



November 10, 2010

A *Brucella abortus* biovar 1 isolate (NVSL case No. B11-0011) was obtained from tissues submitted by the Montana Department of Livestock Diagnostic Lab Division from a bison (animal ID 9-273/FC 0124) owned by the Flying D Ranch, Montana. Tissues were received at the NVSL on October 19, 2010 and the isolation reported out on November 1, 2010. Genotyping was performed using VNTR (variable number tandem repeat; also referred to as MLVA) analysis of 10 individual loci within the *Brucella* genome.

Results:

Only one other isolate in the NVSL database provided an exact match to the VNTR profile (2-4-4-2-3-3-4-3-4-12) of the *B. abortus* isolate recovered from the Flying D Ranch bison: that of an elk *B. abortus* biovar 1 isolate (NVSL case No. 10-0091, animal ID BR090731) cultured in December 2009 from a tissue specimen submitted by Neil Anderson.

Since differences in VNTR profile can be observed even among multiple isolates recovered from the same animal, the fact that these bison and elk profiles are identical, despite being recovered almost one year apart and from different species, is strong evidence that both animals were involved in the same transmission cycle.

Other 'nearest neighbor' isolates with VNTR profiles that differ slightly from that of the bison and elk include *B. abortus* obtained from Montana elk in 2009 – 1010 (Case Nos. B10-0514-S1, 09-0443-S1, 09-0295-S1 and 09-0354). These are depicted in Figure 1, which shows the clustering of isolates generated by a UPGMA (unweighted pair-group method with arithmetic mean) analysis of the VNTR profiles. [A total of 29 cattle, 35 elk, and 43 bison isolates from the NVSL database were incorporated into this analysis; the Flying D isolate is indicated in the Figure by the light blue highlighting]. The placement of elk-derived isolates in the clusters generated by the UPGMA analysis supports a hypothesis that the lineage of *B. abortus* recovered from the Flying D Ranch bison has been circulating in elk in Montana since 2009.

To confirm that the groupings generated by this statistical method are valid, a second method, termed MST (minimum spanning tree) analysis, also was utilized. The tree diagram generated by the MST analysis is presented in Figure 2 (below); this diagram incorporates the same isolates as were used in the UPGMA analysis. The spheres in the diagram represent different VNTR profiles and are color-coded according to the species of animal the isolate was recovered from. Note that larger spheres contain more than one isolate, with all isolates within that sphere sharing the same VNTR profile. Spheres with a bi-colored appearance indicate that the isolates share the same VNTR profile, but originate from different host animals. The pink box in the bottom portion of the diagram indicates the location of the sphere containing the Flying D Ranch *B. abortus* isolate.



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For ease in locating individual entries, full epidemiologic data for all isolates is categorized by species (cattle, bison, and elk) and provided in Tables 1 - 3 (below). The entries for the Flying D Ranch bison, and the Neil Anderson / Montana Fish Wildlife and Parks elk isolate from 2009, are highlighted in light blue in these tables.

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Table 1. List of cattle isolates from the NVSL BioNumerics database used in the clustering analysis

