National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388   Fax (515) 337-7315

Brucella Genotyping Final Report - Confidential

November 22, 2011
(Revised / Corrected February 21, 2012)

This is a genotyping report for NVSL Accession No. 11-055107, an isolate of *Brucella abortus* biovar 1 recovered from a 3 year-old, male bison owned by Turner Enterprises / Snowcrest Ranch, Alder, MT. The animal (ID 982000096475197) was a quarantine herd reactor sampled on October 27, 2011, by Ryan Clarke. The submission was received at the NVSL on October 28, and genotyping performed on an isolate (Case No. B12-0028) during the week of November 13.

The genotyping analysis included 374 *B. abortus* strains of cattle, elk, and bison origin (as well as isolates associated with a llama, a reindeer, a pig, a human, and reference strains), in the NVSL BioNumerics database for which different VNTR (Variable Number Tandem Repeat) analysis (also referred to as MLVA) profiles were available. The VNTR analysis targeted 10 individual loci, or ‘hoofprints’, within the *B. abortus* genome.

Results:

The isolate (B12-0028) from the Turner Enterprises bison possessed a VNTR profile (2-5-4-2-3-1-7-2-3-10) not shared by any other isolate in the NVSL genotyping database.

A minimum spanning tree (MST) analysis of the VNTR profiles is depicted in Figure 1 (below). Note that this is the left-side portion of a larger MST (inset, Figure 1) comprised of all 375 isolates in the analysis. [Note that each smaller node, or circle, in the Figure represents an individual isolate with a distinct VNTR profile, while larger nodes represent multiple isolates, all with the same VNTR profile. Also note that the nodes are color-coded according to the species of host animal.]

The B12-0028 Turner Enterprises isolate (node C in Figure 1) segregated into a cluster occupied by *B. abortus* bv1 isolates recovered from Montana and Wyoming bison and elk in 2005 and 2010 (nodes A, B, D, E, and F in Figure 1). Serving as the ‘root’ isolate for the cluster occupied by the October 2011 Turner herd isolate, is a 2009 elk isolate (09-0443) from a Montana animal examined by Neil Anderson (node I in Figure 1).

There are two other field strain *B. abortus* isolates recovered from Turner / Flying D Ranch bison, present in the NVSL genotyping database. These are:

●Accession No. 10-053081, Case No. B11-0011, October 2010, adult cow[1]

●Accession No. 10-020278, Case No. B11-0473, April 2011, adult male reactor

---

[1] In the original genotyping report of November 22, 2011, this isolate was mistakenly attributed to a fetus aborted by this bison cow, rather than the cow herself

Isolate B11-0473 from the April, 2011 adult male reactor segregated into a cluster close to that of the October Turner Enterprises bison (isolate / node G in Figure 1).

Isolate B11-0011 from the October 2010 cow segregated at a further remove, but still within the 'left side' portion of the overall MST. This isolate shared the exact same VNTR profile as an isolate obtained in 2009 from a hunter-killed elk in Montana (animal ID BR090731) (node H in Figure 1).

Additional analysis of the genetic relationship of the October 2011 Turner Enterprises isolate with other *B. abortus* in the NVSL database was performed using an unweighted pair-group method with arithmetic mean (UPGMA) analysis. An excerpt from this large (i.e., 8-page) UPGMA dendrogram is provided in Figure 2 (below) and includes 'nearest neighbors', and other Turner / Flying D – associated field isolates.

The placement of 'nearest neighbors' to the October 2011 Turner / Snowcrest Ranch *B. abortus* is somewhat different from those selected in the MST analysis, reflecting the different algorithms used by these methods. As indicated by the UPGMA, the October 2011 Turner / Snowcrest herd isolate occupied a unique branch not shared with any other isolates. Nearest neighbors were 2010 Wyoming bison isolates (B11-0249 and B11-0256) provided to the NVSL in 2011 by Hank Edwards, and a 2010 elk isolate (B10-0517) recovered by the Montana FWP.

As with the MST analysis, the two previously characterized Turner Flying D Ranch bison field isolates segregated into clusters / branches distinct from those occupied by the Snowcrest / October 2011 isolate (Figure 2).

In summary, the October 2011 isolate (B12-0028) recovered from an adult, male, seropositive bison located at the Snowcrest Ranch possesses a unique VNTR profile; this profile clusters with other isolates associated with GYA elk and bison. However, two archived field isolates recovered from other Turner herd bison, located at the Flying D Ranch, display modest genetic similarity to B12-0028.

The absence of close genetic similarity between the October 2011 / Snowcrest isolate, and isolates previously recovered from Turner bison at the Flying D Ranch, suggests that the bison at the two ranches are infected with two independent lineages of *B. abortus*.

James Higgins
Mycobacteria and Brucella Section
USDA/APHIS/NVSL
1920 Dayton Ave
Ames, IA 50010
515-337-7034 (fax 7315)

Figure 1. Minimum Spanning Tree (MST) of *B. abortus* isolates from elk, bison, and cattle from the NVSL database. This is the left-side portion of a larger diagram (inset, lower right). The October 2011 Turner Enterprises / Snowcrest Ranch bison isolate (B12-0028), and nearest neighbors, are represented by the nodes labeled with uppercase letters.

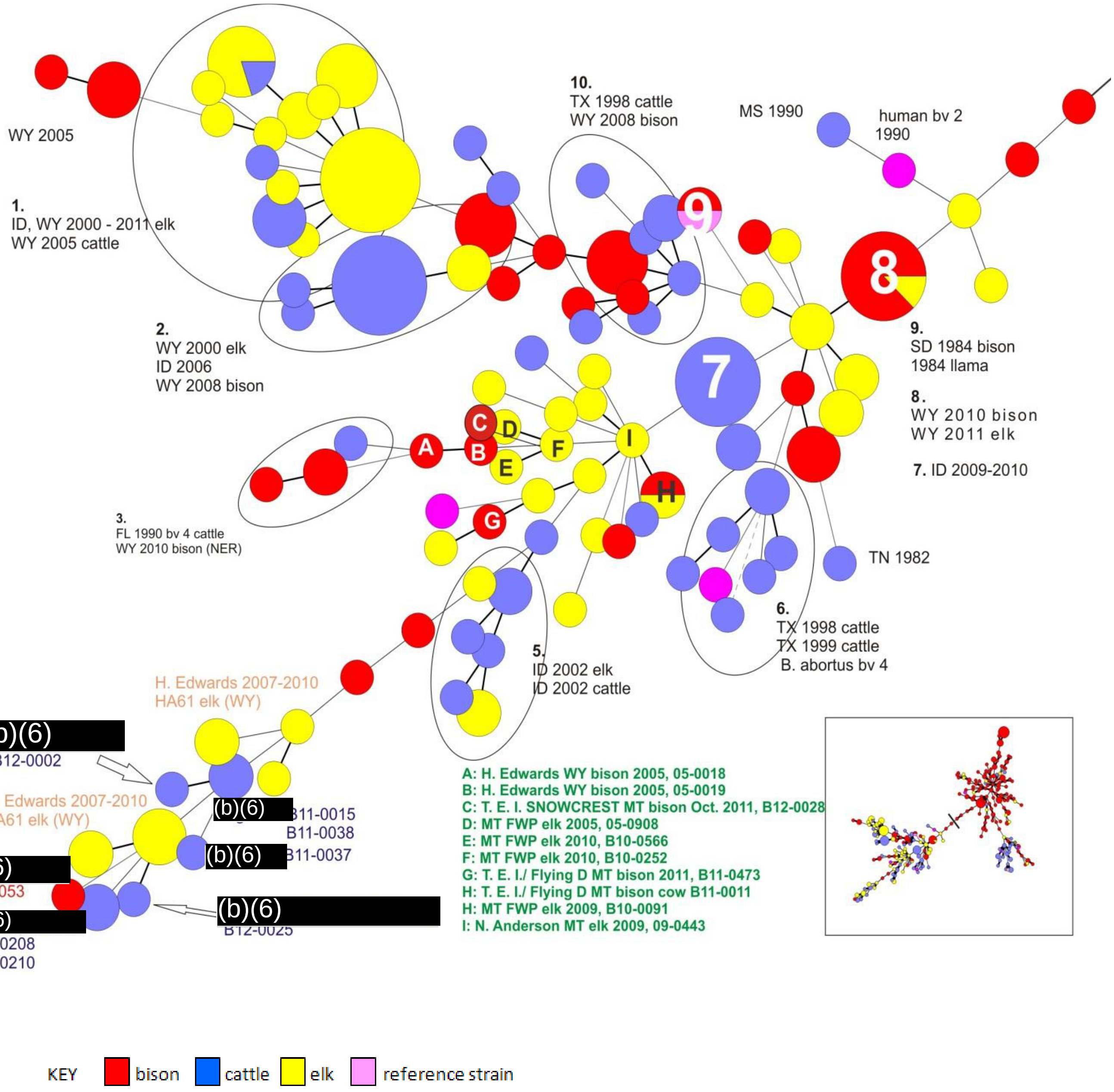
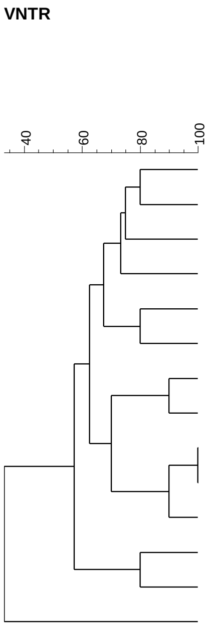


Figure 2. UPGMA clustering dendrogram of *B. abortus* isolates displaying 'nearest neighbor' status with the October 2011 Turner Enterprises / Snowcrest Ranch bison isolate (B12-0028, outlined in red box). (Note that this is an excerpt from a larger diagram). Included with 'nearest neighbors' are two other *B. abortus* field isolates recovered from Turner / Flying D Ranch bison in the Fall of 2010 and the Spring of 2011 (Cases B11-0011 and B11-0473). The host animal species is coded by color: yellow = elk, red = bison, pink = reference strain.



