This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	***2014.009a-dM***	(to be completed by ICTV officers)

Short title: One (1) new genus in the family *Orthomyxoviridae*
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 9 are required)	**1** ☒	**2** ☒	**3** ☒	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☒	

Author(s) with e-mail address(es) of the proposer:

Ben Hause (bhause@vet.k-state.edu)
Feng Li (feng.li@sdstate.edu)

List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at http://www.ictvonline.org/subcommittees.asp. If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	*Orthomyxoviridae* Study Group

ICTV-EC or Study Group comments and response of the proposer:

EC46 decision:

Decision: Uc. Provide details of method used for phylogenetic analysis; improve BLAST presentation in Table 2; highlight isolates of proposed new species in the phylogenetic tree; await SG comments before progressing further.

Date first submitted to ICTV:	07/02/2014
Date of this revision (if different to above):	06/01/2016

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for one isolate of each new species proposed.

Code	***2014.009aM***	(assigned by ICTV officers)

To create one new species within: *Influenzavirus D*

Genus:	*Influenzavirus D* (new)
Subfamily:	
Family:	***Orthomyxoviridae***
Order:	

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

Name of new species:	**Representative isolate:**	**GenBank sequence accession number(s)**
Influenza D virus	D/swine/Oklahoma/1334/2011	JQ922305-JQ922311, relating to segments 1–7, respectively

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.

Further material in support of this proposal may be presented in the Appendix, Module 9

1. Influenza D Viruses (FLUDV) are distantly related to Influenza C Viruses (FLUCV), with an overall genetic distance similar to that observed between influenza A and B viruses (FLUAV and FLUBV, respectively) (Hause et al., PloS Pathogens, 2013; Hause et al., mBio, 2014; and Sheng *et al*., Archives of Virology, 2014)
2. FLUDV does not productively reassort with FLUCV as determined by *in vitro* co-infection (reassortment) experiments (Hause *et al*., mBio, 2014)
3. FLUDV does not cross react with FLUAV, FLUBV or FLUCV antisera in the agar gel immunodiffusion assay (AGID) (Hause *et al*., mBio, 2014)
4. FLUDV does not reassort with FLUCV based on phylogenetic analysis of ≈10 field isolates (Collin et al., Journal of Virology, 2015)
5. The highly conserved non-coding region of the genome segments of FLUDV are similar to FLUCV however possess a single nucleotide difference (position 5 from the 3'-terminus) and polymorphism at the first nucleotide at the 3'-terminus (Hause *et al*., Plos Pathogens, 2013)
6. FLUDV exists in a bovine reservoir. Bovids are rarely infected by FLUAV, FLUBV or FLUCV (Hause *et al*., mBio 2014)
7. The transcriptional splicing event to generate the M1 protein of FLUDV differs from that of FLUCV (Hause *et al*., mBio 2014)
8. FLUDV has been found prevalent in sheep and goats (Quast et al., Veterinary Microbiology, 2015)
9. FLUDV is not prevalent in human populations (Hause et al., Plos Pathogens, 2013 and Smith et al., Journal of Clinical Virology, 2016)

10. FLUDV has been also found in cattle and swine in Europe and Asia (Ducatez et al., Emerging infectious diseases, 2016; Chiapponi et al., Emerging infectious diseases, 2016; and Jiang et al., Virus Genes, 2014).

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2014.009bM***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Orthomyxoviridae***
Order:	

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2014.009cM***	(assigned by ICTV officers)

To name the new genus: *Influenzavirus D*

Assigning the type species and other species to a new genus

Code	***2014.009dM***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Influenza D Virus	Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

One (1)

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

See our justification listed above in module 2

Origin of the new genus name:

Convention

Reasons to justify the choice of type species:

FLUDV D/swine/Oklahoma/1334/2011 is the original isolate and founding members of this proposed new genus and has been extensively studied *in vitro* and *in vivo*

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

Currently, all viruses known to belong to this novel group belong into a single species; i.e. for now the proposed new genus is monogeneric.

MODULE 9: **APPENDIX**: supporting material

Note: C/Swine/Oklahoma/1334/2011, C/bovine/Oklahoma/660/2013, and other C/bovine viruses are members of the proposed genus that would also harbor FLUDV. These names are not changed in results below to be consistent with our published works. However, they are refered to in the phylogenetic trees and PBlst comparisons as D/Swine/Oklahoma/1334/2011, D/bovine/Oklahoma/660/2013, etc.