VNTR

Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	NVSL Acc.	Case No.	Owner	State	Animal ID	Epidemiology
2	3	4	2	3	3	5	3	4	10	179599	02-0235	Albertsons	ID	82VIU1921	2002 ID herd
2	3	10	2	3	3	5	3	4	10	179599	02-0236	Albertsons	ID	82ALK6225	2002 ID herd
2	3	10	2	3	3	5	3	4	9	179599	02-0237	Albertsons	ID	82AKN7632	2002 ID herd
2	3	9	2	3	4	7	1	4	9	179599	02-0239	Albertsons	ID	AKN7636	2002 ID herd
2	3	6	2	3	3	5	3	4	10	179599	02-0241	Albertsons	ID	82VME6918	2002 ID herd
2	6	3	2	3	3	3	3	4	4	406666	06-0034	Traughber, Chuck	ID	409	2005 - ID herd
2	6	3	2	3	3	3	3	4	4	434125	06-0423	Aborted fetus from Bruce.	ID	409 fetus	2005 - ID herd
2	4	3	2	3	3	3	3	4	5	10-011124	B10-0654	3 L Land & Cattle	ID	82APC7447 (W-133)	field isolate
2	3	3	2	3	3	3	3	4	6	09-033482	B10-0173	3-L Cattle (Heath Levis)	ID	82VTT9873	3718 milk
5	3	7	2	4	3	5	3	6	6	496198	07-0266	Barry Ellis (later TransO.	MT	81 TSK 4771	new herd - Montana 2
7	3	9	2	4	3	5	3	6	8	555145	08-0416	Art Burns	MT	1-1340 / #601 / 81TUG0218	new herd Montana - 2
2	4	6	2	3	3	5	3	5	5	324739	04-0418	Cimpl Packing Co.	SD	83VNY6729	Triple U isolate - SDS
2	6	4	2	4	3	5	3	5	12	354997	05-0444	7S Cattle Co.	TX	74EXP6760	2005 - TX herd # 1
5	3	7	2	3	3	3		5	6	375152	05-1065	Mendoza, Nedra	TX	fetus C	MX origin ?
4	5	4	2	4	3	7	3	5	6	392810	05-1285	Lawrence, Wayne	TX	74FAA0148 (milk)	2005 - TX herd # 2
7	4	2	2	4	3	2	3	5	10	451292	06-0861 A	B. abortus strain 19	TX	74EDJ9879	Lester Farms
7	4	4	2	4	3	2	3	7	4	586532	09-0269	B. abortus RB51	TX	Sample 1 / 74SQY3675	Blanche, Todd
9	3	5	2	3	3	3	1	5	9	20370	98-0559	Carlo Salvato	TX	74EAF0421	1998 - TX herd # 1
12	3	5	2	3	3	3	3	5	7	20370	98-0564	Carlo Salvato	TX	74EAF0453	1998 - TX herd # 1
2	4	5	1	4	3	3	3	5	4	22062	98-0614	Kyle Parrish	TX	74DRA8471	1998 - TX herd # 2
2	5	6	2	4	3	3	1	5	4	22062	98-0617	Kyle Parrish	TX	74DNY4218	1998 - TX herd # 2
8	3	5	2	3	3	3	3	5	10	31486	99-0978	None Listed	TX	None Listed	1999 - TX herd # 1
11	3	6	2	3	3	3	1	5	7	20370	98-0563	Carlo Salvato	TX	74EAF0470	1998 - TX herd # 1
8	3	5	2	3	3	3	1	5	9	31486	99-0983	None Listed	TX	None Listed	1999 - TX herd # 1
6	4	2	2	4	3	2	3	5	6	396213	05-1317	King Ranch Inc.	TX	NL	field isolate
6	7	2	2	4	3	2	3	5	6	405615	06-0025	Petri, Ernest	TX	NL	field isolate
5	4	2	2	4	3	2	3	5	7	436983	06-0513	W.H.R.G.	TX	NL	field isolate
2	7	3	2	4	3	3	1	5	7	348993	05-0338	R & B Porter Trust LTD	WY	83XX 4453	reactors
2	7	3	2	4	3	3	3	5	10	557690	08-0666	Zachary Roberts	WY	83VTH1249/ Y-113/ sample .	seropositive herd 200



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Table 2. List of bison isolates from the NVSL BioNumerics database used in the clustering analysis