VNTR														
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B		Case No.	Owner	State	Epi info
2	5	4	2	3	1	5	2	3	12	🟨	B10-0517	MT FWP	MT	field isolate
0	5	4	2	3	1	5	2	3	7	🟥	B11-0249	Edwards, Hank / WY Ga.	WY	NER
0	5	4	2	3	1	3	2	3	5	🟥	B11-0256	Edwards, Hank / WY Ga.	WY	NER
2	5	4	2	3	1	7	2	3	10	🟥	B12-0028	Turner Enterprises	MT	quarantine herd reactor
3	5	4	2	3	1	4	2	3	6	🟨	09-0295	Anderson, Neil (MFWP)	MT	field isolate
3	5	4	2	3	1	5	2	4	6	🟨	09-0354	Anderson, Neil	MT	field isolate
1	2	4	2	3	1	6	2	3	8	🟥	05-0018	Edwards, Hank	WY	field isolate NER 2001
1	2	4	2	3	1	7	2	3	8	🟥	05-0019	Edwards, Hank	WY	field isolate NER 2001
0	2	4	2	3	1	8	2	3	6	🟥	B11-0141	WY Game and Fish Dept. .	WY	bison from NER (adult)
0	2	4	2	3	1	8	2	3	6	🟥	B11-0248	Edwards, Hank / WY Ga.	WY	NER
0	2	4	2	3	1	9	2	3	6	🟥	B11-0257	Edwards, Hank / WY Ga.	WY	NER
2	4	4	2	3	1	4	2	3	11	🟥	B11-0011	TEI- Flying D Ranch	MT	field isolate, Oct 2010
2	4	5	2	3	1	4	2	3	10	🟥	B11-0473	Turner-Flying D	MT	field isolate, male 2 yr
5	4	2	2	4	1	2	2	4	5	🟪	Strain 19	NVSL ctrl		

National Veterinary Services Laboratories
1800 Dayton Rd. , Ames, IA 50010
Phone (515) 663-7266   Fax (515) 663-7397

Brucella Genotyping Final Report - Confidential

January 27, 2010

Four *Brucella abortus* biovar 1 isolates were recovered and genotyped from two adult cows (NVSL Acc #'s 09-030933 and 09-030410), owner listed as (b)(6) from Rigby, ID. The cases were received at NVSL for culture and identification on 11/20 and 11/24, 2009, respectively. Genotyping was performed using VNTR [Variable Number Tandem Repeat] Analysis of 10 individual loci within the Brucella genome.

Results:

Genotyping results of these isolates indicate that the *B. abortus* strain recovered from the (b)(6) herd appears to be similar to strains recovered from a previous outbreak in a cattle herd from Idaho during 2002 (b)(6) owners), a single bison isolate from South Dakota (NVSL Acc # 337359, Owner, Dave Zeman) and an elk from Idaho 9NVSL Acc # 163343). Another cattle isolate (NVSL Acc # 324789; Owners Cimpl Packing Co, SD) appears to be similar as well. However, no epidemiological data is available for this isolate, making it difficult to interpret the relationship of this strain to the 2010 Idaho cattle strains.

Figure 1 below shows the VNTR data generated from all strains used for the comparison, and a phenogram generated via UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) to show general relatedness of the strains included in this comparison. To confirm that the groupings generated by this statistical modeling method are valid, a second method, termed Maximum Parsimony, was also utilized. Figure 2 below provides a visual interpretation of this data.

It is worth noting that strains recovered from different animals in the same herd tend to not produce identical VNTR genotypes (examples: NVSL Acc # 179599, (b)(6) herd, 2002, ID; NVSL Acc # 22062, (b)(6) herd, 1998, TX). This phenomenon is generally caused by the inherent instability of the microsatellite loci which generate the repeat units, and underscores the necessity of evaluating multiple strains from the same herd or outbreak whenever possible.

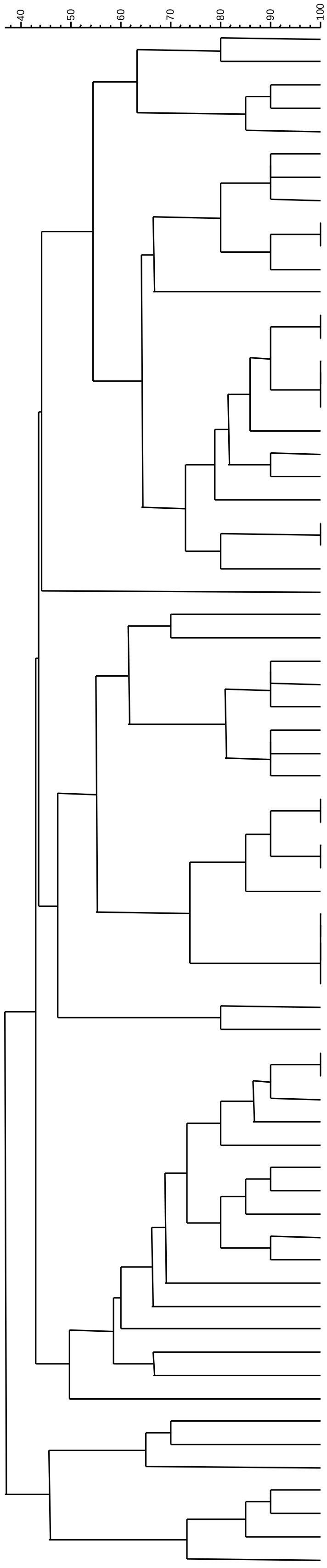
Full epidemiological information for all strains in Figure 1 is found in Table 1.

Beth Harris, M.S., Ph.D.
USDA/APHIS/NVSL
Head, Mycobacteria and Brucella Section
1920 Dayton Ave.
Ames, IA 50010

Table 1. List of isolates depicted in Figures 1 and 2.

