GTP (0.1 mM), dUTP (0.2 mM), rTth DNA polymerase (0.1U/mL), and bovine serum albumin (0.1 µg/µL). Reactions were run and products detected using the Smart Cycler<sup>®</sup> (Cepheid, Inc., Sunnyvale, California, USA). Cycling conditions consisted of a 10-min RT step at 60 C followed by 45 cycles at 95 C for 2 s and 60 C for 30 s. Positive samples were as described in Callahan et al. (2002).

#### Histology

Formalin-fixed specimens were routinely processed and 5-µm sections stained with H&E for histopathologic examination. Immunohistochemistry (IHC) and multichannel immunofluorescence (MIF) microscopy were performed on select formalin-fixed, paraffin-embedded tissues using techniques and reagents described by Arzt et al. (2009) with few modifications. Due to limited available resources for IHC screening, a targeted approach was pursued for the purpose of confirming vesicular lesions were FMDV-associated and investigating the causality of pancreatic lesions. For IHC and MIF, antigen was retrieved by incubating deparaffinized slides for 15 min at 90 C while immersed in Diva Decloaker (Biocare Medical, Concord, California, USA) followed by 5 min of immersion in 1× Hot Rinse (Biocare Medical). Primary detection of FMDV was achieved with a mouse monoclonal antibody, F1412SA, raised against a linear epitope of the 12S fragment of FMDV-A24 but demonstrated to recognize all seven serotypes of FMDV (Yang et al. 2007). For IHC, secondary detection was performed with a micropolymer-based, alkaline phosphatase system (MACH 3, Biocare Medical),

and Vector Red substrate (Vector Laboratories, Burlingame, California, USA). For MIF, cytokeratin was detected with rabbit polyclonal anti-pancytokeratin (Invitrogen, Carlsbad, California, USA). Secondary detection was performed with goat, anti-rabbit IgG-AF488, and anti-mouse IgG1-AF594 fluorescently labeled secondary antibodies (Molecular Probes, Eugene, Oregon, USA). Each IHC experiment included a positive tissue control (tongue vesicle from pronghorn) and a negative tissue control (normal tongue from cattle). Additionally, for every tissue processed through IHC, a serial section received the exact same treatment, with the exception that the primary anti-FMDV antibody was replaced with an isotype control reagent.

Data are presented without statistical analyses due to the limited number of animals in each group.

## RESULTS

### Clinical course of FMD in experimental animals

All inoculated and exposed cattle and pronghorn developed clinical signs and lesions typical of FMD in most ungulates. At 20 h postinoculation (hpi) the inoculated steer and one of the inoculated pronghorn were examined. The steer had vesicles at the four tongue inoculation sites receiving more concentrated virus and the pronghorn at three sites. Temperatures of the inoculated steer and two pronghorn peaked at 41.0 C, 40.1 C, and 40.5 C at 34 hpi, 35 hpi, and 37 hpi respectively. Temperatures reached a peak within 6 h and remained elevated for 5 d until the animals were euthanized. We observed lameness in the three inoculated animals at 39 hpi. In the contact-exposed cattle and pronghorn, the onset of signs usually occurred 3–4 dpe (range: 2–6 dpe).

Clinical signs and foot and oral lesions in cattle were similar to those previously reported (Barker et al. 1993; Rhyan et al. 2008; Table 1). On histopathology, the inoculated steer necropsied 6 d postinoculation (dpi), had marked, acute, multifocal myocarditis characterized by myofiber necrosis with a lymphoplasmacytic inflammatory cell infiltrate dissecting between myofibers and concentrated between muscle bundles and around blood vessels in connective tissue septae.

