

Table S1. Information about collection date, number of tested animals and samples, high-throughput real-time PCR results, and IDV single-plex real-time RT-PCR results for the influenza D virus positive herds. IDV, influenza D virus; BCoV, bovine coronavirus.

Herd ID	Collection date	High-throughput real-time PCR analysis				Single-plex real-time RT-PCR analysis	
		No. of animal tested	Tested as pool (no. of samples in the pool)	Pool tested positive for	No. of samples tested as individual sample	Individual samples tested positive for	No. of individual samples from pool tested positive for IDV
1	06-02-19	8	Yes (8/8)	IDV <i>Mycoplasma</i> <i>P. multocida</i>	4	1. IDV, <i>P. multocida</i> 2. IDV 3. IDV, <i>Mycoplasma</i> , <i>P. multocida</i> 4. IDV	4/4 One sample (3) sequenced D/bovine/Denmark/6171103755-1/2019
1	06-03-19	4	Yes (4/4)	IDV <i>P. multocida</i> <i>T. pyogenes</i>	0	NA	1/4 No sample sequenced
2	01-03-19	10	Yes (10/10)	IDV <i>M. bovis</i> <i>M. haemolytica</i> <i>P. multocida</i>	0	NA	10/10 One sample sequenced D/bovine/Denmark/5246407608-2/2019
3	12-03-19	8	Yes (8/8)	IDV <i>M. haemolytica</i> <i>H. somni</i> <i>P. multocida</i>	0	NA	3/8 One sample sequenced D/bovine/Denmark/5358904609-3/2019
4	05-04-19	10	Yes (10/10)	BCoV IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>M. haemolytica</i> <i>P. multocida</i> <i>T. pyogenes</i>	0	NA	6/10 One sample sequenced D/bovine/Denmark/1173303525-4/2019
5	15-01-19	10	Yes (10/10)	IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>M. haemolytica</i> <i>P. multocida</i>	0	NA	3/10 Three samples sequenced D/bovine/Denmark/4559602762-5/2019 D/bovine/Denmark/5022903340-5/2019 D/bovine/Denmark/5161205135-5/2019
5	15-01-19	15	Yes (9/15)	IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>M. haemolytica</i> <i>P. multocida</i>	15	1. IDV, <i>M. haemolytica</i> , <i>H. somni</i> , <i>P. multocida</i> , <i>T. pyogenes</i> 2. <i>M. bovis</i> , <i>Mycoplasma</i> , <i>M. haemolytica</i> , <i>H. somni</i> , <i>P. multocida</i> 3. IDV, <i>M. haemolytica</i> , <i>H. somni</i> , <i>P. multocida</i> 4. <i>H. somni</i> , <i>P. multocida</i> 5. IDV, <i>M. bovis</i> , <i>P. multocida</i> 6. IDV, <i>Mycoplasma</i> , <i>M. haemolytica</i> , <i>H. somni</i> , <i>P. multocida</i> 7. IDV 8. IDV, <i>P. multocida</i> 9. IDV, <i>Mycoplasma</i> , <i>H. somni</i> , <i>P. multocida</i>	7/8 One sample (10) sequenced D/bovine/Denmark/5195604714-5/2019