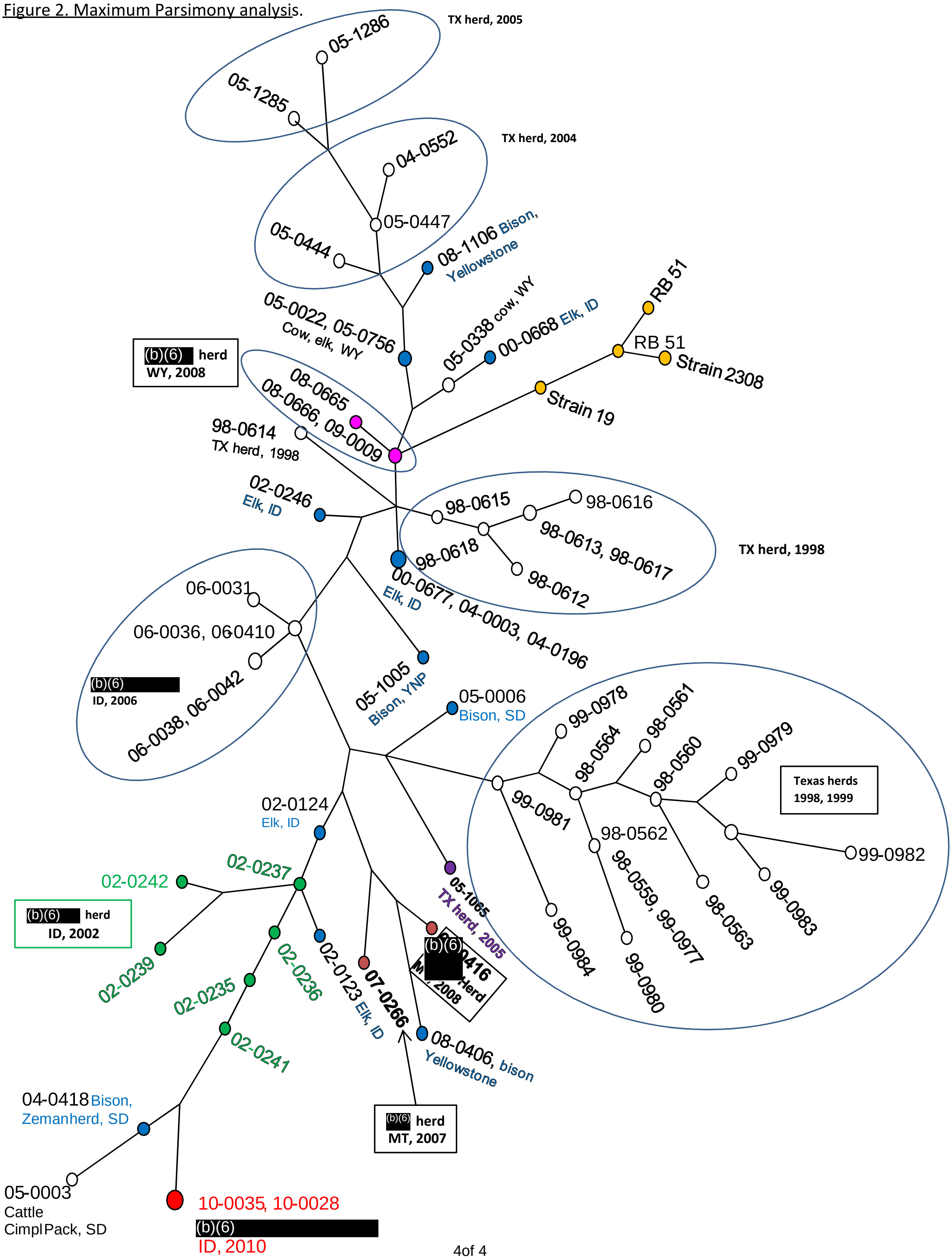
NVSL Acc #	NVSL ID	Animal Species	State	Animal ID	Owner
20370	98-0559	Cattle	TX	74EAF0421	(b)(6)
20370	98-0560	Cattle	TX	74EAF0492	(b)(6)
20370	98-0561	Cattle	TX	74EAF0410	(b)(6)
20370	98-0562	Cattle	TX	74EAF0526	(b)(6)
20370	98-0563	Cattle	TX	74EAF0470	(b)(6)
20370	98-0564	Cattle	TX	74EAF0453	(b)(6)
22062	98-0612	Cattle	TX	74DSF4190	(b)(6)
22062	98-0613	Cattle	TX	74DNA2041	(b)(6)
22062	98-0614	Cattle	TX	74DRA8471	(b)(6)
22062	98-0615	Cattle	TX	74DUV9998	(b)(6)
22062	98-0616	Cattle	TX	74DUV9999	(b)(6)
22062	98-0617	Cattle	TX	74DNY4218	(b)(6)
22062	98-0618	Cattle	TX	74DUV1204	(b)(6)
31486	99-0977	Cattle	TX	None listed	None listed
31486	99-0978	Cattle	TX	None listed	None listed
31486	99-0979	Cattle	TX	None listed	None listed
31486	99-0980	Cattle	TX	None listed	None listed
31486	99-0981	Cattle	TX	None listed	None listed
31486	99-0982	Cattle	TX	None listed	None listed
31486	99-0983	Cattle	TX	None listed	None listed
30291	99-0984	Cattle	TX	None listed	(b)(6)
51837	00-0677	Elk	ID	60237	None listed
51837	00-0668	Elk	ID	60237	None listed
163343	02-0123	elk	ID	60218/327	ID Dept Fish & Game
163343	02-0124	Elk	ID	60218/327	ID Dept Fish & Game
179599	02-0235	cattle	ID	82VIU1921	(b)(6)
179599	02-0236	cattle	ID	82ALK6225	(b)(6)
179599	02-0237	cattle	ID	82AKN7632	(b)(6)
179599	02-0239	Cattle	ID	None listed	(b)(6)
179599	02-0241	cattle	ID	82VME6918	(b)(6)
179599	02-0242	cattle	ID	82ALK223	(b)(6)
180373	02-0246	elk	ID	7217 fetus # 2 215	ID Dept Fish & Game
243781	03-0072	elk	ID	yellow 332 (N03-060)	B. abortus Strain 2308
278724	04-0003	elk	ID	None listed	None listed
300438	04-0196	elk	ID	ERO3-177	ID Dept Fish & Game
324789	04-0418	cattle	SD	None listed	None listed
337359	05-0003	Bison	SD	David Zeman	None listed
337359	05-0006	Bison	SD	David Zeman	None listed
342040	05-0022	Cattle	WY	(b)(6)	None listed
348993	05-0338	Cattle	WY	(b)(6)	None listed
327720	04-0552	cattle	TX	74EME0146	None listed
354997	05-0444	cattle	TX	74EXP6760	(b)(6)
355805	05-0447	Cattle	TX	74EXP6761	(b)(6)
365387	05-0756	elk	ID	4985031104	ID Dept Fish & Game
372442	05-1005	Bison	WY	None listed	Yellowstone Nat'l Park
375152	05-1065	Cattle	TX	None listed	(b)(6)
392810	05-1285	cattle	TX	74FAA0148 (milk)	(b)(6)
392810	05-1286	cattle	TX	74FAA0148 (tissue)	(b)(6)
408269	06-0031	cattle	ID	432	(b)(6)
408269	06-0036	cattle	ID	437	(b)(6)
408269	06-0038	cattle	ID	403	(b)(6)
408270	06-0042	cattle	ID	409	(b)(6)
433600	06-0410	cattle	ID	421 (milk)	(b)(6)
451292	06-0861	Cattle	TX	74EDJ9879	B. abortus Strain 19
496198	07-0266	cattle	MT	81 TSK 4771	(b)(6)
553707	08-0406	bison	MT	8-346 / P08-011 / Bison	Yellowstone Nat'l Park
555145	08-0416	cattle	MT	1-1340 / #601 / 81TUG0218	(b)(6)
557690	08-0665	Cattle	WY	83VUL1990 / BL-313	(b)(6)
557690	08-0666	Cattle	WY	83VTH1249/ Y-113	(b)(6)
563907	08-1106	Bison	MT	YNP-365	Yellowstone Nat'l Park
575556	09-0009	Cattle	WY	08B6838	(b)(6)
585093	09-0268	Cattle	TX	1017	B. abortus RB 51
586532	09-0269	Cattle	TX	74SQY3675	B. abortus RB 51
09-030933	10-0035	Cattle	ID	82VWC2669	(b)(6))
09-030410	10-0028	Cattle	ID	82ANM7926, W-204	(b)(6)

Figure 1. MLVA locus data and UPGMA phylogram of strains included in this analysis.



Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B					
4	5	4	2	4	3	7	3	5	6	392810	05-1285	cattle	(b)(6) (b)(6)	TX
3	5	4	2	4	3	9	3	5	6	392810	05-1286	cattle	[redacted]	TX
2	6	5	2	4	3	5	3	5	6	327720	04-0552	cattle	None Listed	TX
2	6	4	2	4	3	5	3	5	6	355805	05-0447	cattle	(b)(6) .	TX
2	6	4	2	4	3	5	3	5	12	354997	05-0444	cattle	(b)(6) .	TX
2	3	6	2	4	3	3	3	5	4	22062	98-0612	cattle	(b)(6)	TX
2	7	6	2	4	3	3	3	5	4	22062	98-0615	cattle	(b)(6)	TX
2	5	6	2	4	3	3	3	5	4	22062	98-0618	cattle	(b)(6) (b)(6)	TX
2	5	6	2	4	3	3	1	5	4	22062	98-0613	cattle	[redacted]	TX
2	5	6	2	4	3	3	1	5	4	22062	98-0617	cattle	(b)(6) h	TX
2	5	5	2	4	3	3	1	5	4	22062	98-0616	cattle	(b)(b)(6)	TX
2	4	5	1	4	3	3	3	5	4	22062	98-0614	cattle	(b)(6) (b)(6)	TX
2	7	3	2	4	3	3	3	5	10	557690	08-0666	cattle	[redacted]	WY
2	7	3	2	4	3	3	3	5	10	575556	09-0009	Cattle	(b)(6)	WY
2	7	3	2	4	3	3	3	5	8	51837	00-0677	elk	None Listed	ID
2	7	3	2	4	3	3	3	5	8	278724	04-0003	elk	IDFG Wildlife Health Lab-Caldwell	ID
2	7	3	2	4	3	3	3	5	8	300438	04-0196	elk	Idaho Dept. of Fish & Game	ID
2	7	3	2	4	3	3	3	4	8	180373	02-0246	elk	ID. Dept. of Fish & Game Wildlife Lab	ID
2	7	3	2	4	3	3	1	5	8	51837	00-0668	elk	None Listed	ID
2	7	3	2	4	3	3	1	5	7	348993	05-0338	Cattle	(b)(6) (b)(6)	WY
1	7	3	2	4	3	3	3	5	10	557690	08-0665	Cattle	[redacted]	WY
2	6	3	2	4	3	3	3	5	7	342040	05-0022	cattle	J(b)(6)	WY
2	6	3	2	4	3	3	3	5	7	365387	05-0756	elk	ID Dept of Fish & Game, Caldwell	ID
2	6	4	2	4	3	3	3	5	4	563907	08-1106	Bison	Yellowstone National Park	MT
8	2	3	2	4	3	4	3	4	6	372442	05-1005	Bison	Yellowstone National Park	WY
2	3	9	2	3	4	7	1	4	9	544000	02-0239	cattle	(b)(6)	ID
2	3	10	2	3	4	7	3	4	10	179599	02-0242	cattle	[redacted]	ID
2	3	4	2	3	3	5	3	4	10	179599	02-0235	cattle	[redacted]	ID
2	3	10	2	3	3	5	3	4	10	179599	02-0236	cattle	[redacted]	ID
2	3	6	2	3	3	5	3	4	10	179599	02-0241	cattle	[redacted]	ID
2	3	13	2	3	3	5	3	4	9	163343	02-0123	elk	Idaho Fish & Game Wildlife Lab	ID
2	3	7	2	3	3	5	3	4	9	163343	02-0124	elk	Idaho Fish & Game Wildlife Lab	ID
2	3	10	2	3	3	5	3	4	9	179599	02-0237	cattle	(b)(6) , (b)(6)	ID
2	6	2	2	3	3	3	3	4	4	408269	06-0038	cattle	(b)(6)	ID
2	6	2	2	3	3	3	3	4	4	408270	06-0042	cattle	[redacted]	ID
2	6	3	2	3	3	3	3	4	4	408269	06-0036	cattle	[redacted]	ID
2	6	3	2	3	3	3	3	4	4	433600	06-0410	cattle	[redacted]	ID
3	6	3	2	3	3	3	3	4	4	406666	06-0031	cattle	[redacted]	ID
2	4	3	2	3	3	3	3	4	5	09-030933	10-0035	Catlle	[redacted]	ID
2	4	3	2	3	3	3	3	4	5	09-030933	10-0035	Catlle	[redacted]	ID
2	4	3	2	3	3	3	3	4	5	09-030410	10-0028	Catlle	(b)(6)	ID
2	4	3	2	3	3	3	3	4	5	09-030410	10-0028	Catlle	[redacted]	ID
2	4	6	2	3	3	5	3	5	5	324789	04-0418	cattle	Cimpl Packing Co., Yankton	SD
1	4	6	2	3	3	5	1	5	5	337359	05-0003	Bison	Zeman, David	SD
9	3	5	2	3	3	3	1	5	9	20370	98-0559	cattle	(b)(6) (b	TX
9	3	5	2	3	3	3	1	5	9	31486	99-0977	cattle	None Listed	TX
9	3	5	2	3	3	3	1	5	10	31486	99-0979	cattle	None Listed	TX
8	3	5	2	3	3	3	1	5	9	31486	99-0983	cattle	None Listed	TX
13	3	5	2	3	3	3	1	5	7	20370	98-0560	cattle	(b)(6)	TX
14	3	5	2	3	3	3	3	5	7	20370	98-0561	cattle	[redacted]	TX
12	3	5	2	3	3	3	3	5	7	20370	98-0564	cattle	(b)(6)	TX
12	3	5	2	3	3	3	3	5	9	20370	98-0562	cattle	[redacted]	TX
8	3	5	2	3	3	3	3	5	10	31486	99-0978	cattle	None Listed	TX
8	3	5	2	3	3	3	3	5	8	31486	99-0981	cattle	None Listed	TX
4	3	5	3	3	3	3	3	5	9	31486	99-0980	cattle	None Listed	TX
11	3	6	2	3	3	3	1	5	7	20370	98-0563	cattle	(b)(6)	TX
9	5	5	2	1	3	3	3	5	9	31486	99-0982	cattle	None Listed	TX
5	3	7	2	3	3	3		5	6	375152	05-1065	cattle	(b)(6)	TX
1	3	7	2	3	3	8	3	5	5	337359	05-0006	Bison	Zeman, David	SD
8	5	5	2	3	3	3	2	6	8	30291	99-0984	cattle	(b) (6)	TX
5	3	7	2	4	3	5	3	6	6	496198	07-0266	cattle	TransOva Genetics (b) (6)	MT
7	3	9	2	4	3	5	3	6	8	555145	08-0416	cattle	(b)(6)	MT
7	3	4	2	4	3	6	3	6	12	553707	08-0406	bison	Yellowstone National Park	MT
6	4	2	2	4	3	2	3	7	4	243781	03-0072	elk	B. abortus strain 2308	ID
7	4	2	2	4	3	2	3	7	4	585093	09-0268	Cattle	B. abortus RB51	TX
7	4	4	2	4	3	2	3	7	4	586532	09-0269	Cattle	B. abortus RB51	TX
7	4	2	2	4	3	2	3	5	10	451292	06-0861 A	Cattle	B. abortus strain 19	TX

Figure 2. Maximum Parsimony analysis.



APHIS

National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388   Fax (515) 337-7315

Brucella Genotyping Final Report - Confidential

This genotyping analysis was conducted on *Brucella abortus* isolates recovered from cattle at the (b)(6) (b)(6). owned by the (b)(6) in Rigby, ID. Genotyping was performed using VNTR (Variable Number Tandem Repeat) analysis (also referred to as MLVA). This updated analysis includes 10 isolates not present in the first genotyping report issued on January 27, 2010.

Results:

The majority of the newer isolates, from animal No. 82VTT9873 (NVSL Acc. No. 09-034536, December 2009), No. 82APC7447 and its fetus (Acc. No. 10-011124, March 2010), and No. 82VUF4578 (Acc. No. 10-020661, May 2010), all displayed the same VNTR profile as that of the previous isolates from animals No. 82ANM7926 (Acc. No. 09-029070, 09-030410) and No. 82VWC2669 (Acc. No. 09-030933, 09-032249).

However, two of the three *B. abortus* isolates cultured from animal No. 82VTT9873 (Acc. No. 09-033482, 09-034681, December 2009) displayed a VNTR profile that differed from the other (b)(6) (b)(6) isolates by one repeat each at two loci, ‘hoof 3’ and ‘VNTR5B’. Despite these altered VNTR profiles, all (b)(6) isolates displayed greater genetic similarity to each other, than to other isolates in the database.

The updated genotyping analysis confirms observations made in the January 2010 report, that the (b)(6) (b)(6) *B. abortus* strains are similar to those recovered in 2002 from cattle owned by the (b)(6), and to strains recovered in 2006 from the (b)(6) herd. Other isolates displaying relatedness are *B. abortus* recovered from an elk from Idaho (Acc. No. 163343), and an elk from Montana (Acc. No. 09-033518).

Full epidemiological information for all strains in included in the genotyping analysis is found in Table 1 (below). [To aid in identifying isolates in the Figures, note the use of a color code to indicate State of origin]. The (b)(6) isolates are indicated by the red outline; the older isolates used in the January 2010 genotyping analysis are demarcated by the light blue outline.

Figure 1 (below) shows the VNTR (‘MLVA 21’) profiles generated from all strains used for the comparison, and a phenogram (the far left side of the Figure) generated via UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) to show general relatedness of the strains included in the comparison. The (b)(6) isolates are indicated by the red outline; the older isolates used in the January 2010 genotyping analysis are indicated by the light blue outline. Different VNTR profiles among the (b)(6) isolates are demarcated by the horizontal red lines.

To confirm that the groupings generated by this statistical modeling method are valid, a second method, termed Maximum Parsimony, was also utilized. Figure 2 provides a visual interpretation of this analysis (note that larger orbs represent multiple isolates with the same VNTR profile, and bi-colored orbs represent different isolates with the same profile). Since the Maximum Parsimony method uses a slightly different algorithm to calculate relatedness among VNTR profiles, direct comparisons between it and the UPGMA method should be performed with caution. However, the Maximum Parsimony analysis indicates that *B. abortus* strains obtained from the (b)(6) cattle, the Idaho elk, a bison from Wyoming / Yellowstone National Park, and a Montana elk, constitute the ‘nearest neighbors’ to the (b)(6) (b)(6) isolates.