In contact-exposed pronghorn, the time of onset of clinical disease varied between 3 and 6 dpe (Table 1). Clinical signs were similar in all pronghorn and included fever, depression, shifting-leg lameness with reluctance to stand, foot shaking, and occasional chewing movements. Body temperatures peaked (range=40.0–40.9 C) between 3 and 6 dpe and remained elevated for up to 3 wk. Physical examination revealed moderate oral vesicle development, primarily on the ball of the tongue. Some animals developed gingival vesicles. Oral vesicles usually ruptured within a day forming erosions which usually healed within 2 wk. Foot lesions were often present on the day lameness developed consisting of blanching of the coronary bands first detectable at the bulbs of the heels and progressing to involve the lateral aspects of the feet. Vesicles formed that ruptured usually in 2 d, and by 6 dpe, the entire coronary band was circumferentially eroded with separation of heels from denuded, red, moist, underlying tissue. Animals were often initially lame on one foot, but lameness and lesions usually progressed to involve all feet. Lameness was more severe in the rear feet. Lameness responded well to analgesics, and following their administration, animals would usually move about and eat.

Four pronghorn were euthanized during the first 9 dpi/dpe. One was found dead in the morning 28 dpe; necropsy revealed marked fibrinopurulent pleuropneumonia with associated bacteria (not cultured). Two developed decubital ulcers due to sternal recumbency and were euthanized for humane reasons 24 and 32 dpe, and three were euthanized at the conclusion of the study 50 and 51 dpe. All animals were necropsied.

### Post mortem gross and histologic examination

Gross lesions varied depending on the time interval between the onset of disease and necropsy. In pronghorn necropsied 6 dpi and 9 dpe, oral lesions in three animals consisted of irregular erosions/ulcers measuring 1.5–3 cm in diameter usually located on the torus lingua (Fig. 2A). Smaller erosions (5–8 mm)

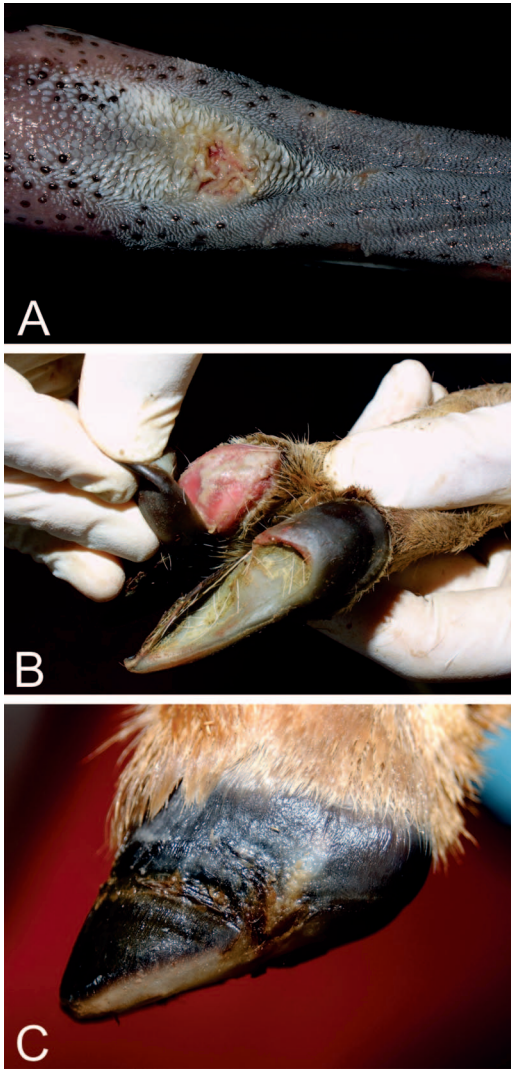


FIGURE 2. Gross lesions of foot-and-mouth disease in experimentally infected pronghorn (*Antilocapri americana*). (A) Tongue of pronghorn necropsied 9 d postexposure to foot-and-mouth disease virus (FMDV) with erosion on the torus linguae. (B) Foot of pronghorn necropsied 9 dpe to FMDV with severe undermining of heels and sole of foot. (C) Foot of pronghorn necropsied 51 dpe to FMDV with hoof deformity and regrowth.