VNTR

Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	NVSL Acc.	Case No.	Owner	State	Animal ID	Epidemiology
8	3	7	2	4	3	4	2	6	8	371106	05-0911	Yellowstone National Par.	MT	81APF6425	surveillance
6	6	8	2	4	3	8	3	7	5	378788	05-1132	USDA	MT	81APF6442	reactor
9	4	5	2	4	3	5	2	6	11	391747	05-1282	APHIS, VS	MT	81 APH 5970 (1 yo M)	Corwin Springs
7	3	3	2	4	3	5	3	5	5	426558	06-0279	Bison Quarantine Study	MT	14-06/bison	seropositive YNP bison calf
6	3	3	2	4	3	5	3	5	5	426555	06-0281	Bison Quarantine Study	MT	07-06/bison	seropositive YNP bison calf
6	3	3	2	4	3	5	3	5	5	436720	06-0429	Bison Quarantine Feasibil.	MT	sample 29-06	YNP B. abortus-exposed bi
6	3	3	2	4	3	5	3	5	5	453922	06-0887	USDA/APHIS/VS	MT	77-06 (1.5 yo F)	Corwin Springs, Belgrade
5	3	6	2	4	3	5	3	6	10	553656	08-0382	University of Montana	MT	#27 / Bison	Yellowstone National Park
7	3	6	2	4	3	6	3	6	11	553663	08-0386	University of Montana	MT	#33 / Bison	Yellowstone National Park
10	3	7	2	4	3	4	3	6	12	553670	08-0389	University of Montana	MT	#36 / Bison	Yellowstone National Park
6	3	8	2	4	3	5	3	7	13	553671	08-0390	University of Montana	MT	#37 / Bison	Yellowstone National Park
8	3	6	2	4	3	5	3	6	6	553674	08-0392	University of Montana	MT	#39 / Bison	Yellowstone National Park
7	3	4	2	4	3	6	3	6	12	553707	08-0404	YNP	MT	8-340 / P08-010	Yellowstone National Park
9	4	8	2	4	3	6	3	6	11	554884	08-0410	YNP	MT	8-407-08 / P08-071 / Bis.	Mammoth Hot Springs
5	3	6	2	4	3	5	3	6	9	563110	08-1019	USDA/APHIS/VS/BQFS	MT	20-08 / 81ARG3869	Bison quarantine study
7	3	4	2	4	3	6	3	6	12	563110	08-1080	USDA/APHIS/VS/BQFS	MT	91-08 / 81APM1605	Bison quarantine study
14	3	9	2	4	3	5	3	6	9	563912	08-1119	BQFS	MT	50-08 / 81AYE4071	Bison quarantine study
7	3	4	2	4	3	6	3	6	12	563912	08-1120	BQFS	MT	54-08 / 81AYE4054	Bison quarantine study
14	3	9	2	4	3	5	3	6	9	563912	08-1121	BQFS	MT	6 / 81AYE4053	Bison quarantine study
8	3	4	2	4	3	6	3	6	12	563912	08-1122	BQFS	MT	91-08 / 81APM1605	Bison quarantine study