James Higgins, MS, PhD
Mycobacteria and Brucella Section
USDA/APHIS/NVSL
1920 Dayton Ave
Ames, IA 50010
515-337-7034 (fax 7315)

National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388 Fax (515) 337-7315

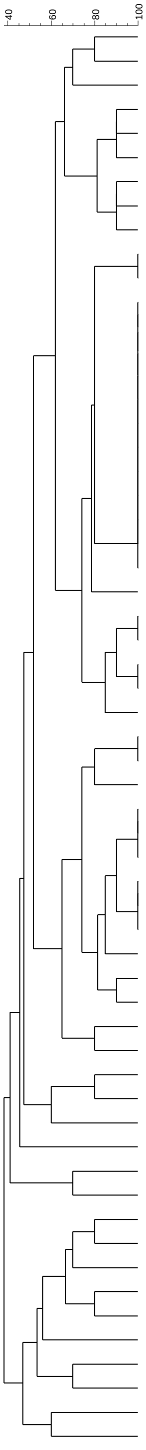
Brucella Genotyping Final Report - Confidential

Table 1. List of isolates depicted in Figures 1 and 2

Color Code (State)	NVSL Acc. #	Case #	Species	State	Animal ID	Owner	Epidemiology
🟩	179599	02-0235	cattle	ID	82VIU1921	(b)(6) [redacted]	2002 ID herd
🟩	179599	02-0236	cattle	ID	82ALK6225	[redacted]	2002 ID herd
🟩	179599	02-0241	cattle	ID	82VME6918	[redacted]	2002 ID herd
🟩	163343	02-0123	elk	ID	60217	Idaho Fish & Game Wildlife Lab	Conant Creek
🟩	163343	02-0124	elk	ID	60218	Animal Health Labs	Conant Creek
🟩	179599	02-0237	cattle	ID	82AKN7632	(b)(6) [redacted]	2002 ID herd
🟩	09-033482	B10-0173	Cattle	ID	82VTT9873	[redacted]	3718 milk
🟩	09-034681	B10-0474	Cattle	ID	82VTT9873 LH	[redacted]	3718 #1 culture
🟩	09-030410	B10-0028	Cattle	ID	82ANM7926, W-204 (LRQ.	[redacted]	culture
🟩	09-030410	B10-0028	Cattle	ID	82ANM7926, W-204 (LRQ.	[redacted]	culture
🟩	09-030933	B10-0035	Cattle	ID	82VWC2669	[redacted]	milk
🟩	09-030933	B10-0035	Cattle	ID	82VWC2669	[redacted]	milk
🟩	09-034536	B10-0407	Cattle	ID	82VTT9873	(b)(6) [redacted]	tissue
🟩	10-011124	B10-0654	Cattle	ID	82APC7447	[redacted]	milk
🟩	10-011124	B10-0655	Cattle	ID	fetus of 82APC7447	[redacted]	tissue 1
🟩	10-011124	B10-0655	Cattle	ID	fetus of 82APC7447	[redacted]	tissue 2
🟩	10-011124	B10-0655	Cattle	ID	fetus of 82APC7447	[redacted]	tissue 3
🟩	10-020661	B10-0948	Cattle	ID	82VUF4578	[redacted]	from Lewis (ID) herd
🟩	09-029070	B10-0018	Cattle	ID	82ANM7926	[redacted]	milk
🟩	09-032249	B10-0141	Cattle	ID	82VWC2669	[redacted])	culture3540 #1RF&#2RR
🟩	408269	06-0038	cattle	ID	403	(b)(6) [redacted]	2005 - ID herd
🟩	408270	06-0042	cattle	ID	409	[redacted]	2005 - ID herd
🟩	408269	06-0036	cattle	ID	437	[redacted]	2005 - ID herd
🟩	433600	06-0410	cattle	ID	421 (milk)	[redacted]	2005 - ID herd
🟩	406666	06-0031	cattle	ID	432	[redacted]	2005 - ID herd
🟩	365387	05-0756	elk	ID	4985031104	ID Dept of Fish & Game, Caldwell	Rainey Creek - Feb 2005
🟩	51837	00-0677	elk	ID	60237	None Listed	Rainey Creek - Feb 2000
🟩	278724	04-0003	elk	ID	ER03-177 / 2233-160	IDFG Wildlife Health Lab-Caldwell	Rainey Creek - Jun 2002
🟩	300438	04-0196	elk	ID	ERO3-177	Idaho Dept. of Fish & Game	Rainey Creek - Jun 2002
🟩	180373	02-0246	elk	ID	7217 fetus # 2 215	ID. Dept. of Fish & Game Wildlife Lab	
🟩	51837	00-0668	elk	ID	60237	None Listed	Rainey Creek - Feb 2000
🟩	179599	02-0239	cattle	ID	AKN7636	(b)(6) [redacted]	2002 ID herd
🟩	179599	02-0242	cattle	ID	82ALK223	[redacted]	2002 ID herd
🟩	243781	03-0072	elk	ID	yellow 332 (N03-060)	B. abortus strain 2308	Canyon, ID
🟥	10-001635	B10-0514	Elk	MT	BR090381	MT FWP	
🟥	10-008688	B10-0566	Elk	MT	BR090476	MT FWP	
🟥	589401	09-0328	Elk	MT	BR081200	Anderson, Neil (MFWP)	
🟥	09-033518	B10-0203	Elk	MT	BR092077	MT FWP	
🟥	563907	08-1106	Bison	MT	YNP-365	Yellowstone National Park	John Treanor - Submitter
🟥	496198	07-0266	cattle	MT	81 TSK 4771	(b) (6) [redacted] (later TransOva)	new herd - Montana 2007
🟥	555145	08-0416	cattle	MT	1-1340 / #601 / 81TUG0218	(b)(6) [redacted]	new herd Montana - 2008
🟥	553707	08-0406	bison	MT	8-346 / P08-011 / Bison	Yellowstone National Park	Mammoth Hot Springs
🟥	539969	08-0151	elk	MT	#17 / Madden / 08/03/1982.	Montana Fish Wildlife & Parks	Bozeman
🟥	563907	08-1107	Bison	MT	YNP-363	Yellowstone National Park	John Treanor - Submitter
🟦	324739	04-0418	cattle	SD	83VNY6729	Cimpl Packing Co., Yankton	(b)(6) [redacted] isolate - SDSU possible x-contam c.
🟦	337359	05-0003	Bison	SD	None Listed	Zeman, David	
🟦	337359	05-0006	Bison	SD	None Listed	Zeman, David	
🟨	22062	98-0612	cattle	TX	74DSF4190	(b)(6) [redacted]	1998 - TX herd # 2
🟨	22062	98-0617	cattle	TX	74DNY4218	(b)(6) [redacted]	1998 - TX herd # 2
🟪	342040	05-0022	cattle	WY	None Listed	(b)(6) [redacted]	
🟪	557690	08-0665	Cattle	WY	83VUL1990 / BL-313 / Sa.	[redacted]	
🟪	557690	08-0666	cattle	WY	83VTH1249/ Y-113/ sampl.	[redacted]	positive herd 2008
🟪	575556	09-0009	Cattle	WY	08B6838	[redacted]	see NVSL Acc# 557690
🟪	348993	05-0338	Cattle	WY	None Listed	(b)(6) [redacted]	
🟪	372442	05-1005	Bison	WY	None Listed	Yellowstone National Park	
🟪	560761	08-0876	Bison	WY	YNP-289	Yellowstone National Park	
🟪	562880	08-0970	Bison	WY	YNP- 401	Yellowstone National Park	
🟪	562879	08-0962	Bison	WY	YNP- 300	Yellowstone National Park	
🟪	567494	08-1247	Bison	WY	YNP-254	Yellowstone National Park	

Figure 1. VNTR (MLVA) locus data and UPGMA phenogram of strains included in this analysis