were also present on the anterior third of tongues of two pronghorn and a larger erosion on the gingiva and lip of one. A single ruptured vesicle (5 mm diameter) was the only oral lesion present on the soft palate of the fourth. Oral lesions were not present in pronghorn necropsied 24–51 dpe. Foot le-

sions in inoculated pronghorn necropsied at 6 dpi consisted of partially ruptured, nearly circumferential vesicles of the coronary band with partial undermining and detachment of the bulbs of the heel. At 9 dpe, coronary bands of all feet were eroded or ulcerated, and there was severe detachment of the soles (Fig. 2B). Pronghorn necropsied 24 to 32 dpe had decubital ulcers, foot lesions, and lesions associated with secondary infections (Table 1). The three pronghorn necropsied 50 and 51 dpe had hoof wall deformities with regrowth (Fig. 2C). All but three pronghorn had erosions, ulcers, or scars on the rumen pillars.

Histopathologic examination of pronghorn foot (coronary band), mouth, and rumen lesions revealed changes typical of FMD in other species consisting of vesicle and microabscess formation at the stratum spinosum, characterized by focal ballooning degeneration, edema, cell necrosis, and focal infiltrates of neutrophils (Fig. 3A). Larger lesions consisted of intraepithelial bullae formation with superficial epithelial caps. Overlying and adjacent epithelium displayed acanthosis and hyperkeratosis. Erosions were overlaid with coagula of detached keratinocytes, amorphous eosinophilic material, neutrophils, and bacteria. Basal epithelium was usually preserved, but some lesions extended through the basement membrane with marked mixed inflammatory cell infiltrates in the submucosa or dermis.

Marked diffuse pancreatitis was noted in the pronghorn necropsied 9 dpe. Pancreatitis involved all lobules examined and was characterized by loss of acinar cells and normal acinar architecture, moderate, diffuse, and marked focal interstitial cellular infiltrates of lymphocytes and plasmacells, and focal early fibrosis (Fig. 3B). Islets were present and often hypercellular. Other lesions noted in one or more pronghorn included focal myositis in the tongue characterized by myofiber hyaline degeneration and necrosis accompanied by sarcolemmal proliferation, pleuritis and bronchopneumonia with associated bacterial colonization, decubital ulcers, and tenosynovitis.