Additional material in support of this proposal

References:

Hause BM, Ducatez M, Collin EA, Ran Z, Liu R, Sheng Z,Armien A, Kaplan B, Chakravarty S, Hoppe AD, Webby RJ, Simonson RR, Li F. 2013. Isolation of a novel swine influenza virus from Oklahoma in 2011 which is distantly related to human influenza C viruses. PLoS Pathog. 9:e1003176. doi:10.1371/journal.ppat.1003176.

Hause BM, Collin EA, Liu R, Huang B, Sheng Z, Lu W, Wang D, Nelson EA, Li F. 2014. Characterization of a novel influenza virus in cattle and swine: proposal for a new genus in the *Orthomyxoviridae* family. mBio 5(2):e00031-14. doi:10.1128/mBio.00031-14.

Zizhang Sheng, Zhiguang Ran, Dan Wang, Adam D. Hoppe, Randy Simonson, Suvobrata Chakravarty, Ben M. Hause, Feng Li. 2014. Genomic and evolutionary characterization of a novel influenza C-like virus from swine. Archives of Virology, Volume 159:2, pp 249-255

Emily Collin, Zizhang Sheng, Yukun Lang, Wenjun Ma, Ben Hause, **Feng Li**. 2014. Co-circulation of two distinct genetic and antigenic lineages of proposed influenza D virus in cattle. *J. Virol*. 89(2):1036-42. doi: 10.1128/JVI.02718-14. Epub 2014 Oct 29

Quast M, Sreenivasan C, Sexton G, Nedland H, Singrey A, Fawcett L, Miller G, Lauer D, Voss S, Pollock S, Cunha CW, Christopher-Hennings J,Nelson E, Feng Li. 2015. Serological evidence for the presence of influenza D virus in small ruminants. Veterinary Microbiology. 2015 Sep 14. pii: S0378-1135(15)30023-7. doi: 10.1016/j.vetmic.2015.09.005. [Epub ahead of print]

Smith DB, Gaunt ER, Digard P, Templeton K, Simmonds P. 2016. Detection of influenza C virus but not influenza D virus in Scottish respiratory samples. J Clin Virol., 2016 Jan;74:50-3. doi: 10.1016/j.jcv.2015.11.036. Epub 2015 Nov 28.

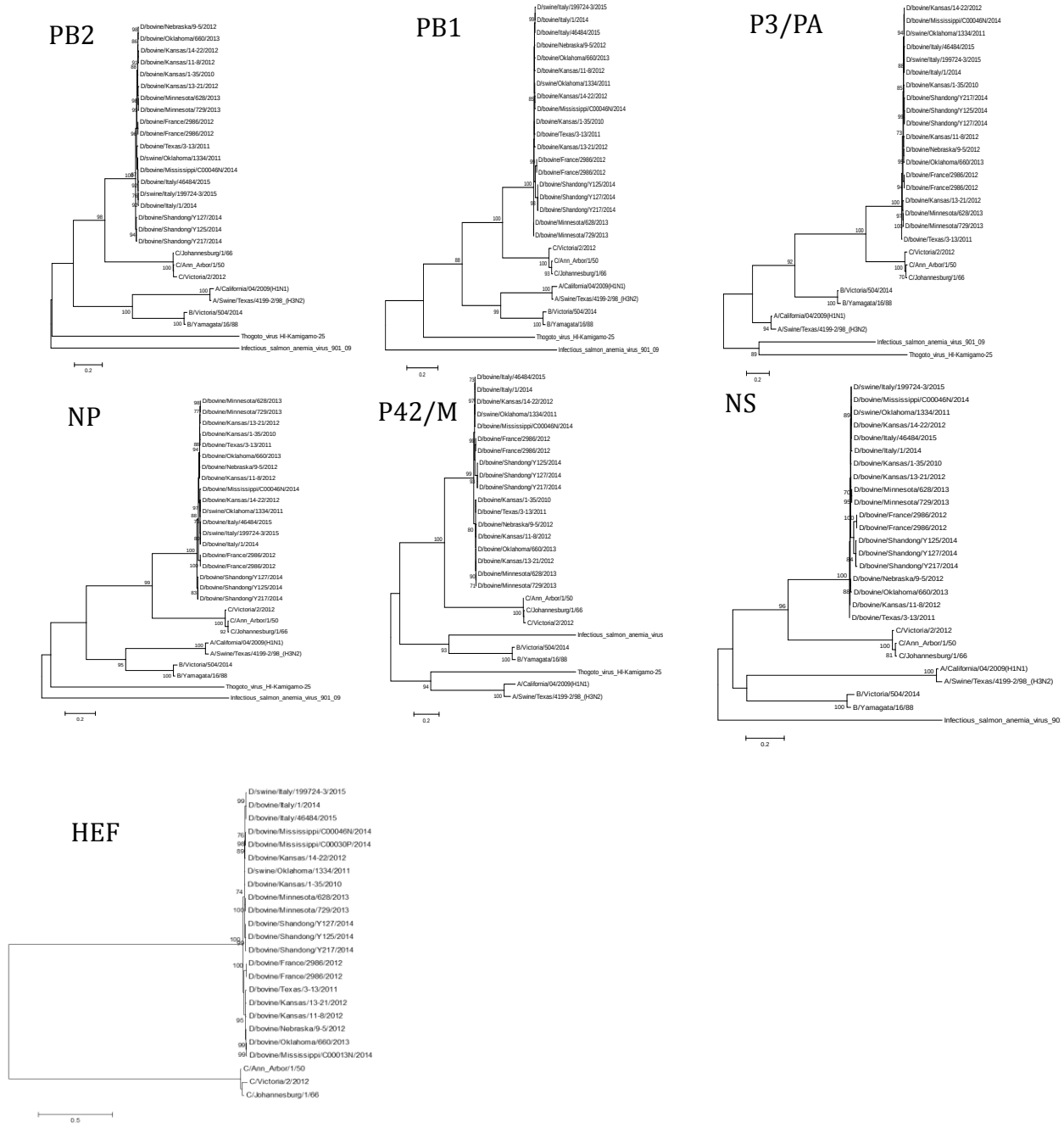
Ducatez MF, Pelletier C, Meyer G. Influenza D virus in cattle, France, 2011–2014. Emerg Infect Dis [Internet]. 2015 Feb [date cited]. http://dx.doi.org/10.3201/eid2102.141449