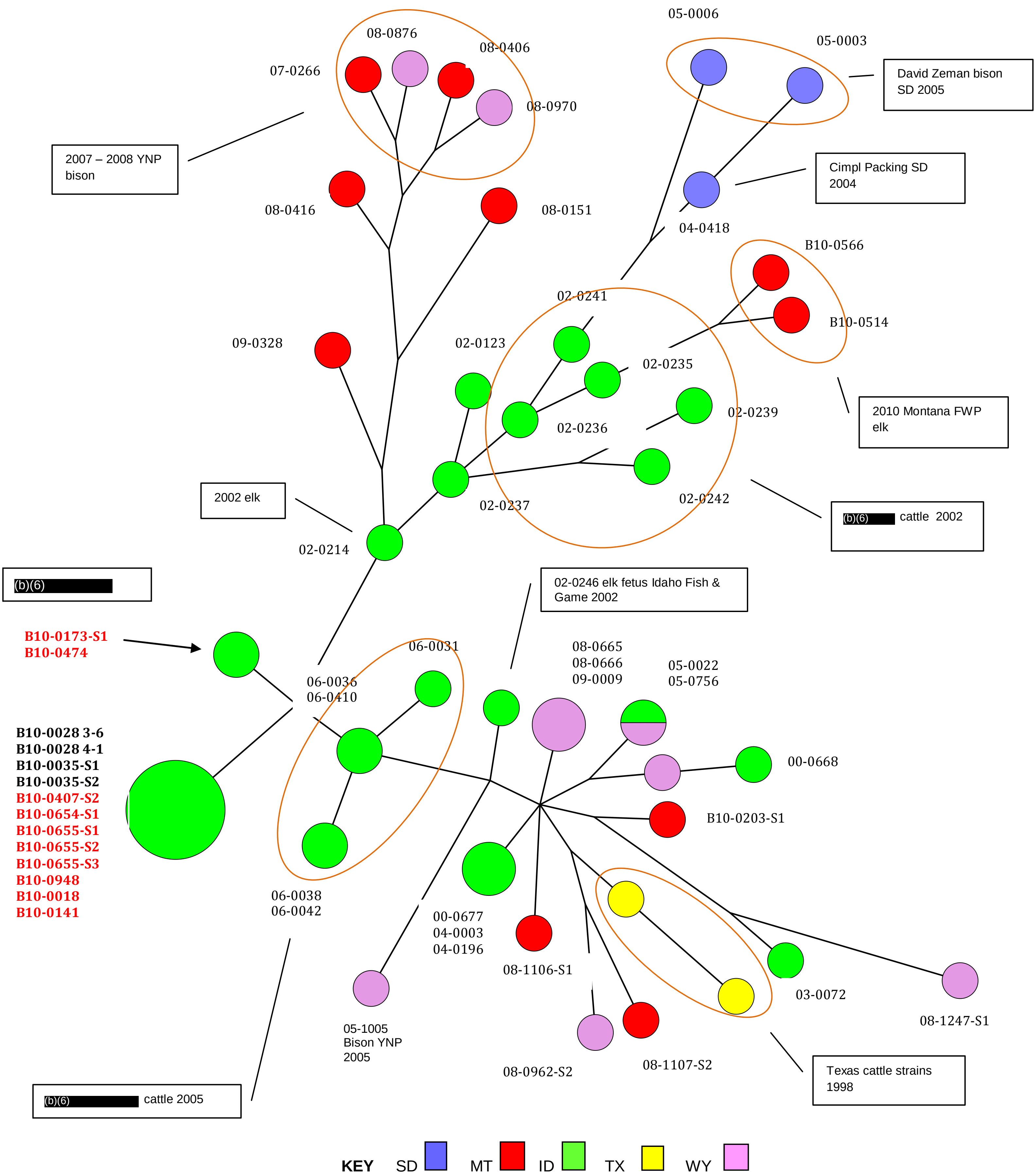
MLVA 21



MLVA 21

Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B		NVSL Acc. #	Case #	Species	Animal ID	Owner	Epidemiology
2	4	4	2	3	3	4	3	4	10	🟥	10-001635	B10-0514	Elk	BR090381	MT FWP	
2	4	4	2	3	3	7	3	4	14	🟥	10-008688	B10-0566	Elk	BR090476	MT FWP	
2	4	5	2	3	3	5	3	4	11	🟥	589401	09-0328	Elk	BR081200	Anderson, Neil (MFWP)	
2	3	4	2	3	3	5	3	4	10	🟩	179599	02-0235	cattle	82VIU1921	(b)(6)	2002 ID herd
2	3	10	2	3	3	5	3	4	10	🟩	179599	02-0236	cattle	82ALK6225	(b)(6)	2002 ID herd
2	3	6	2	3	3	5	3	4	10	🟩	179599	02-0241	cattle	82VME6918	(b)(6)	2002 ID herd
2	3	13	2	3	3	5	3	4	9	🟩	163343	02-0123	elk	60217	Idaho Fish & Game Wildlife Lab	Conant Creek
2	3	7	2	3	3	5	3	4	9	🟩	163343	02-0124	elk	60218	Animal Health Labs	Conant Creek
2	3	10	2	3	3	5	3	4	9	🟩	179599	02-0237	cattle	82AKN7632	(b)(6)	2002 ID herd
2	3	3	2	3	3	3	3	4	6	🟩	09-033482	B10-0173	Cattle	82VTT9873	(b)(6)	3718 milk
2	3	3	2	3	3	3	3	4	6	🟩	09-034681	B10-0474	Cattle	82VTT9873 LH	[redacted]	3718 #1 culture
2	4	3	2	3	3	3	3	4	5	🟩	09-030410	B10-0028	Cattle	82ANM7926, W-204 (L.	(b)(6)	culture
2	4	3	2	3	3	3	3	4	5	🟩	09-030410	B10-0028	Cattle	82ANM7926, W-204 (L.	[redacted]	culture
2	4	3	2	3	3	3	3	4	5	🟩	09-030933	B10-0035	Cattle	82VWC2669	(b)(6)	milk
2	4	3	2	3	3	3	3	4	5	🟩	09-030933	B10-0035	Cattle	82VWC2669	[redacted]	milk
2	4	3	2	3	3	3	3	4	5	🟩	09-034536	B10-0407	Cattle	82VTT9873	(b)(6)	tissue
2	4	3	2	3	3	3	3	4	5	🟩	10-011124	B10-0654	Cattle	82APC7447	[redacted]	milk
2	4	3	2	3	3	3	3	4	5	🟩	10-011124	B10-0655	Cattle	fetus of 82APC7447	(b)(6)	tissue 1
2	4	3	2	3	3	3	3	4	5	🟩	10-011124	B10-0655	Cattle	fetus of 82APC7447	[redacted]	tissue 2
2	4	3	2	3	3	3	3	4	5	🟩	10-011124	B10-0655	Cattle	fetus of 82APC7447	(b)(6)	tissue 3
2	4	3	2	3	3	3	3	4	5	🟩	10-020661	B10-0948	Cattle	82VUF4578	[redacted]	from(b)(6) herd
2	4	3	2	3	3	3	3	4	5	🟩	09-029070	B10-0018	Cattle	82ANM7926	(b)(6)	milk
2	4	3	2	3	3	3	3	4	5	🟩	09-032249	B10-0141	Cattle	82VWC2669	[redacted])	culture3540 #1RF&#2RR
2	4	3	2	3	3	3	3	5	4	🟥	09-033518	B10-0203	Elk	BR092077	MT FWP	
2	6	2	2	3	3	3	3	4	4	🟩	408269	06-0038	cattle	403	(b)(6)	2005 - ID herd
2	6	2	2	3	3	3	3	4	4	🟩	408270	06-0042	cattle	409	[redacted]	2005 - ID herd
2	6	3	2	3	3	3	3	4	4	🟩	408269	06-0036	cattle	437	(b)(6)	2005 - ID herd
2	6	3	2	3	3	3	3	4	4	🟩	433600	06-0410	cattle	421 (milk)	[redacted]	2005 - ID herd
3	6	3	2	3	3	3	3	4	4	🟩	406666	06-0031	cattle	432	[redacted]	2005 - ID herd
2	6	3	2	4	3	3	3	5	7	🟪	342040	05-0022	cattle	None Listed	(b)(6) (b)(6)	
2	6	3	2	4	3	3	3	5	7	🟩	365387	05-0756	elk	4985031104	ID Dept of Fish & Game, Caldwell	Rainey Creek - Feb 2005
2	6	4	2	4	3	3	3	5	4	🟥	563907	08-1106	Bison	YNP-365	Yellowstone National Park	John Treanor - Submitter
2	7	3	2	4	3	3	3	5	10	🟪	557690	08-0665	Cattle	83VUL1990 / BL-313 / .	(b)(6)	
2	7	3	2	4	3	3	3	5	10	🟪	557690	08-0666	cattle	83VTH1249/ Y-113/ sa.	[redacted]	positive herd 2008
2	7	3	2	4	3	3	3	5	10	🟪	575556	09-0009	Cattle	08B6838	(b)(6)	see NVSL Acc# 557690
2	7	3	2	4	3	3	3	5	8	🟩	51837	00-0677	elk	60237	None Listed	Rainey Creek - Feb 2000
2	7	3	2	4	3	3	3	5	8	🟩	278724	04-0003	elk	ER03-177 / 2233-160	IDFG Wildlife Health Lab-Caldwell	Rainey Creek - Jun 2002
2	7	3	2	4	3	3	3	5	8	🟩	300438	04-0196	elk	ERO3-177	Idaho Dept. of Fish & Game	Rainey Creek - Jun 2002
2	7	3	2	4	3	3	3	4	8	🟩	180373	02-0246	elk	7217 fetus # 2 215	ID. Dept. of Fish & Game Wildlife L.	
2	7	3	2	4	3	3	1	5	8	🟩	51837	00-0668	elk	60237	None Listed	Rainey Creek - Feb 2000
2	7	3	2	4	3	3	1	5	7	🟪	348993	05-0338	Cattle	None Listed	(b)(6)	
2	3	6	2	4	3	3	3	5	4	🟨	22062	98-0612	cattle	74DSF4190	(b)(6)	1998 - TX herd # 2
2	5	6	2	4	3	3	1	5	4	🟨	22062	98-0617	cattle	74DNY4218	[redacted]	1998 - TX herd # 2
2	4	6	2	3	3	5	3	5	5	🟦	324739	04-0418	cattle	83VNY6729	Cimpl Packing Co., Yankton	(b)(6) isolate - SDSU possible x-contam case
1	4	6	2	3	3	5	1	5	5	🟦	337359	05-0003	Bison	None Listed	Zeman, David	
1	3	7	2	3	3	8	3	5	5	🟦	337359	05-0006	Bison	None Listed	Zeman, David	
8	2	3	2	4	3	4	3	4	6	🟪	372442	05-1005	Bison	None Listed	Yellowstone National Park	
2	3	9	2	3	4	7	1	4	9	🟩	179599	02-0239	cattle	AKN7636	(b)(6)	2002 ID herd
2	3	10	2	3	4	7	3	4	10	🟩	179599	02-0242	cattle	82ALK223	[redacted]	2002 ID herd
5	3	7	2	4	3	5	3	6	6	🟥	496198	07-0266	cattle	81 TSK 4771	(b) (6) (later TransOva)	new herd - Montana 2007
5	3	6	2	4	3	5	3	6	12	🟪	560761	08-0876	Bison	YNP-289	Yellowstone National Park	
7	3	9	2	4	3	5	3	6	8	🟥	555145	08-0416	cattle	1-1340 / #601 / 81TUG.	(b)(6)	new herd Montana - 2008
7	3	4	2	4	3	6	3	6	12	🟥	553707	08-0406	bison	8-346 / P08-011 / Bison	Yellowstone National Park	Mammoth Hot Springs
8	3	7	2	4	3	6	3	6	12	🟪	562880	08-0970	Bison	YNP- 401	Yellowstone National Park	
9	4	8	2	4	3	5	3	6	11	🟥	539969	08-0151	elk	#17 / Madden / 08/03/1.	Montana Fish Wildlife & Parks	Bozeman
4	3	3	2	4	3	6	3	5	5	🟪	562879	08-0962	Bison	YNP- 300	Yellowstone National Park	
9	3	4	2	4	3	6	3	5	4	🟥	563907	08-1107	Bison	YNP-363	Yellowstone National Park	John Treanor - Submitter
6	4	2	2	4	3	2	3	7	4	🟩	243781	03-0072	elk	yellow 332 (N03-060)	B. abortus strain 2308	Canyon, ID
10	4	6	2	4	3	4	3	6	4	🟪	567494	08-1247	Bison	YNP-254	Yellowstone National Park	