9	3	4	2	4	3	5	3	5	4	563912	08-1123	BQFS	MT	38-08 / 81ARG3848	Bison quarantine study
8	3	9	2	4	3	6	3	7	13	567648	08-1256	Corwin Springs	MT	24-08	surveillance
10	3	4	2	4	3	6	3	5	4	571693	08-1354	Corwin Springs	MT	92-08 / 8-161	field isolate
12	6	7	2	4	3	6	3	6	5	602350	09-0553	BQFS- Bison Quarantine.	MT	85-08	Bison quarantine study
7	3	6	2	4	3	5	3	6	5	10-023707	B10-0973	Clarke, P. Ryan-bull biso.	MT	YNP95021	field study
2	4	4	2	3	3	4	3	4	12	10-053081	B11-0011	TEI- Flying D Ranch	MT	9-273/FC 0124	field isolate
8	2	3	2	4	3	4	3	4	6	372442	05-1002	Yellowstone National Park	WY	3203	surveillance
8	2	3	2	4	3	4	3	4	6	372442	05-1005	Yellowstone National Park	WY	3221	surveillance
9	3	9	2	4	3	5	3	6	9	557001	08-0638	Yellowstone National Park	WY	YNP-82	field isolate
10	3	4	2	4	3	6	3	5	4	560173	08-0863	Yellowstone National Park	WY	YNP-180	field isolate
11	3	6	2	4	3	5	3	6	6	560760	08-0869	Yellowstone National Park	WY	YNP-292	field isolate
5	3	6	2	4	3	5	3	6	12	560761	08-0876	Yellowstone National Park	WY	YNP-289	field isolate
8	3	6	2	4	3	5	3	6	13	561953	08-0921	Rhyan, Dr. Jack (Nat'l .	WY	32-08	Bison quarantine study
9	3	4	2	4	3	6	3	5	4	561953	08-0923	Rhyan, Dr. Jack (Nat'l .	WY	3877	Bison quarantine study
10	3	4	2	4	3	6	3	5	4	561953	08-0923	Rhyan, Dr. Jack (Nat'l .	WY	3877	Bison quarantine study
12	5	8	2	3	6	3	3	5	4	561953	08-0924	Rhyan, Dr. Jack (Nat'l .	WY	3892	Bison quarantine study
4	3	3	2	4	3	4	3	6	10	561953	08-0928	Rhyan, Dr. Jack (Nat'l .	WY	4058	Bison quarantine study
12	3	8	2	4	3	5	3	7	11	561953	08-0929	Rhyan, Dr. Jack (Nat'l .	WY	45-08	Bison quarantine study
7	3	4	2	4	3	6	3	6	12	561953	08-0933	Rhyan, Dr. Jack (Nat'l .	WY	81-08	Bison quarantine study
11	3	12	2	4	3	6	3	6	8	561953	08-0934	Rhyan, Dr. Jack (Nat'l .	WY	97-08	Bison quarantine study
12	3	7	2	4	3	5	3	6	12	562880	08-0966	Yellowstone National Park	WY	YNP- 398	field isolate
5	3	6	2	4	3	5	3	6	10	563918	08-1147	Yellowstone National Park	WY	YNP-206	field isolate
12	5	8	2	4	3	6	3	7	5	567486	08-1221	Yellowstone National Park	WY	YNP-347	surveillance