Chiapponi C, Faccini S, de Mattia A, Baioni L, Barbieri I, Rosignoli C, et al. Detection of influenza D virus among swine and cattle, Italy [letter]. Emerg Infect Dis. 2016 Feb [date cited]. http://dx.doi.org/10.3201/eid2202.151439.

Jiang WM, Wang SC, Peng C, Yu JM, Zhuang QY, Hou GY, . Identification of a potential novel type of influenza virus in Bovine in China. Virus Genes. 2014;49:493–6

Annex:

1. **Influenza D Viruses (FLUDV) are distantly related to Influenza C Viruses (FLUCV), with an overall genetic distance similar to that observed between Influenza A and B Viruses (FLUAV and FLUBV, respectively).**



Note that the complete segment nucleotide sequences were aligned by Clustal. Phylogeny inferred using the Maximum Likelihood algorithm with the best-fitting general-time reversible model of nucleotide substitution with gamma distribution. Tree topology was assessed using 500 bootstrap replicates.

2. BlastP analysis of the eight putative open reading frames of FLUDV C/swine/Oklahoma/1334/2011

ORF (Amino acid)	Best blast hit (virus variant; accession number)	Identity (%)	Positive (%)[a]
772	PB2 (C/Johannesburg/1/66; Q9IMP3)	53	71
758	PB1 (C/Johannesburg/1/66; AF170575)	72	85
710	P3 (C/Ann Arbor/1/50; NC_006309)	50	66
664	HEF (C/Catalonia/1318/2009; HM748631)	53	69
552	NP (C/Johannesburg/4/67; BAL72794)	39	59
246	M1 (C/Taylor/1233/47; BAA05545)	42	62
243	NS1 (C/Hiroshima/248/2000; AB099621)	33	48
184	NS2 (C/Sao Paulo/378/82; AB035366)	31	50

[a]Positive value indicates the degree of similarity between proteins

	1	2	3	4	5	6
D.OK PB2 translation of CDS PB2	1		50.56	23.14	16.43	13.40	14.20
C.JHB PB2 translation of CDS PB2	2	50.56		22.50	15.14	13.86	11.88
B.FA.PB1 translation of CDS PB2	3	23.14	22.50		26.75	13.52	12.95
A.TX.PB2 translation of CDS PB2	4	16.43	15.14	26.75		9.53	10.92
ISAV PB2 translation of CDS PB2	5	13.40	13.86	13.52	9.53		9.35
a PB2 translation of CDS THOV_s1gp1	6	14.20	11.88	12.95	10.92	9.35	