Figure 2. Maximum Parsimony analysis (isolates labeled by Case #; newer (b)(6) solates are indicated by red font)



National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388 Fax (515) 337-7315

Brucella Genotyping Final Report - Confidential

December 20, 2010

This genotyping analysis is updated to include a set of 15 isolates recently provided to the NVSL by Mike O'Brien and Matt Gruber of the Montana Conservation Genetic Laboratory of the University of Montana. The samples were originally collected by Hank Edwards from elk fetuses and adults, as well as a bison, from the Grand Teton National Park, the National Elk Refuge, and various feeding grounds, from 1999 - 2009.

Information about the 15 isolates is provided in Table 1, below (note that the entries colored yellow were received earlier this year and were already in the NVSL genotyping database).

Genotyping was performed using VNTR (variable number tandem repeat; also referred to as MLVA) analysis of 10 individual loci within the *Brucella* genome.

__Results:__

Figure 1 (below) shows the clustering of isolates generated by a UPGMA (unweighted pair-group method with arithmetric mean) analysis of the VNTR profiles. A total of 38 cattle, 48 elk, and 48 bison isolates from the NVSL database were incorporated into this analysis. The Wyoming elk/bison isolates are highlighted in light blue.

[Note that, because I was unable to split the BioNumerics dendrogram into defined single-paged segments, entries at the bottom of the dendrogram on page 6 are repeated at the top of the dendrogram on page 7, and likewise entries at the bottom of the dendrogram on page 7 are repeated at the top of the dendrogram on page 8; these duplicated entries are highlighted in light green.]

Also note that dendrogram entry B11-0141, animal ID GF101408, is the same Wyoming bison isolate from Table 1, Animal ID GF101408B (the 'B' was absent from the entry on the former's submission form). This isolate presented with a rare VNTR profile marked by the absence of any tandem repeats for the 'Hoof Print 1' locus (i.e. 0-2-4-2-3-3-8-3-4-7). Another aliquot of *B. abortus* cells from this isolate was separately extracted and subjected to VNTR and displayed the same pattern, thus, we are confident this profile does not represent an artifact.

As is indicated in Figure 1, the majority of the newly genotyped isolates from Wyoming elk and bison cluster with other *B. abortus* recovered from elk from Wyoming, Montana, and Idaho. Notable cattle isolates joining this cluster include animals from the (b)(6) (b)(6) herds.

Of the newly genotyped Hank Edwards / Wyoming isolates, only the bison isolate mentioned above –Case No. B11-0141, Animal ID GF101408 (aka GF101408B) - clusters in proximity to the *B. abortus* strains associated with the Meeteetse area (i.e., cattle owned by (b)(6) / Worland Livestock

Auction, or a bison owned by (b)(6). However, the presence of differences in repeat number at four loci between the Wyoming elk isolates and the (b)(6) isolates (e.g., 0-2-4-2-3-3-8-3-4-7 Vs 4-8-4-2-3-3-3-3-4-8) suggests that there is limited genetic similarity among these lineages of *B. abortus* , and does not provide strong support to a hypothesis of shared transmission among these cohorts of animals.

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.

Although the MST analysis uses a different algorithm than the UPGMA analysis, it does separate the *B. abortus* isolates into two major clusters (demarcated by the black bar). The upper cluster primarily contains Greater Yellowstone region bison isolates, as well as field isolates from Texas and Montana cattle. The newly genotyped Hank Edwards Wyoming isolates segregate into the bottom cluster of the MST. This bottom cluster includes most of the elk strains in the analysis, as well as field isolates of vaccine strain-derived brucellosis cases from cattle; field isolates from the 2002 outbreak in the (b)(6) cattle herd; and the more recent outbreak in the (b)(6) cattle herd.

James Higgins, MS PhD
Mycobacteria and Brucella Section
NVSL / APHIS / USDA
1920 Dayton Ave.
Ames, IA 50010
515-337-7034 (fax 7315)
James.A.Higgins@aphis.usda.gov

Table 1. Archived Wyoming / Hank Edwards elk and bison *B. abortus* isolates newly added to the NVSL BioNumerics database

Wyoming Brucella abortus isolates 10/08/2010

Animal/Vial ID	Species	Date	Sample	Location	Isolate	Biovar	VNTR Profile
NER-1999	Elk	1999	?	NER	*B. abortus*	1/4*	2932333354
00W5798	Elk	5/23/2000	Fetus	Black Butte FG	*B. abortus*	1/4	2442334355
01W12371	Elk	12/4/2001	Joint	GTNP	*B. abortus*	1	2732433359
06W16362	Elk	2/23/2006	Fetus	Soda Lake FG	*B. abortus*	1/4	2432334354
06W5404	Elk	4/6/2006	Fetus	Franz FG	*B. abortus*	4	2732433359
06W2080	Elk	11/14/2006	Joint	NER	*B. abortus*	1/4	0352334345
07W2402	Elk	3/12/2007	Fetus	NER	*B. abortus*	1/4	2732434367
07W4970	Elk	5/1/2007	Fetus	Cody	*B. abortus*	1	245233434[10]
GF031008C	Elk	3/10/2008	Fetus	South Park FG	*B. abortus*	1/4	2632433358
GF031408	Elk	3/14/2008	VIT*	Dell Creek FG	*B. abortus*	1	2432535353
GF031908	Elk	3/19/2008	VIT	Dell Creek FG	*B. abortus*	1	2532534364
GF031008B	Elk	3/27/2008	Fetus	Horse Creek FG	*B. abortus*	1/4	2732434354
GF032808	Elk	3/28/2008	VIT	Grey's River FG	*B. abortus*	1/4	2732434356
GF042208	Elk	4/22/2008	Fetus	Franz FG	*B. abortus*	4	2732433359
GF042708	Elk	4/27/2008	Fetus	Franz FG	*B. abortus*	4	2632433357
GF042908	Elk	4/29/2008	Fetus	Franz FG	*B. abortus*	1	2632433357
GF101408B	Bison	10/14/2008	Lymph node	NER	*B. abortus*	1	0242338347
GF040609	Elk	4/11/2009	Fetus	Franz FG	*B. abortus*	1	2732433355
GF040709	Elk	4/11/2009	Fetus	Horse Creek FG	*B. abortus*	1	2432434364
GF042009	Elk	4/23/2009	Fetus	Horse Creek FG	*B. abortus*	4	2632433358

