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Short communication

Serological evidence for the presence of influenza D virus in small ruminants

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ABSTRACT

Influenza D virus (FLUDV) was isolated from diseased pigs with respiratory disease symptoms in 2011, and since then the new virus has also been spread to cattle. Little is known about the susceptibility of other agricultural animals and poultry to FLUDV. This study was designed to determine if other farm animals such as goats, sheep, chickens, and turkey are possible hosts to this newly emerging influenza virus. 648 goat and sheep serum samples and 250 chicken and turkey serum samples were collected from 141 small ruminant and 25 poultry farms from different geographical locations in the United States and Canada. Serum samples were examined using the hemagglutination inhibition (HI) assay and the sheep and goat samples were further analyzed using the serum neutralization assay. Results of this study showed FLUDV antibodies were detected in 13.5% (17/126) of the sampled sheep farms, and 5.2% (29/557) of tested sheep serum samples were positive for FLUDV antibodies. For the goat results, the FLUDV antibodies were detected in 13.3% (2/15) of the sampled farms, and 8.8% (8/91) of the tested goat serum samples were positive for FLUDV antibodies. Furthermore, all tested poultry serum samples were negative for FLUDV antibodies. Our data demonstrated that sheep and goat are susceptible to FLUDV virus and multiple states in U.S. have this virus infection already in these two species. This new finding highlights a need for future surveillance of FLUDV virus in small ruminants toward better understanding both the origin and natural reservoir of this new virus.

1. Introduction

Influenza viruses are divided into three types: A–C, and this classification is based on their antigenic differences in the matrix and nucleoprotein (Palese and Shaw, 2007; Treanor, 2000). We recently identified a novel influenza virus with bovine as a primary reservoir. Phylogenetic analysis suggests that it is most closely related to influenza C (FLUCV), rather than to influenza A (FLUAV) and influenza B (FLUBV) viruses. However, the distance between the new virus and FLUCV is similar to the differences between FLUAV and FLUBV for most of the genomic segments (Hause et al., 2013). In addition to bovine, this new virus is also isolated from U.S. swine exhibiting severe influenza-like illness as well as from diseased cattle in France and China (Ducatez et al., 2015; Jiang et al., 2014). Its intercontinental transmission and prevalence in both cattle and swine highlight its potential threat to other agricultural animals and humans. We recently proposed that this new group of viruses represents a new genus, designated influenza D, in *Orthomyxoviridae* family. Henceforth, we refer to this virus as influenza D virus (FLUDV) (Hause et al., 2014).

Since FLUDV was discovered, pigs, cows, ferrets, and guinea pigs have been found susceptible to the virus (Hause et al., 2014; Hause et al., 2013). Humans and multiple animal species are susceptible to influenza virus; therefore, other potential hosts of this virus need to be determined. The primary objective of this study is to investigate the seroprevalence of FLUDV in agricultural animals such as small ruminants (sheep and goats) and poultry (chicken and turkey) by conducting a serological survey.

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2. Materials and methods

2.1. Cell culture and virus production

Swine testicular (ST) cells (ATCC CRL-1746) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (PAA Laboratories Inc., Dartmouth, MA, USA) and 1% penicillin and streptomycin (Life Technologies, Carlsbad, CA, USA). Influenza D/bovine/Oklahoma/660/2013 (D/660) and D/swine/Oklahoma/1334/2011 (D/OK) were previously isolated from bovine or swine with respiratory disease symptoms. The virus was grown on ST cells at 0.01 multiplicity of infection (MOI) and incubated at 37 °C with ~5% CO_2 for 5 days. For virus growth/maintenance media, DMEM with 0.1 μg/mL exogenous tosylsulfonyl phenylalanyl chloromethyl ketone (TPCK) trypsin (Sigma, St. Louis, MO, USA) was used. Virus titer was determined using Madin–Darby canine kidney (MDCK) cells (ATCC CCL-34) according to Reed and Meunch's method (Reed and Meuench, 1938).