	1	2	3	4	5	6
D.OK PB1 translation of CDS PB1		75.00	28.04	28.96	22.29	16.48
C.JHB PB1 translation of CDS PB1	75.00		29.22	28.38	21.90	15.02
A.TX.PB1 translation of CDS PB1	28.04	29.22		18.47	11.72	8.90
thogoto PB1 translation of CDS THOV_s2gp1	28.96	28.38	18.47		18.27	10.97
ISAV PB1 translation of CDS PB1	22.29	21.90	11.72	18.27		8.67
B.FA.PB1 translation of CDS PB2	16.48	15.02	8.90	10.97	8.67	

	1	2	3	4	5	6
D.OK P3 translation of CDS P3		50.00	26.28	7.02	18.26	10.98
C.JHB P3 translation of CDS P3	50.00		24.87	7.15	15.73	10.39
B.FA.P3 translation of CDS PA	26.28	24.87		9.75	15.31	10.52
A.TX.PA translation of CDS PA	7.02	7.15	9.75		4.58	5.90
thogoto PA translation of CDS THOV_s3gp1	18.26	15.73	15.31	4.58		8.10
ISAV PA translation of CDS PA	10.98	10.39	10.52	5.90	8.10	

	1	2	3	4	5	6
D.OK HEF translation of CDS HEF		52.70	19.69	12.24	10.44	6.17
C.JHB HEF translation of CDS HEF	52.70		18.57	12.39	11.33	7.14
B.FA.HA translation of CDS HA	19.69	18.57		15.67	10.55	7.01
A.TX.HA translation of CDS hemagglutinin	12.24	12.39	15.67		8.52	8.65
thogoto GP translation of CDS THOV_s4gp1	10.44	11.33	10.55	8.52		6.44
ISAV HE translation of CDS HE	6.17	7.14	7.01	8.65	6.44	

	1	2	3	4	5	6
D.OK NP translation of CDS NP		39.86	20.00	11.51	12.69	13.50
C.JHB.NP translation of CDS NP	39.86		20.13	13.54	13.23	11.66
B.FA.NP translation of CDS NP	20.00	20.13		28.50	14.25	14.32
A.TX.NP translation of CDS nucleoprotein	11.51	13.54	28.50		7.95	8.55
thogoto NP translation of CDS THOV_s5gp1	12.69	13.23	14.25	7.95		11.58
ISAV NP translation of CDS NP	13.50	11.66	14.32	8.55	11.58	

	1	2	3	4	5	6
D.OK P42 translation of CDS P42		41.60	13.01	13.58	12.55	14.45
C.JHB.P42 translation of CDS M	41.60		14.23	10.70	14.51	11.72
thogoto M translation of CDS THOV_s6gp2	13.01	14.23		10.00	9.38	8.98
ISAV M translation of CDS M1	13.58	10.70	10.00		9.88	6.61
B.FA.M translation of CDS M1	12.55	14.51	9.38	9.88		30.95
A.TX.M translation of CDS M1	14.45	11.72	8.98	6.61	30.95	

	1	2	3	4	5
D.OK NS translation of CDS NS1	1		32.95	14.53	12.21	6.87
C.JHB.NS translation of CDS NS1	2	32.95		13.49	13.19	6.59
B.FA.NS translation of CDS NS1	3	14.53	13.49		12.67	8.04
A.TX.NS translation of CDS NS1	4	12.21	13.19	12.67		6.87
ISAV NS translation of CDS NS1	5	6.87	6.59	8.04	6.87	

	1	2	3	4	5
D.OK NS translation of CDS NS2	1		30.27	13.04	10.70	8.04
C.JHB.NS translation of CDS NS2	2	30.27		10.93	6.99	12.12
A.TX.NS translation of CDS NS2	3	13.04	10.93		18.44	8.14
B.FA.NS translation of CDS NEP	4	10.70	6.99	18.44		8.94
ISAV NS translation of CDS putative nuclear export protein	5	8.04	12.12	8.14	8.94	

Note that the above table shows percent pairwise amino acid identity for proteins encoded by representative Orthomyxoviruses.

3. **FLUDV do not productively reassort with FLUCV as determined by *in vitro* co-infection experiments.**

Genotypes identified in plaque purified viruses isolated following infection of cells with influenza C virus(es). C/Johannesburg/1/66 and C/Taylor/1233/1947 are reference human FLUCV. C/Swine/Oklahoma/1334/2011 and C/bovine/Oklahoma/660/2013 are members of the proposed genus that also harbors FLUDV.