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Table 2. List of elk isolates from the NVSL BioNumerics database used in the clustering analysis

VNTR											NVSL	Case No.	Owner	State	Animal ID	Epidemiology
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	Acc.						
2	7	3	2	4	3	3	1	5	9		00-0669		ID	60237-89	Rainey Creek - Feb 2000	
2	3	13	2	3	3	5	3	4	9	163343	02-0123	Idaho Fish & Game Wildlife L.	ID	60217	Conant Creek	
2	3	7	2	3	3	5	3	4	9	163343	02-0124	Animal Health Labs	ID	60218	Conant Creek	
2	7	3	2	4	3	3	3	4	8	180373	02-0246	ID. Dept. of Fish & Game Wild.	ID	7217 fetus # 2 215	field isolate	
6	4	2	2	4	3	2	3	7	4	243781	03-0072	B. abortus strain 2308	ID	yellow 332 (N03-060)	Canyon, ID	
2	7	3	2	4	3	3	3	5	8	278724	04-0003	IDFG Wildlife Health Lab-Cal.	ID	ER03-177 / 2233-160	Rainey Creek - Jun 2002	
2	6	3	2	4	3	3	3	5	7	365387	05-0757	ID Dept of Fish & Game	ID	4985031105	Rainey Creek - Feb 2005	
2	4	4	2	3	3	5	3	4	9	370796	05-0908	Fish, Wildlife & Parks	MT	unavail	South Fork Indian Creek	
9	4	8	2	4	3	5	3	6	11	539969	08-0151	Montana Fish Wildlife & Parks	MT	#17 / Madden / 08/03/19.	Bozeman	
7	5	8	2	4	3	8	3	8	6	539969	08-0187	Montana Fish Wildlife & Parks	MT	#53 / Rawlins / 12/24/19.	Bozeman	
10	4	7	2	4	3	7	3	6	11	588409	09-0276	Anderson, Neil (MFWP)	MT	BR081145	field isolate	
9	3	7	2	4	3	4	3	6	6	588409	09-0286	Anderson, Neil (MFWP)	MT	BR081161	field isolate	
3	5	4	2	3	3	4	3	4	7	588409	09-0295	Anderson, Neil (MFWP)	MT	BR081174	field isolate	
2	9	3	2	3	3	3	3	5	4	588409	09-0298	Anderson, Neil (MFWP)	MT	BR081181	field isolate	
2	4	5	2	3	3	5	3	4	11	589401	09-0328	Anderson, Neil (MFWP)	MT	BR081200	field isolate	
3	5	4	2	3	3	5	3	5	7	590672	09-0354	Anderson, Neil	MT	BR081308	field isolate	
2	4	4	2	3	3	4	3	4	5	596921	09-0443	Neil Anderson	MT	BR080548	field isolate	
2	4	4	2	3	3	4	3	4	12	09-032078	B10-0091	MT FWP	MT	BR090731	field isolate	
6	3	7	2	4	3	6	3	6	10	09-032080	B10-0118	MT FWP	MT	BR091887	field isolate	
2	4	3	2	3	3	3	3	5	4	09-033518	B10-0203	MT FWP	MT	BR092077	field isolate	
2	4	4	2	3	3	7	3	4	9	09-033523	B10-0252	MT FWP	MT	BR092578	field isolate	
9	3	8	2	4	3	5	3	6	8	09-033525	B10-0273	MT FWP	MT	BR092770	field isolate	
2	4	3	2	3	3	3	3	5	4	09-033531	B10-0325	MT FWP	MT	BR092992	field isolate	
2	4	4	2	3	3	4	3	4	10	10-001635	B10-0514	MT FWP	MT	BR090381	field isolate	
2	5	4	2	3	3	5	3	4	13	10-001635	B10-0517	MT FWP	MT	BR090384	field isolate	
7	3	9	2	4	3	6	3	6	8	10-001635	B10-0529	MT FWP	MT	BR092820	field isolate	
2	4	4	2	3	3	7	3	4	14	10-008688	B10-0566	MT FWP	MT	BR090476	field isolate	
9	3	5	2	4	3	5	3	5	9	10-008689	B10-0584	MT FWP	MT	BR090822	field isolate	
2	8	3	2	3	3	3	3	5	4	339944	05-0014	Edwards, Hank	WY	00W2173	undetermined	
1	2	4	2	3	3	6	3	4	9	339944	05-0018	Edwards, Hank	WY	01W307	field isolate	
1	2	4	2	3	3	7	3	4	9	339944	05-0019	Edwards, Hank	WY	01W311	field isolate	
2	7	3	2	4	3	3	3	5	9	575556	09-0005	Wyoming Game and Fish Dep.	WY	GF042208	elk fetus from Franz fee.	
2	6	3	2	4	3	3	3	5	7	575556	09-0006	Wyoming Game and Fish Dept	WY	GF042908	elk fetus from Franz fee.	
2	5	3	2	5	3	4	3	6	4	575556	09-0007	Wyoming Game and Fish Dep.	WY	GF031908	from vaginal implant tran.	
2	4	3	2	5	3	5	3	5	4	575556	09-0008	Wyoming Game and Fish Dep.	WY	GF031408	from vaginal implant tran.	