					10. IDV, <i>M. haemolytica</i> , <i>H. somni</i> 11. Neg 12. IDV, <i>M. haemolytica</i> , <i>P. multocida</i> 13. IDV, <i>M. bovis</i> , <i>Mycoplasma</i> , <i>H. somni</i> , <i>P. multocida</i> 14. IDV, <i>M. bovis</i> , <i>M. haemolytica</i> , <i>P. multocida</i> , <i>T. pyogenes</i> 15. <i>H. somni</i> , <i>P. multocida</i>		
5	15-01-19	3	Yes (2/3)	IDV <i>Mycoplasma</i> <i>M. haemolytica</i> <i>H. somni</i> <i>P. multocida</i>	3	1. IDV 2. <i>Mycoplasma</i> , <i>M. haemolytica</i> , <i>H. somni</i> , <i>P. multocida</i> 3. IDV, <i>M. haemolytica</i> , <i>P. multocida</i>	2/3 No sample sequenced
5	20-02-19	9	Yes (9/9)	IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>P. multocida</i>	0	NA	2/9 One sample sequenced D/bovine/Denmark/5195604790-5/2019
5	24-04-19	6	Yes (6/6)	IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>M. haemolytica</i> <i>H. somni</i> <i>P. multocida</i>	0	NA	2/6 One sample sequenced D/bovine/Denmark/5224403563-5/2019
6	08-01-19	5	Yes (5/5)	IDV <i>M. haemolytica</i> <i>H. somni</i> <i>P. multocida</i>	5	1. IDV, <i>T. pyogenes</i> 2. IDV, <i>M. bovis</i> , <i>Mycoplasma</i> , <i>M. haemolytica</i> , <i>H. somni</i> , <i>P. multocida</i> 3. <i>M. bovis</i> , <i>H. somni</i> 4. <i>Mycoplasma</i> , <i>M. haemolytica</i> 5. <i>P. multocida</i>	1/1 One sample (1) sequenced D/bovine/Denmark/3313408142-6/2020
6	08-01-19	2	No	NA	2	1. IDV, <i>Mycoplasma</i> , <i>H. somni</i> , <i>P. multocida</i> , <i>T. pyogenes</i> 2. IDV, <i>M. haemolytica</i> , <i>H. somni</i> , <i>P. multocida</i>	0/0 No sample sequenced
6	05-02-19	5	Yes (5/5)	IDV <i>M. haemolytica</i> <i>P. multocida</i>	0	NA	0/0 No sample sequenced
6	05-02-19	5	Yes (5/5)	IDV <i>Mycoplasma</i> <i>M. haemolytica</i> <i>P. multocida</i>	0	NA	0/0 No sample sequenced
7	08-01-20	1	No	NA	1	1. IDV	1/1 No sample sequenced
7	05-02-20	5	Yes (5/5)	IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>M. haemolytica</i> <i>P. multocida</i>	0	NA	0/0 No sample sequenced
7	05-02-20	5	Yes (5/5)	IDV <i>M. bovis</i> <i>Mycoplasma</i>	0	NA	0/0 No sample sequenced

			<i>M. haemolytica</i> <i>P. multocida</i>				
7	05-02-20	5	Yes (5/5)	IDV <i>M. haemolytica</i> <i>P. multocida</i>	0	NA	0/0 No sample sequenced
7	12-03-20	5	Yes (5/5)	BCoV IDV <i>Mycoplasma</i> <i>M. haemolytica</i> <i>P. multocida</i>	0	NA	5/5 One sample sequenced D/bovine/Denmark/3727003200-7/2020
8	06-03-20	5	Yes (5/5)	IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>M. haemolytica</i> <i>H. somni</i> <i>P. multocida</i> <i>T. pyogenes</i>	1	1. Neg	3/5 One sample sequenced D/bovine/Denmark/5256205576-8/2020
9	17-12-19	1	No	NA	1	1. IDV, <i>H. somni</i> , <i>P. multocida</i>	1/1 No sample sequenced
9	17-12-19	5	Yes (5/5)	IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>M. haemolytica</i> <i>P. multocida</i> <i>T. pyogenes</i>	2	1. <i>M. haemolytica</i> , <i>P. multocida</i> 2. <i>M. bovis</i> , <i>Mycoplasma</i> , <i>H. somni</i>	0/0 No sample sequenced
9	17-12-19	5	Yes (5/5)	IDV <i>P. multocida</i>	2	1. <i>M. bovis</i> , <i>Mycoplasma</i> , <i>H. somni</i> , <i>P. multocida</i> 2. <i>M. haemolytica</i>	0/0 No sample sequenced
9	11-02-20	5	Yes (5/5)	IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>P. multocida</i>	0	NA	4/5 One sample sequenced D/bovine/Denmark/5995505714-9/2020
9	11-02-20	5	Yes (5/5)	IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>P. multocida</i>	0	NA	0/0 No sample sequenced
9	05-03-20	5	Yes (5/5)	IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>M. haemolytica</i> <i>H. somni</i> <i>P. multocida</i> <i>T. pyogenes</i>	0	NA	0/0 No sample sequenced
10	16-01-20	1	No	NA	1	1. IDV, <i>Mycoplasma</i> , <i>H. somni</i> , <i>P. multocida</i>	1/1 No sample sequenced
10	16-01-20	6	Yes (4/6)	IDV <i>M. bovis</i> <i>Mycoplasma</i>	6	1. <i>Mycoplasma</i> , <i>H. somni</i> , <i>P. multocida</i> 2. IDV, <i>M. haemolytica</i> , <i>H. somni</i> , <i>P. multocida</i> 3. IDV, <i>M. bovis</i> , <i>Mycoplasma</i> , <i>M. haemolytica</i> , <i>H. somni</i>	1/1 One sample sequenced