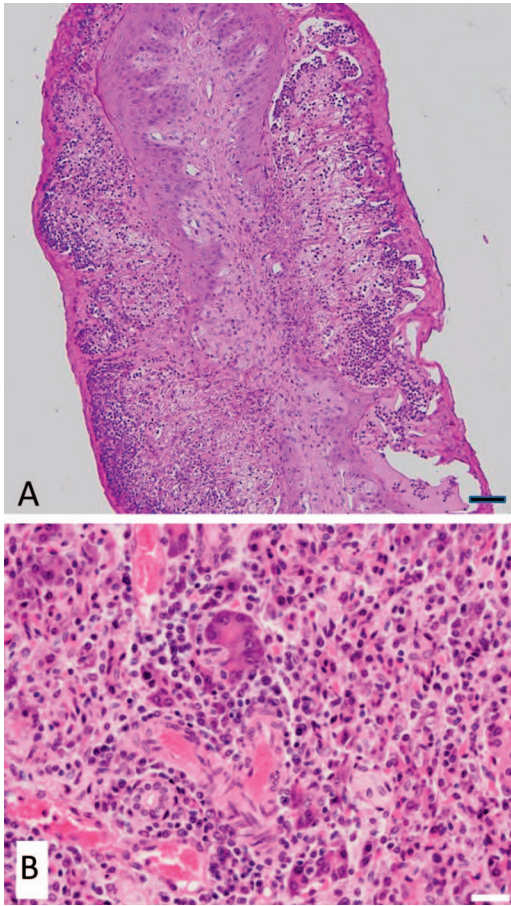


FIGURE 3. Photomicrographs of histologic lesions of foot-and-mouth disease virus (FMDV) experimentally infected pronghorn (*Antilocapri americana*). (A) Rumen papilla from pronghorn necropsied 9 d postexposure (dpe) to FMDV. Note forming vesicle with elevation of superficial epithelium, marked edema, a mixed inflammatory cell infiltrate (predominantly neutrophils), focal necrosis of basal epithelium, and focal detachment of epithelial cap (right side). H&E. Bar=100  $\mu$ m. (B) Pancreas from pronghorn necropsied 9 dpe to FMDV. There is acinar cell necrosis with loss of much of the acinar architecture and a mild to moderate diffuse, focally concentrated, inflammatory cell infiltrate consisting primarily of lymphocytes and plasma cells. H&E. Bar=30  $\mu$ m.

#### Immunomicroscopy

Epithelial lesions from FMDV-infected pronghorn were further characterized by IHC and MIF microscopy. The FMDV capsid antigen was localized to intraepithelial vesicles/microabscesses within rumen (Fig. 4) and

coronary band. Using IHC, viral antigen was detected in the rumen as regionally diffuse, pale staining across most of the vesicle with multifocal intensely stained individual cells predominantly at the periphery of the lesion cavity (Fig. 4B, C). No immunopositivity was detected anywhere else in the tissue other than the vesicles. Multichannel immunofluorescence microscopy confirmed a similar distribution of FMDV and allowed further characterization of host cell morphology and phenotypes (Fig. 4D). A thin rim of cytokeratin-positive keratinocytes circumscribed the lesion confirming the intra-epithelial location. Numerous degenerate keratinocytes were present within the vesicle (acanthocytes) and were variably FMDV-positive. Sections of pancreas with pancreatitis were negative for FMDV on IHC.

#### Virus isolation, rRT-PCR, and serology

Laboratory results confirmed FMDV in all pronghorn and cattle in the study by VI or rRT-PCR. Among contact-infected pronghorn, virus was detected in oral and nasal swabs as early as 1 dpe and persisted in some animals over 1 wk (Table 2). Oropharyngeal (probing) samples collected at 21, 32, 48, and 56 dpe, however, were negative on VI and FMDV rRT-PCR. Serology revealed antibodies against structural proteins in pronghorn and cattle 11 dpe, and against nonstructural proteins 18 and 11 dpe in pronghorn and cattle respectively (Table 3). Results of the 3ABC ELISA and the 3D ELISA were almost identical (Table 3).

#### DISCUSSION

Pronghorn demonstrated similar high susceptibility as cattle to infection with FMDV, with all animals in both species developing clinical signs following inoculation or contact exposure to O1 Manisa strain. Intraspecies transmission and transmission from cattle to pronghorn and pronghorn to cattle occurred when animals were housed together. Time of onset of clinical signs following inoculation or exposure was similar in pronghorn and cattle