Virus(es)[a]	PB2[b]	PB1[b]	P3[b]	HEF[b]	NP[b]	P42[b]	NS[b]
C/JHB C/Tay C/OK C/660	C/OK (8) C/660 (2)	C/660(9) *(1)	C/OK(6) C/660(4)	C/OK(7) C/660(1) *(2)	C/OK(4) C/660(4) *(2)	C/OK(1) C/660(4) *(5)	C/OK(7) C/660(3)
C/JHB C/Tay	*(10)	*(10)	C/JHB(7) C/Tay(3)	C/Tay(4) *(6)	*(10)	C/Tay(10)	*(10)
C/JHB C/OK	C/OK(10)	C/OK(10)	C/OK(10)	C/OK(10)	C/OK(10)	C/OK(10)	C/OK(10)
C/JHB C/660	C/660(10)	C/660(10)	C/660(10)	C/660(10)	C/660(10)	C/660(10)	C/660(10)
C/Tay C/OK	C/OK(10)	C/OK(10)	C/OK(10)	C/OK(10)	C/OK(10)	C/OK(10)	C/OK(10)
C/Tay C/660	C/660(10)	C/660(10)	C/660(10)	C/660(10)	C/660(10)	C/660(10)	C/660(10)
C/OK C/660	C/OK(10)	C/OK(3) C/660(7)	C/OK(8) C/660(2)	C/OK(10)	C/OK(6) C/660(4)	C/OK(4) C/660(5) *(1)	C/OK(6) C/660(1) *(3)

[a]Virus(es) used for (co)-infection
[b]Parentage of viral genome segments present in virus plaques from co-infected cells. Ten plaque purified viruses were analyzed from each co-infection experiment. Number of plaques from each donor indicated in parentheses
*Some viral segment donors could not be identified

4. FLUDV do not cross react with FLUAV, FLUBV or FLUCV antisera in agar gel immunodiffusion (AGID) and hemagglutination inhibition (HI) assays.

Results of Agar Gel Immunodiffusion (AGID) assay

Antigen (virus or mock control)	Antiserum			
	A/NWS/34(H1)-A/Equine/Prague/1/56(N7)	B/Hong Kong/8/73(Matrix)	C/Taylor/1233/47	C/swine/OK/1334/2011
A/WSN/1933	+[a]	–[b]	–	–
B/Brisbane/60/2008	–	+	–	–
C/Taylor/1233/1947	–	–	+	–
C/Johannesburg/1/1966	–	–	+	–
C/swine/OK/1334/2011	–	–	–	+
C/bovine/OK/660/2013	–	–	–	+
MDCK Mock[c]	–	–	–	–
HRT-18G Mock	–	–	–	–
PBS	–	–	–	–

[a]indicates the presence of a visible white precipitation line between antigen and antiserum wells

[b]means the absence of a visible white precipitation line between antigen and antiserum wells