			<i>M. haemolytica</i> <i>H. somni</i> <i>P. multocida</i>		4. <i>M. bovis</i> , <i>M. haemolytica</i> , <i>H. somni</i> , <i>P. multocida</i> 5. Neg 6. <i>M. bovis</i> , <i>H. somni</i> , <i>P. multocida</i>	D/bovine/Denmark/1052304114-10/2020	
10	10-03-20	4	Yes (4/4)	IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>M. haemolytica</i> <i>H. somni</i> <i>P. multocida</i>	0	NA	0/0 No sample sequenced
10	10-03-20	5	Yes (5/5)	IDV <i>Mycoplasma</i> <i>M. haemolytica</i> <i>H. somni</i> <i>P. multocida</i>	0	NA	0/0 No sample sequenced
11	09-10-19	5	Yes (5/5)	IDV <i>Mycoplasma</i> <i>H. somni</i>	0	NA	4/5 One sample sequenced D/bovine/Denmark/3058506689-11/2019
12	16-01-19	10	Yes (10/10)	IDV <i>M. bovis</i> <i>Mycoplasma</i> <i>M. haemolytica</i> <i>P. multocida</i> <i>T. pyogenes</i>	0	NA	6/10 No sample sequenced
13	03-11-15	0	No	NA	0	NA	1/1 One sample sequenced D/bovine/Denmark/47398-3622/2015

Table S2. Accession numbers for the sequences of this study.

Strain	PB2	PB1	P3	HEF	NP	P42	NS
D/bovine/Denmark/6171103755-1/2019	OM468189	OM468201	OM468224	OM468243	OM468260	OM468270	OM468297
D/bovine/Denmark/5246407608-2/2019	OM468185	OM468202	OM468217	OM468246	OM468253	OM468265	OM468283
D/bovine/Denmark/5358904609-3/2019	OM468188	OM468203	OM468227	OM468247	OM468259	OM468269	OM468281
D/bovine/Denmark/1173303525-4/2019	OM468187	OM468207	OM468226	OM468242	OM468254	OM468267	OM468282
D/bovine/Denmark/4559602762-5/2019	OM468196	OM468211	OM468218	OM468240	OM468258	OM468274	OM468290
D/bovine/Denmark/5022903340-5/2019	OM468193	OM468208	OM468219	OM468237	OM468256	OM468275	OM468287

D/bovine/Denmark/5161205135-5/2019	OM468194	OM468209	OM468220	OM468239	OM468257	OM468273	OM468288
D/bovine/Denmark/5195604714-5/2019	OM468195	OM468210	OM468221	OM468238	OM468255	OM468276	OM468289
D/bovine/Denmark/5195604790-5/2019	OM468190	OM468212	OM468225	OM468245	OM468261	OM468272	OM468284
D/bovine/Denmark/5224403563-5/2019	OM468191	OM468213	OM468223	OM468244	OM468262	OM468271	OM468285
D/bovine/Denmark/3313408142-6/2020	OM468186	OM468206	OM468228	OM468241	OM468264	OM468266	OM468286
D/bovine/Denmark/3727003200-7/2020	OM468197	OM468214	OM468229	OM468233	OM468252	OM468277	OM468292
D/bovine/Denmark/5256205576-8/2020	OM468198	OM468215	OM468230	OM468234	OM468249	OM468278	OM468293
D/bovine/Denmark/5995505714-9/2020	OM468200	OM468216	OM468232	OM468236	OM468251	OM468280	OM468295
D/bovine/Denmark/1052304114-10/2020	OM468199	OM468205	OM468231	OM468235	OM468250	OM468279	OM468294
D/bovine/Denmark/3058506689-11/2019	OM468192	OM468204	OM468222	OM468248	OM468263	OM468268	OM468291
D/bovine/Denmark/47398-3622/2015	-	-	-	OM468296	-	-	-

Table S3. Predicted N-linked glycosylation sites of HEF for D/660 influenza D viruses (from Figure 1).

