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Influenza D Virus Infection in Dromedary Camels, Ethiopia

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DOI: https://doi.org/10.3201/eid2506.181158

Influenza D virus has been found to cause respiratory diseases in livestock. We surveyed healthy dromedary camels in Ethiopia and found a high seroprevalence for this virus, in contrast to animals co-existing with the camels. Our observation implies that dromedary camels may play an important role in the circulation of influenza D virus.

Influenza D virus (IDV) was first isolated from pigs with respiratory symptoms in the United States in 2011 (*1*). Epidemiologic analyses revealed that the most likely main host of IDV is cattle, because the seropositivity rate in these animals is higher than that for other livestock (*2–4*). In a recent report, dromedary camels (*Camelus dromedaries*) exhibited substantially high seroprevalence (99%) for IDV in Kenya (*5*), suggesting that this animal is a potential reservoir of IDV. We examined seroprevalence of IDV in dromedary camels in Ethiopia and in Bactrian camels (*Camelus bactrianus*) in Mongolia.

We collected serum samples from dromedary camels (n = 38; average age 4.3 years, range 1–13 years), goats (n = 20; average age 3.9 years, range 1–8 years), sheep (n = 20; average age 2.7 years, range 1–4 years), cattle (n = 15; average age 6.7 years, range, 1–11 years), and donkeys (n = 2; ages 1 and 6) from 2 herds in Bati district, Amhara region, and 1 herd in Fafen district, Somali region, Ethiopia. All animals were apparently healthy, shared the same pasturage during the day, and stayed in barns specific for each animal species at night. To detect influenza D infection, we titrated the serum samples by hemagglutination inhibition (HI) assay using 3 antigenically distinct influenza D strains: D/swine/Oklahoma/1334/2011 (D-OK lineage; D/OK) (*1*), D/bovine/Nebraska/9–5/2013 (D/660-lineage; D/NE) (*6*), and D/bovine/Yamagata/10710/2016 (D/Japan-lineage; D/Yamagata) (*7*). For the HI test, we treated the samples with receptor-destroying enzyme (RDEII; Denka Seiken, http://www.keyscientific.com) at 37°C for 16 h, followed by heat inactivation at 56°C for 30 min. We then reacted serially diluted samples with each virus (4 HAU) at room temperature for 30 min and incubated them with a 0.6% suspension of turkey red blood cells at room temperature for 30 min. The HI titer of each sample was expressed as the reciprocal of the highest sample dilution that completely inhibited HA. We considered samples with HI titer ≥1:40 positive, to eliminate nonspecific reactions at low dilutions (*4,8,9*).

Of the 21 dromedary camel samples from Bati, 10 were positive for D/OK, 11 for D/NE, and 19 for D/Yamagata (Figure). Of other animal samples, only 1 goat sample was positive (titer 1:40), indicating that the prevalence rate of influenza D antibodies was higher in dromedary camels than in co-grazing ruminants in the tested herd. The data on the camels' age indicated that the HI antibodies were not detected due to maternal antibodies, which is only stable for 5–6 months in dromedary camels (*10*). Much closer face-to-face contact may be required for virus transmission among different animal species. The HI titers in camel samples were higher for D/Yamagata (range 1:40–1:160) than those for D/OK and D/NE (1:40–1:80). Meanwhile, we found several positives in dromedary camel samples from Fafen, albeit at lower positive rates and titers compared with those in Bati (Figure).

We confirmed the specificity of the HI reaction with a viral neutralizing test using HI-positive samples (data not shown). HI titers obtained were not high, suggesting that the infections may have occurred in these animals some time ago or that results might have been due to the variance of HI

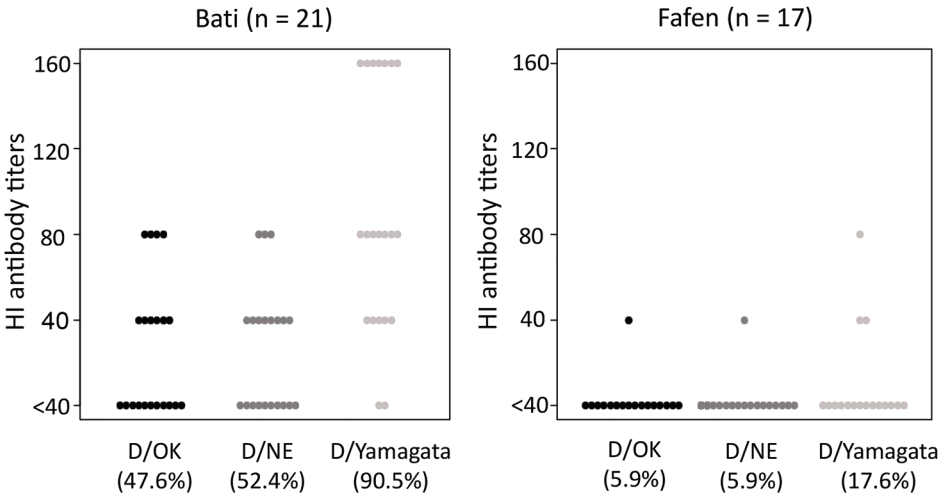

Figure. Hemagglutination inhibition (HI) antibody titers for influenza D viruses in serum samples from dromedary camels, Bati and Fafen Districts, Ethiopia. Each dot represents 1 camel. HI assay was performed with RDE(II)-treated serum samples and turkey red blood cells (0.6%) against D/swine/Oklahoma/1334/2011 (D/OK), D/bovine/Nebraska/9–5/2013 (D/NE), and D/bovine/Yamagata/10710/2016 (D/Yamagata). HI-positive rate for each virus is shown below the virus name.

methods used in each laboratory. For example, turkey red blood cell was used in this study, whereas horse red blood cell was used in a previous study (*5*). Nonetheless, these data suggest that the virus antigenically related to D/Yamagata was circulating in dromedary camels in this region.