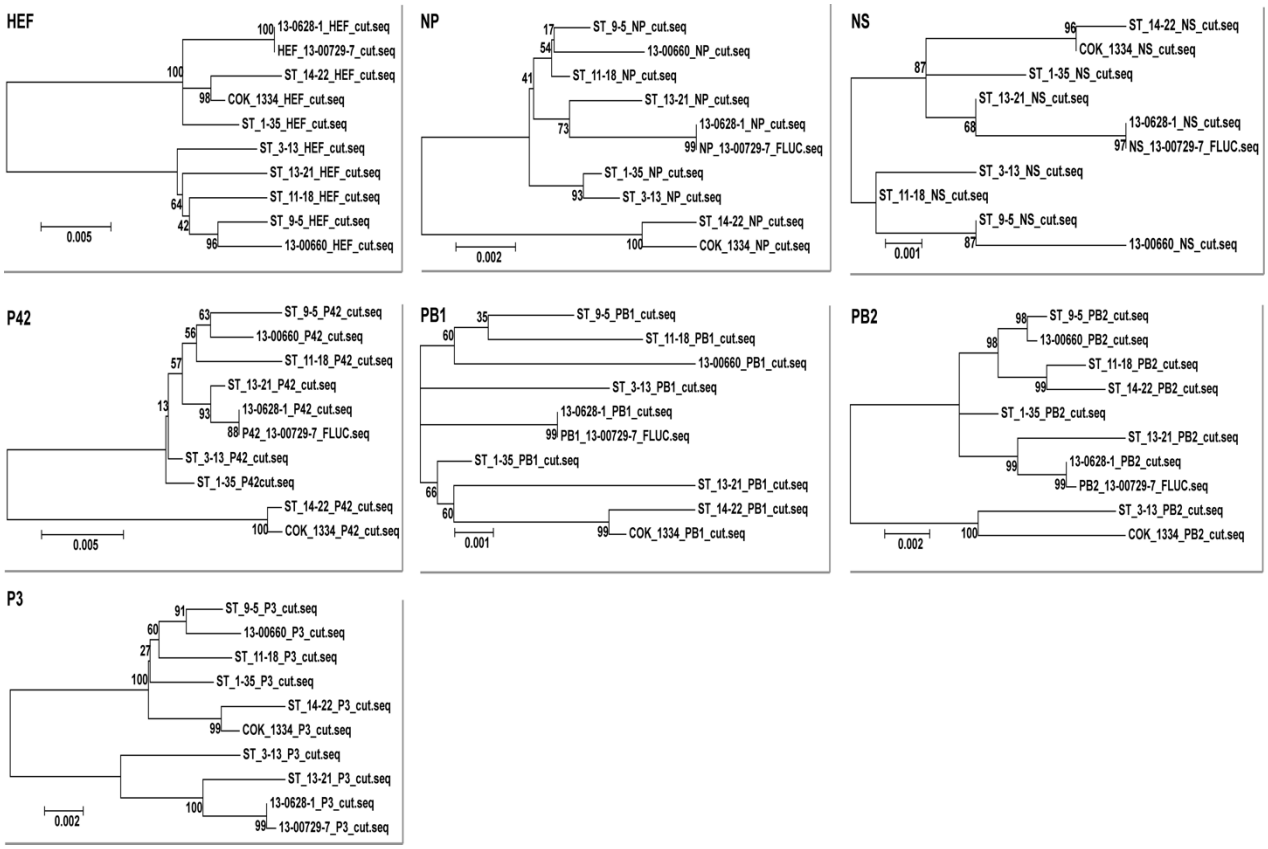
[c]indicates the same protocol to produce viral antigens employed to prepare supernatants collected from uninfected cells.

Cross-reactivity of antibodies to influenza A, B and C viruses and C/swine/Oklahoma/1334/2011 virus as measured by hemagglutination inhibition assay using turkey red blood cells.

Virus	**A/CA**	**A/NC**	**A/MN**	**B/Florida**	**C/OK**	**C/Taylor**
A/CA/04/2009(H1N1)	**160**	<10	<10	<10	<10	<10
A/swine/NC/6300-1/2010(H1N2)	<10	**320**	<10	<10	<10	<10
A/swine/MN/3793/2008(H1N1)	<10	<10	**320**	<10	<10	<10
B/Florida/2006	<10	<10	<10	**160**	<10	<10
C/swine/OK/1334/2011	<10	<10	<10	<10	**≥1280**	<10
C/Taylor/1233/1947	<10	<10	<10	<10	<10	**320**

5. FLUDV does not reassort with FLUCV based on phylogenetic analysis of ≈10 field isolates

See phylogenetic analysis above in appendix 1. The figure below represents the phylogenetic analysis of 10 proposed FLUDV strain. All segments are closely related to the proposed type species D/swine//Oklahoma/1334/2011.



6. The highly conserved non-coding region of the genome segments of FLUDV were similar to FLUCV however possessed a single nucleotide difference (position 5 from the 3'-terminus) and polymorphism at the 1st nucleotide at the 3'-terminus