2.2. Serology

The hemagglutination inhibition (HI) and the microneutralization (MN) assays were performed as described in the WHO standard manual (W.H.O., 2011). Turkey red blood cells (Lampire Biological Laboratories, Pipersville, PA, USA) were used for the HI assay, while MDCK cells were employed for the MN assay. For the HI assay, an antibody titer of 40 was used as a threshold, i.e., a sample with a titer of less than 40 was judged as negative, and those with a titer equal to or higher than 40 were viewed as positive. For the HI and MN assays, serial 2-fold dilutions of serum sample were tested in duplicate. HI or MN titers were expressed as the reciprocal of the highest dilution of serum that gave complete hemagglutination or 50% neutralization, respectively. All samples were assayed in three separate experiments and the mean antibody titers were calculated from these triplicate data.

2.3. Serum sample collection

250 chicken and turkey serum samples were acquired from Minnesota Poultry Testing Laboratory in Willmar, Minnesota and were taken from 25 poultry farms in Minnesota and Iowa in April 2014. Among them, 100 samples were chickens and 150 samples were turkeys. A total of 499 serum samples from small ruminants (27 from goats and 472 from sheep) were collected through Animal Disease Research and Diagnostic Laboratory at South Dakota State University (SDSU) from March to September 2014. Goat and sheep farms are located in the Midwest region including South Dakota (SD), Minnesota (MN), Iowa (IA), Nebraska (NE), Missouri (MO), and North Dakota (ND). Washington State University (WSU) at Pullman, Washington, provided an additional 64 goat serum samples and 85 sheep serum samples for this study. Serum samples from WSU were collected from various age groups and breeds of animals from 2001 to 2007, and farms that derived these goat and sheep samples are located in Canada and various states of the United States including California (CA), Montana (MT), North Dakota (ND), Missouri (MO), Illinois (IL), Texas (TX), North Carolina (NC), South Carolina (SC), Maryland (MD), New York (NY), Massachusetts (MA), and Maine (ME).

3. Results and discussion

Avian species are known hosts and reservoirs of FLUAV. To determine if chickens and turkeys are susceptible to FLUDV, we analyzed 100 serum samples from chickens and 150 from turkeys for the presence of virus-specific antibodies by the standard HI assay. Poultry farms that derived serum samples are located in the Midwest region where we frequently isolated FLUDV from bovine herds. All 250 samples had titers <10 for FLUDV antibody in the HI assay (data not shown). Absence of FLUDV-specific antibody indicated that the tested population was not exposed previously to the virus. On the other hand, it can be envisioned that both chicken and turkey may not be susceptible to this newly emerged influenza

Table 1
Summary of the serological survey from the goats and sheep farms in the Midwest by the HI assay.

Animal	Farm	Location	No. of seropositive[a] for FLUDV[b]	HI titer range	Date of sample taken
Goat	A	Montana	0/7	0	March 2014
	B	South Dakota	0/2	0	March 2014
	C	South Dakota	0/1	0	March 2014
	D	South Dakota	0/3	0	May 2014
	E	South Dakota	7/14 (77.1)[c]	40–80	May 2014
Sheep[d]	F	South Dakota	1/1 (60)	60	July 2014
	G	South Dakota	1/4 (80)	80	July 2014
	H	Iowa	1/2 (40)	40	July 2014
	I	South Dakota	4/19 (50)	40–80	July 2014
	J	North Dakota	1/1 (40)	40	July 2014
	K	South Dakota	1/3 (40)	40	July 2014
	L	South Dakota	1/2 (160)	160	July 2014
	M	South Dakota	1/1 (80)	80	August 2014
	N	South Dakota	3/20 (60)	40–80	August 2014
	O	South Dakota	1/16 (40)	40	August 2014
	P	South Dakota	1/7 (240)	240	August 2014
	Q	Minnesota	1/5 (320)	320	August 2014
	R	South Dakota	4/4 (65)	40–80	August 2014
	S	South Dakota	2/20 (50)	40–80	August 2014
	T	South Dakota	1/15 (80)	80	August 2014
	U	North Dakota	1/5 (320)	320	September 2014
	V	South Dakota	4/17 (50)	40–80	September 2014

[a] Data are the number of positive samples out of the total number tested.
[b] D/bovine/Oklahoma/660/2013 was used.
[c] Numbers in parentheses indicate mean HI titers for samples with a value of ≥40.
[d] Data shown are the only positive sheep serum samples.