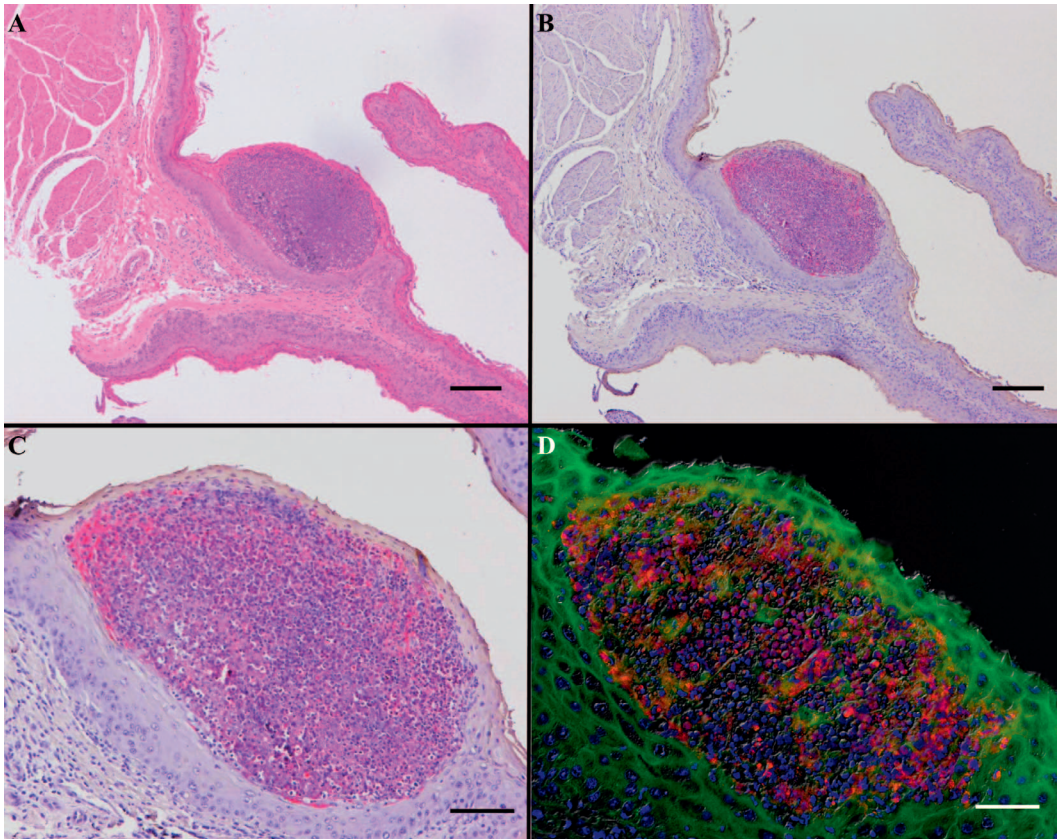


FIGURE 4. Rumen vesicle/microabscess from pronghorn (PC) 9 d postexposure to foot-and-mouth disease virus (FMDV). (A) Intraepithelial lesion at the base of papilla; H&E, 4 $\times$ . (B) FMDV capsid antigen is localized exclusively within vesicle; anti-FMDV immunohistochemistry (IHC), 4 $\times$ . (C) Vesicle lumen consists of densely packed degenerate epithelial cells and mixed leukocytes; anti-FMDV IHC, 10 $\times$ . (D) Simultaneous multichannel immunofluorescence microscopy of serial section of A–C, 20 $\times$ . Green cytokeratin labeling identifies intact keratinocytes surrounding lesion and acanthocytes within. Red labeling of FMDV capsid demonstrates localization of FMDV within acanthocytes, intact keratinocytes, and mononuclear leukocytes. Scale bars: A–B=200  $\mu$ m, C=100  $\mu$ m, D=50  $\mu$ m.

TABLE 2. Virus isolation (VI) and real-time reverse transcription PCR results from contact-infected pronghorn (*Antilocapra americana*) in an experimental infection with O1 Manisa strain of foot-and-mouth disease virus.

Days postexposure	Blood (no. positive/no. tested)		Oral swab (no. positive/no. tested)		Nasal swab (no. positive/no. tested)	
	VI	PCR <sup>a</sup>	VI	PCR	VI	PCR
0	0/8	0/8	0/8	0/8	0/8	0/8
1	0/8	0/8	0/8	1/8	0/8	3/7
2	3/8	2/7	2/8	2/5	3/8	3/7
3	5/8	4/7	2/8	3/5	3/8	3/5
4	4/8	5/7	3/8	3/5	4/8	2/5
5	4/8	4/7	3/8	2/4	2/8	3/5
6	4/8	3/6	4/8	2/4	2/8	4/5
7	1/8	ND	5/8	1/1	1/8	1/3

<sup>a</sup> ND = no data.

TABLE 3. Serology test (3ABC and 3D enzyme-linked immunosorbent assay [ELISA] and virus neutralization [VN]) results for pronghorn (*Antilocapra americana*) and cattle (*Bos taurus*) experimentally exposed to the O1 Manisa strain of foot-and-mouth disease virus.