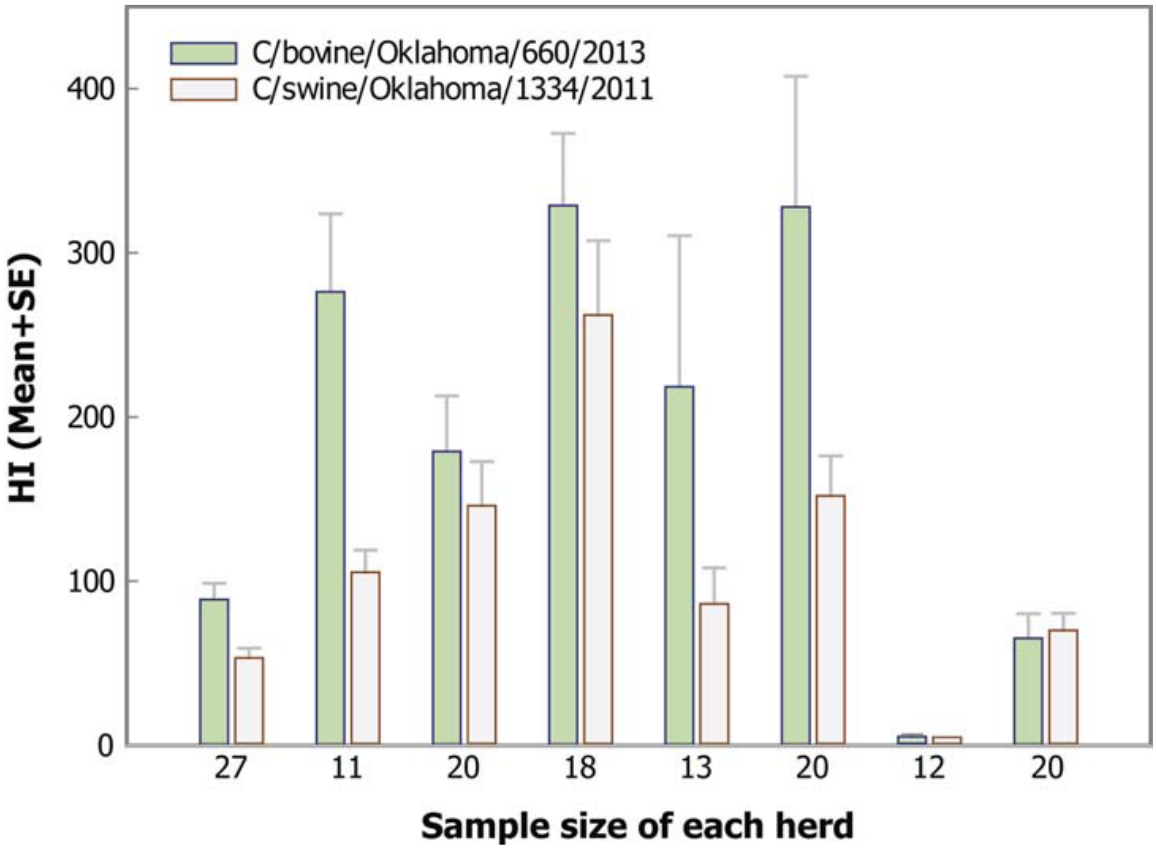
[a]conserved sequences in bold; start and stop codons underlined; poly U stretch in italic

C/swine/Oklahoma/1334/2011		
Segment	3' end non coding sequence[a]	5' end non coding sequence
PB2	C**CGUAUUCGUC**UCCUAC	**AGCAGUAGCAAG**AGG**G***AUUUUUU*CAAUGUGCUUCA
PB1	C**CGUAUUCGUC**UCCUAAAAUAU UGUUAC	**AGCAGUAGCAAG**AGG**G***AUUUUU*CUGUUAUUAAACAACGCAAAGCUUA
P3	C**CGUAUUCGUC**CUCUAAAUCUU UAC	**AGCAGUAGCAAG**GAG**G***AUUUUU*AACAUUACAAGGCCUUUGGUCA
HEF	U**CGUAUUCGUC**CUCUAAAAGUU UCUAC	**AGCAGUAGCAAG**GAG**G***AUUUUUU*CUAAGAUUCUA
NP	C**CGUAUUCGUC**CUCUAAUAAUU CGUUAUAC	**AGCAGUAGCAAG**GAG**G***AUUUUUU*GUUAAAUAAGACAAACCAACAUCUUUAACACCC ACUGGGGACUGCAACAGAACCAUCCAAAGAUGAGUUA
M	U**CGUAUUCGUC**UCCUAUAAAAA CUCGCUUAC	**AGCAGUAGCAAG**AGG**G***AUUUUUU*CGCGAUUA
NS	U**CGUAUUCGUC**CCCACAUGUUA AAGUUAUAC	**AGCAGUAGCAAG**GGG*UUUUUUCA*