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Figure 1. UPGMA clustering dendrogram of *B. abortus* isolates from the Flying D Ranch bison, and selected elk and cattle isolates from the NVSL database

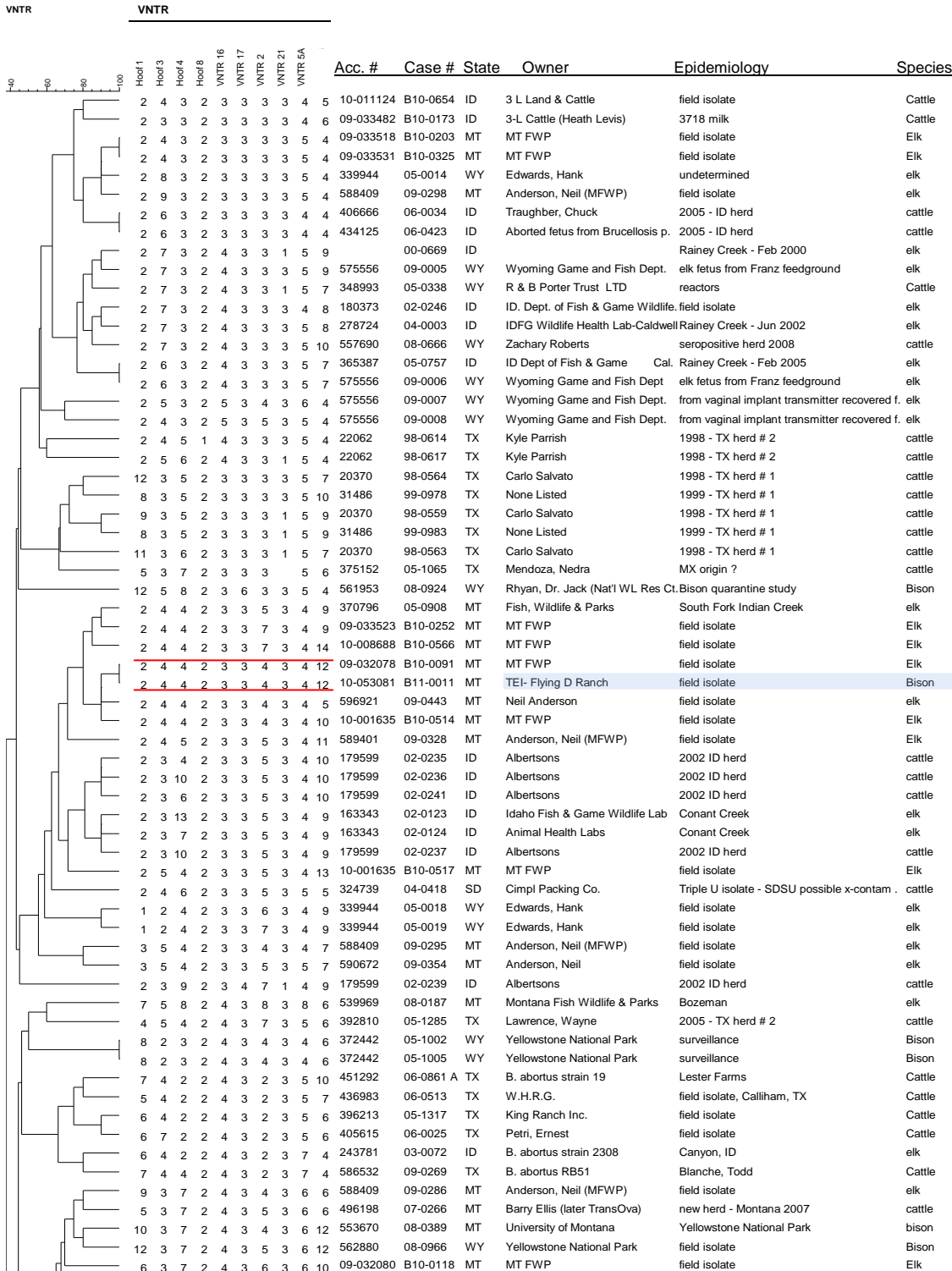




Figure 1. (continued)