D/660 Sequences	Amino acid positions										
	28 NESF	54 NVTK	133 NWSP	146 NWTQ	249 NKTA	254 NTTG	346 NATE	390 NDTV	513 NDTN	562 NISM	613 NGSA
D/bovine/Italy/35490/2019	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Italy/28300/2019	+	+++	---	+	NRTA ++	/	+	-	+	-	+
D/bovine/Italy/28145/2019	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Italy/45097/2019	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Denmark/3727003200-7/2020-03-12	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Italy/19RS176-11/2018	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Denmark/5995505714-9/2020-02-11	+	+++	---	+	++	/	+	-	+	-	+

D/bovine/Denmark/1052304114-10/2020-01-16	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Denmark/5256205576-8/2020-03-06	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Quebec/5E-H/2018	+	+++	---	+	++	-	+	-	+	-	+
D/bovine/Quebec/3E-H/2018	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Quebec/1M-H/2019	+	+++	---	+	/	/	+	-	+	-	+
D/bovine/Mississippi/C00009P/2014	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Mississippi/C00002N/2014	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Nebraska/9-5/2012	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Oklahoma/660/2013	+	+++	---	+	NKTX ++	/	+	-	+	-	+
D/bovine/Mexico/S7/2015	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Mexico/S56/2015	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/Quebec/3M-B/2020	+	+++	---	+	++	/	+	-	+	-	+
D/bovine/France/2986/2012	+	+++	---	+	/	/	+	-	/	-	+

+ indicates prediction of an N-glycosylated site. - indicates a non-glycosylated site. / indicates no prediction of a glycosylated site.
Definition according to <https://services.healthtech.dtu.dk/service.php?NetNGlyc-1.0>:
+ Potential > 0.5, ++ Potential > 0.5 and Jury agreement (9/9) or Potential > 0.75, +++ Potential > 0.75 and Jury agreement, ++++ Potential > 0.90 and Jury agreement.
- Potential < 0.5, -- Potential < 0.5 and Jury agreement (all nine < 0.5), --- Potential < 0.32 and Jury agreement.

Table S4. Predicted N-linked glycosylation sites of HEF for D/OK influenza D viruses (from Figure 1).

D/OK Sequences	Amino acid positions									
	28 NESF	54 NVTK	133 NWSP	146 NWTQ	249 NKTA	346 NATE	390 NDTV	513 NDTN	562 NLMS	613 NGSA
D/bovine/Italy/16RS2359/2016 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Italy/4128547/2018 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Italy/18RS182-14/2017 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Italy/59542/2018 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Italy/50543-3/2018 (+1)	+	+++	---	+	++	+	-	+	-	+
D/swine/Italy/218655/2017 (+1)	+	+++	---	+	++	+	-	/	-	+
D/bovine/Italy/37760/2019 (+1)	+	/	---	NWTH +	++	+	-	+	-	+
D/swine/Italy/173287-4/2016 (+1)	+	+++	---	++	+++	+	-	+	-	+
D/swine/Italy/254578/2015 (+1)	+	+++	---	+	++	+	-	-	-	+