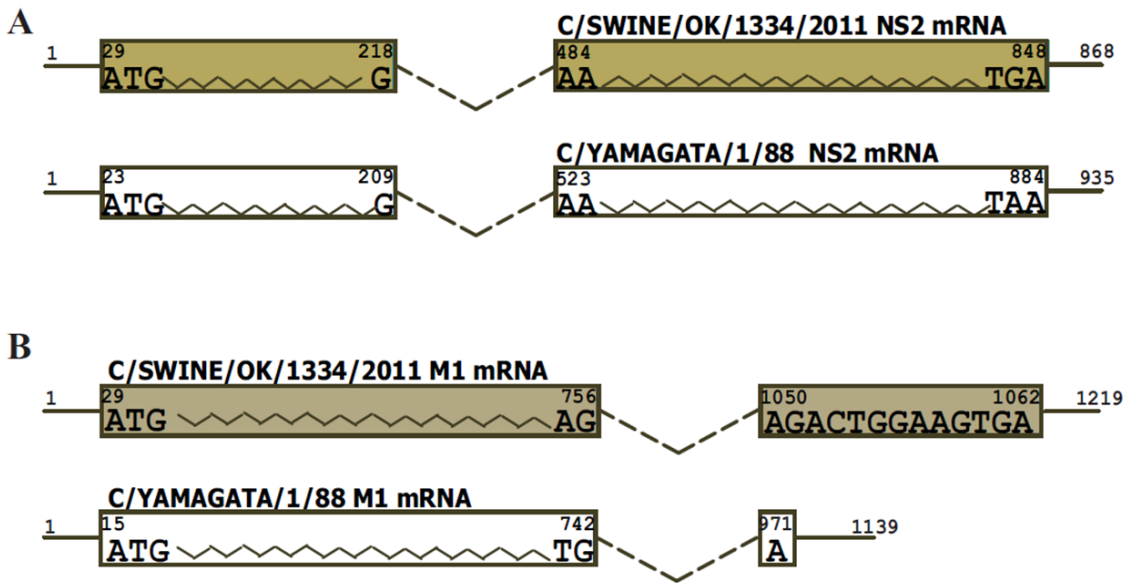
C/JHB/1/66		
Segment	3' end non coding sequence	5' end non coding sequence
PB2	**UCGUCUUCGUC**UCCUAACCU UUAC	**AGCAGUAGCAAG**AGG**G***AUUUUA*
PB1	**UCGUCUUCGUC**UCCUAAUAC	**AGCAGUAGCAAG**AGG**G***AUUUUUU*CAUUUAAUGGAAUAACAAAAAUAUGUGCAAGUA GGAGGAAAGGGUUUAACAGCCCCUCCUCA
P3	**UCGUCUUCGUC**CCCUAGGCU UUAC	**AGCAGUAGCAAG**GGG**G***AUUUUUU*CUUAUAAUGAUCA
HEF	**UCGUCUUCGUC**CCCCAAUUA UUAC	**AGCAGUAGCAAG**GGG**G***AUUUUU*GUUUUUUAUAAAACAGUACAAAAUAUUGACCAAC ACAUUAUCCAUUUUUCAAAAUUGUCUCAAUCA
NP	**UCGUCUUCGUC**CUCUAAACC AAAAGUUUUUAC	**AGCAGUAGCAAG**GAG**A***UUUUU*GAAUUAUAUAUAGCAAUACAACAGUUGAUCAUAA AAUGUGCGAUGAAUUUAAUCUGACUUUAAUUUUCUCCAGGAAUGUUGCUA
M	**UCGUCUUCGUC**CCCUGAAAA UUUGUUAC	**AGCAGUAGCAAG**GGG**A***UUUUUUU*CAAGGUAAUUA
NS	**UCGUCUUCGUC**CCCAUGAAA AAGUUUUAC	**AGCAGGAGCAAG**GGG*UUUUUU*AACUUUGGAAUAACAACUUAAAACAAUUA

7. FLUDV exists in a bovine reservoir. Bovids are rarely infected by FLUAV, FLUBV, or FLUCV

HI assays were run on bovine sera collected from 8 herds. Greater than 90% of animals had titers >10. Mean HI titers for each herd to two different isolates of the proposed FLUDV are shown below



8. The transcriptional splicing event to generate the M1 protein of FLUDV differs from that of FLUCV



Splicing strategies of C/OK virus for NS segment (A) and M segment (B). Panel A schematically illustrate a splicing strategy of C/OK virus NS segment to produce NS2 protein in comparison to its counterpart in human FLUCV, while panel B describes a novel splicing strategy of M segment to produce M1 protein in comparison to human FLUCV's M1 protein synthesis.