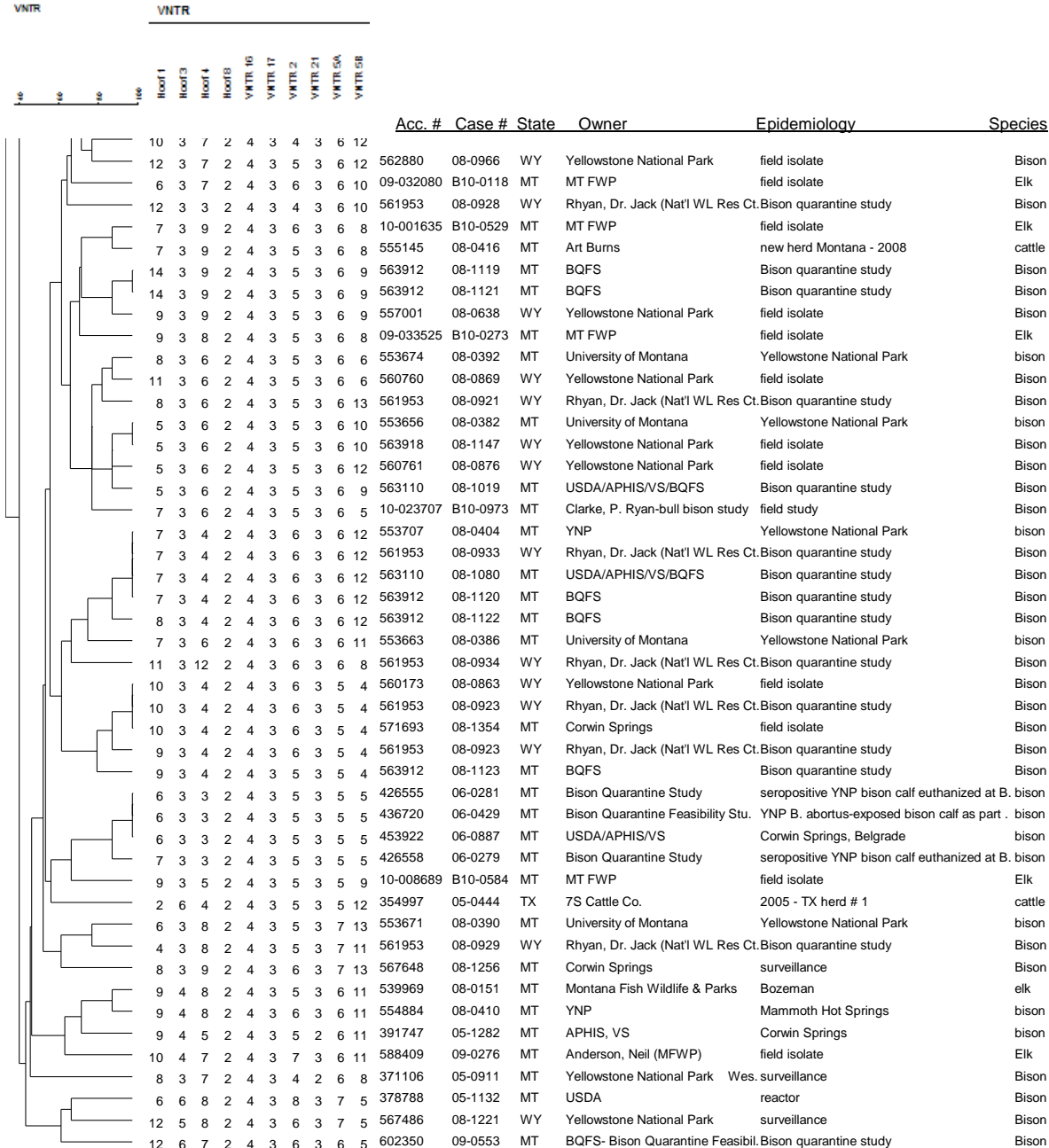




Figure 2. Minimum spanning tree (MST) clustering analysis for bison, elk, and cattle *B. abortus* isolates