D/bovine/Italy/31525/2018 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Italy/18RS182-13/2017 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Italy/18RS182-8/2017 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Italy/16RS2581-9/2016 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/France/5920/2014 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Italy/46484/2015 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Denmark/5195604790-5/2019-02-20	+	/	---	+	++	+	-	+	-	+
D/bovine/Denmark/5224403563-5/2019-04-24	+	+++	---	+	++	+	-	+	-	+
D/bovine/Denmark/6171103755-1/2019-02-06	+	+++	---	+	++	+	-	+	-	+
D/bovine/Denmark/1173303525-4/2019-04-05	+	+++	---	+	++	+	-	+	-	+
D/bovine/Denmark/3313408142-6/2020-01-08	+	+++	---	+	++	+	-	+	-	+
D/bovine/Denmark/3058506689-11/2019-10-09	+	+++	---	+	++	+	-	+	-	+
D/bovine/Denmark/4559602762-5/2019-01-15	+	+++	---	+	++	+	-	+	-	+
D/bovine/Denmark/5195604714-5/2019-01-15	+	+++	---	+	++	+	-	+	-	+
D/bovine/Denmark/5161205135-5/2019-01-15	+	+++	---	+	++	+	-	+	-	+
D/bovine/Denmark/5022903340-5/2019-01-15	+	+++	---	+	++	+	-	+	-	+
D/bovine/Denmark/5358904609-3/2019-03-12	+	+++	---	+	++	+	-	+	-	+
D/bovine/Denmark/5246407608-2/2019-03-01	+	+++	---	+	++	+	-	/	-	+
D/bovine/Denmark/47398-3622/2015-11-03	+	+++	---	+	++	+	-	/	-	+
D/bovine/Italy/1/2014 (+1)	+	+++	---	+	++	+	-	+	-	+
D/swine/Italy/18VIR6833/2015 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Italy/18RS182-16/2018 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Italy/18RS182-10/2017 (+1)	+	+++	---	+	++	+	-	+	-	+
D/swine/Italy/354017/2015 (+1)	+	+++	---	+	++	/	-	+	-	+
D/bovine/Northern Ireland/24280/2017 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Italy/40574/2018 (+1)	+	+++	---	+	++	+	-	+	-	+
D/bovine/Mississippi/C00030P/2014 (+1)	+	+++	---	+	NKTT ++	+	-	+	-	+
D/bovine/Mississippi/C00020N/2014 (+1)	+	+++	---	+	NKTT ++	+	-	+	-	+
D/bovine/Mississippi/C00135N/2014 (+1)	+	+++	---	+	NKTT ++	+	-	+	-	+
D/bovine/Mississippi/C00162N/2015 (+1)	+	+++	---	+	NKTT ++	+	-	+	-	+
D/bovine/Mississippi/C00165P/2015 (+1)	+	+++	---	+	NKTT ++	+	-	+	NISM -	+
D/bovine/Kansas/1-35/2010 (+1)	+	+++	---	+	NKTT ++	+	-	+	NISM -	+
D/bovine/Minnesota/729/2013 (+1)	+	+++	---	+	NKTT ++	+	-	+	NISM -	+
D/bovine/Minnesota/628/2013 (+1)	+	+++	---	+	NKTT ++	+	-	+	NISM -	+
D/bovine/Guangdong/YC/2017 (+1)	+	+++	---	+	NKTT ++	/	-	+	NISM -	+
D/bovine/Guangdong/SK/2018 (+1)	+	+++	---	+	NKTT ++	+	-	+	NISM -	+

D/bovine/Shandong/Y217/2014 (+1)	+	+++	---	+	NKTT ++	+	-	+	NISM -	+
D/bovine/Shandong/Y125/2014 (+1)	+	+++	---	+	NKTT ++	+	-	+	NISM -	+
D/bovine/Mississippi/C00148N/2014 (+1)	+	/	---	+	++	+	-	+	NISM -	+
D/bovine/Texas/72/2017 (+1)	+	+++	---	+	++	+	-	+	NISM -	+

+ indicates prediction of an N-glycosylated site. - indicates a non-glycosylated site. / indicates no prediction of a glycosylated site.

Definition according to <https://services.healthtech.dtu.dk/service.php?NetNGlyc-1.0>:

+ Potential > 0.5, ++ Potential > 0.5 and Jury agreement (9/9) or Potential > 0.75, +++ Potential > 0.75 and Jury agreement, ++++ Potential > 0.90 and Jury agreement.

- Potential < 0.5, -- Potential < 0.5 and Jury agreement (all nine < 0.5), --- Potential < 0.32 and Jury agreement.