In a previous report, a considerable number of dromedary camels in Kenya were seropositive not only for influenza D but also for influenza C virus (ICV) (*5*). Thus, we additionally used C/Ann Arbor/1/1950 virus as an antigen for HI assay. The results suggest a limited circulation of ICV in this area because 0 samples in Bati were positive and only 2 in Fafen, which were negative for IDV, were positive (titers 1:40). In addition, we performed the HI test using selected samples following preadsorption with ICV. We did not observe any significant decrease in HI titers to IDV, suggesting no cross-reactivity between IDV and ICV in our samples.

We also collected serum samples of apparently healthy Bactrian camels (n = 40) in Dundgovi, Zavkhan, and Umnugovi Provinces, Mongolia, and tested for HI antibody for IDV. These samples did not test positive for these IDV strains.

Despite the limited samples tested, this study suggests that dromedary camels in East Africa might play a substantial role in the circulation of IDV. Further studies using additional samples from multiple countries are expected to clarify the role of this animal on the ecology and epidemiology of this virus, including its reservoir potential in nature.

Acknowledgments

We thank Ben M. Hause for the viruses used in the study.

T.H. is supported by Livestock Promotion Funds from the Japan Racing Association. S.M. is supported by a Grant-in-Aid for the Encouragement of Young Scientists (A) (grant no. 17H05042).

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Absence of *Neospora caninum* DNA in Human Clinical Samples, Spain

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DOI: https://doi.org/10.3201/eid2506.181431

Low antibody titers to *Neospora caninum* have been reported in humans, but infection has not been confirmed. We used *N. caninum*–specific PCR to test 600 clinical samples from patients with toxoplasmosis signs but *Toxoplasma gondii*–negative PCR results. We did not detect *N. caninum* DNA, demonstrating it is an unlikely opportunistic zoonotic agent.

The coccidian parasite *Neospora caninum* (Apicomplexa: Sarcocystidae) is a major abortifacient agent in ruminants, especially cattle. It is phylogenetically close to *Toxoplasma gondii* (*1*), a parasite of high prevalence in humans, but biologically different. *N. caninum* parasites have a restricted host range but can infect primates (*2,3*).

N. caninum infection causes neuromuscular disease in dogs and reproductive disorders in ruminants, causing fetal loss due to vertical transfer of parasites during acute infections or reactivation of chronic infections. Clinical neosporosis in animals resembles the disease outcome of toxoplasmosis (*1*).

Table. Types of samples analyzed and demographic and clinical data for 600 patients tested for *Neospora caninum* parasites, Spain*

Characteristics	No. (%)
Sample type	
Amniotic fluid	267 (44.5)
Cerebrospinal fluid	113 (18.8)
Blood	100 (16.7)
Placental tissue	51 (8.5)
Bronchoalveolar lavage	25 (4.2)
Urine	17 (2.8)
Brain biopsy	12 (2.0)
Aqueous humor	4 (0.7)
Fetal tissues and fluids	4 (0.7)
Lymph node aspirate	3 (0.5)
Vitreous humor	2 (0.3)
Bone marrow aspirate	1 (0.2)
Hepatic abscess aspirate	1 (0.2)
Patient demographic information	
Sex	
Unknown	6 (1.0)
M	135 (22.5)
F	459 (76.5)
Of childbearing age, 17–42 y	333 (55.5)
Age, y	
>1, average 36.1	481 (80.2)
<1	116 (19.3)
Native country	
Spain	382 (63.7)
Other†	36 (6.0)
Unknown	182 (30.3)
Patient clinical information	
Immune status	
Immunocompetent	458 (76.3)
Immunodepressed or immunosuppressed	83 (13.8)
HIV/AIDS	19 (22.9)
Chemotherapy	29 (34.9)
Organ transplant	35 (42.2)
Unknown	59 (9.8)
Pregnancy-related disorders	454 (75.7)
Seroconversion–infection suspicion and lymphadenopathy	418 (92.1)
Spontaneous abortion	27 (5.9)
Ophthalmic	4 (0.9)
Other fetal signs‡	5 (1.1)
Neurologic and ocular symptoms and conditions§	108 (18.0)
Neurologic condition with ocular signs	8 (7.4)
Ophthalmic only	3 (2.7)
General signs, n = 38	38 (6.3)
Pneumonia	24 (63.2)
Lymphadenopathy	2 (5.3)
Hematologic or oncologic	12 (31.6)

*Patients were from 12 regions: Andalusia, Aragon, Asturias, Balearic Islands, Cantabria, Castile-La Mancha, Castile and Leon, Extremadura, Galicia, Madrid, Navarre, Valencia.
†Africa, 8; Asia, 1; Europe, 8; Latin America, 19.
‡Anencephaly, malformations, and microcephaly.
§Neurologic symptoms and conditions include ataxia, disorientation, sudden blindness, encephalitis, calcifications, and intracranial space occupying lesions; ophthalmic symptoms and conditions include chorioretinitis, panuveitis, posterior uveitis, and vitritis.

N. caninum parasites have been successfully cultured in human cell lines, but low antibody titers of unconfirmed specificity against *N. caninum* have been reported in human serum samples (*1*,*4*,*5*). The significance