Species	dpi/dpe <sup>a</sup>	3ABC ELISA (no. positive/no. tested)	3D ELISA (no. positive/no. tested)	VN 50% <sup>b</sup>
Pronghorn	0	0/3	0/3	<2
	4	0/3	0/3	<2
	11	0/3	0/3	72, 91, 260
	18	2/2	2/2	57, 230
	46	3/3	2/3	180, 200, 460
	52	2/3	2/3	180, 110
Cattle	0	0/2	0/2	<2
	4	0/2	0/2	<2
	11	1/1	1/1	20
	18	2/2	2/2	75, 200
	46	2/2	2/2	120, 160
	52	1/1	1/1	64

<sup>a</sup> dpi/dpe = days postinoculation or days postexposure.

<sup>b</sup> VN titers reported as the reciprocal of the final serum dilution that neutralized 100 tissue culture infective doses of O1 Manisa FMDV in 50% of the wells.

as was the detection of virus and humoral immune response. Foot lesions in pronghorn were severe; mouth lesions occurred but were generally not severe, often being limited to the ball of the tongue. The viral infection was generally not fatal in pronghorn, and no gross or microscopic evidence of myocarditis was noted. One pronghorn died with an acute bacterial pleuritis and pneumonia, and two were euthanized for humane reasons when they developed decubital ulcers. Three pronghorn that were euthanized 50/51 dpe were clinically normal with marked hoof regrowth. The extent to which stress from containment and repeated handling may have contributed to or amplified primary or secondary lesions in pronghorn is unknown.

Our findings suggest that an outbreak in pronghorn would likely cause high case fatality due to secondary infections and lameness resulting in marked predation. The intra- and interspecies transmission of FMDV demonstrated in this experiment identifies a potential role for pronghorn in the farm-to-farm spread of the infection in the case of an outbreak. In Africa, impala (*Aepyceros melampus*) transmit FMD between African buffalo (*Syncerus caffer*) herds (Bastos et al.

2000) and between buffalo and cattle (Hargreaves et al. 2004; Vosloo et al. 2006).

We observed pancreatitis in one pronghorn and lingual myositis in another. Pancreatitis has been described in FMDV-infected cattle (Barboni and Manocchio 1962) and observed in naturally and experimentally infected mountain gazelles (*Gazella gazelle*; Perl et al. 1989; Berkowitz et al. 2010). The lack of positive IHC results in the affected pancreas suggests there may be other causes for the lesion besides FMDV. Lingual myositis has also been observed in mountain gazelles where it was accompanied by muscle lesions in the heart, diaphragm, and other skeletal muscle (Shimshony 1988). Arzt and others (2011) recently reviewed the myotropic nature of FMDV.

Immunolocalization of FMDV antigens within lesions of acutely affected pronghorn was in general agreement with what has been described in vesicles of cattle and pigs (*Sus scrofa*; Yilma 1980; Arzt et al. 2009). Virus was localized to intact and degenerate cytokeratin-positive epithelial cells and occasional cells of indeterminate histogenesis. Overall this confirms that lesions observed in these pronghorn were caused by FMDV and suggests that

pathogenesis of vesiculation in pronghorn is similar to that described in other species. To our knowledge this is the first microscopic characterization of FMDV vesicles in rumen epithelium in any species.

In summary, results of this limited experiment demonstrate the susceptibility of pronghorn to the O1 Manisa strain of FMDV. The virus was highly virulent in pronghorn and was transmissible among pronghorn and between pronghorn and cattle. Laboratory tests for virus detection and serology were suitable for use in pronghorn, and long-term (persistent) infection was not detected. In nature, FMDV infection would likely result in high mortality among pronghorn due to the severe foot lesions and the resulting immobility and increased susceptibility to predation. Pronghorn should be considered as potential short-term hosts of FMDV, facilitating transmission between herds of domestic animals if an outbreak were to occur in the US, although the rapidly developing lameness would likely limit animal mobility and spatial spread of the disease.

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