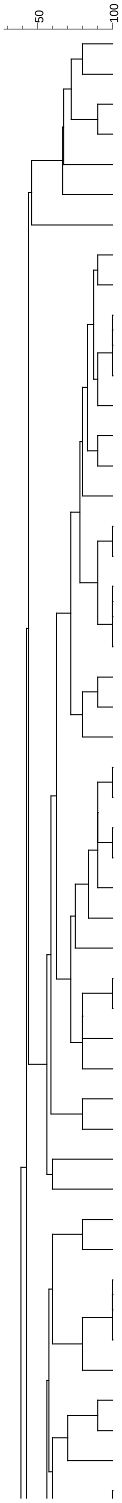
*1/4 = Biovar 1 or 4, dominant antigen not determined

*VIT = vaginal implant transmitter culture

(isolates highlighted in yellow were previously added to the NVSL database earlier in 2010)

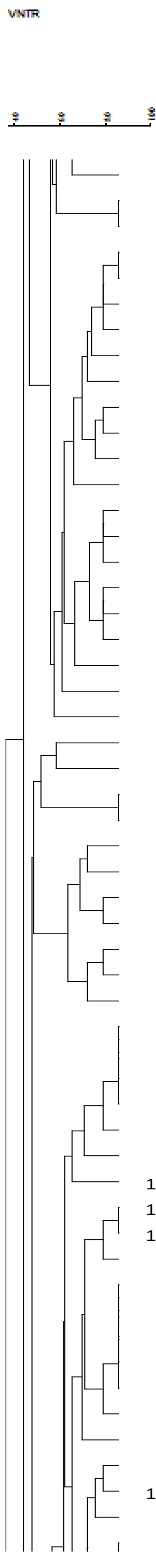
Figure 1. UPGMA clustering dendrogram of *B. abortus* isolates from elk, bison, and cattle isolates from the NVSL database

VNTR VNTR



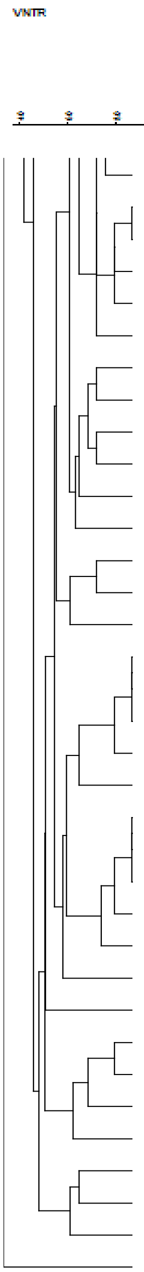
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	Case No	Owner	State	Animal ID	Epidemiology	Species
12	3	5	2	3	3	3	3	5	7	98-0564	(b)(6)	TX	74EAF0453	1998 - TX herd # 1	cattle
8	3	5	2	3	3	3	3	5	10	99-0978	None Listed	TX	None Listed	1999 - TX herd # 1	cattle
9	3	5	2	3	3	3	1	5	9	98-0559	(b)(6)	TX	74EAF0421	1998 - TX herd # 1	cattle
8	3	5	2	3	3	3	1	5	9	99-0983	None Listed	TX	None Listed	1999 - TX herd # 1	cattle
11	3	6	2	3	3	3	1	5	7	98-0563	(b)(6) (b)(6)	TX	74EAF0470	1998 - TX herd # 1	cattle
5	3	7	2	3	3	3		5	6	05-1065	[redacted]	TX	fetus C	MX origin ?	cattle
12	5	8	2	3	6	3	3	5	4	08-0924	Rhyan, Dr. Jack (Nat'l .	WY	3892	Bison quarantine study	Bison
2	7	3	2	4	3	3	3	5	10	08-0666	(b)(6)	WY	83VTH1249/ Y-113/.	seropositive herd 2008	cattle
2	7	3	2	4	3	3	3	5	5	B11-0159	Montana Conservation G.	WY	GF040609	fetus, Franz FG, 2009	Elk
2	7	3	2	4	3	3	3	5	9	09-0005	Wyoming Game and Fis.	WY	GF042208	elk fetus from Franz fee.	elk
2	7	3	2	4	3	3	3	5	9	B11-0144	Montana Conservation G.	WY	01W12371	joint, Gr Teton Ntl Pk, 2.	Elk
2	7	3	2	4	3	3	3	5	9	B11-0146	Montana Conservation G.	WY	06W5404	fetus, Franz FG, 2006	Elk
2	7	3	2	4	3	3	1	5	9	00-0669		ID	60237-89	Rainey Creek - Feb 2000	elk
2	7	3	2	4	3	3	3	4	8	02-0246	ID. Dept. of Fish & Game.	ID	7217 fetus # 2 215	field isolate	elk
2	7	3	2	4	3	3	3	5	8	04-0003	IDFG Wildlife Health Lab.	ID	ER03-177 / 2233-1.	Rainey Creek - Jun 2002	elk
2	7	3	2	4	3	3	1	5	7	05-0338	(b)(6)	WY	83XX 4453	reactors	Cattle
2	6	3	2	4	3	3	3	5	8	B11-0150	Montana Conservation G.	WY	GF031008C	fetus, South Park FG, 2.	Elk
2	6	3	2	4	3	3	3	5	8	B11-0161	Montana Conservation G.	WY	GF042009	fetus, Horse Creek FG, .	Elk
2	6	3	2	4	3	3	3	5	7	05-0757	ID Dept of Fish & Game .	ID	4985031105	Rainey Creek - Feb 2005	elk
2	6	3	2	4	3	3	3	5	7	09-0006	Wyoming Game and Fis.	WY	GF042908	elk fetus from Franz fee.	elk
2	6	3	2	4	3	3	3	5	7	09-0004	Wyoming Game and Fis.	WY	GF042708	elk fetus from Franz fee.	elk
2	7	3	2	4	3	4	3	5	4	B11-0153	Montana Conservation G.	WY	GF031008B	fetus, Horse Creek FG, .	Elk
2	7	3	2	4	3	4	3	5	6	B11-0154	Montana Conservation G.	WY	GF032808	VIT cult., Grey's River F.	Elk
2	7	3	2	4	3	4	3	6	7	B11-0148	Montana Conservation G.	WY	07W2402	fetus, NER, 2007	Elk
2	4	3	2	3	3	3	3	5	4	B10-0203	MT FWP	MT	BR092077	field isolate	Elk
2	4	3	2	3	3	3	3	5	4	B10-0325	MT FWP	MT	BR092992	field isolate	Elk
2	9	3	2	3	3	3	3	5	4	09-0298	Anderson, Neil (MFWP)	MT	BR081181	field isolate	elk
2	9	3	2	3	3	3	3	5	4	B11-0142	Montana Conservation G.	WY	NER-1999	1999 isolate from NER	Elk
2	8	3	2	3	3	3	3	5	4	05-0014	Edwards, Hank	WY	00W2173	elk from NER, Febr 2000	elk
2	4	3	2	3	3	4	3	5	4	B11-0145	Montana Conservation G.	WY	06W16362	fetus, Soda Lake FG, 20.	Elk
2	4	3	2	5	3	5	3	5	4	09-0008	Wyoming Game and Fis.	WY	GF031408	from vaginal implant tran.	elk
2	6	3	2	3	3	3	3	4	4	06-0034	(b)(6)	ID	409	2005 - ID herd	cattle
2	6	3	2	3	3	3	3	4	4	06-0423	Aborted fetus from Bruce.	ID	409 fetus	2005 - ID herd	cattle
2	3	3	2	3	3	3	3	4	6	B10-0173	(b)(6)	ID	82VTT9873	3718 milk	Cattle
2	4	3	2	3	3	3	3	4	5	B10-0654	(b)(6)	ID	82APC7447 (W-13.	field isolate	Cattle
2	5	3	2	5	3	4	3	6	4	09-0007	Wyoming Game and Fis.	WY	GF031908	from vaginal implant tran.	elk
2	4	3	2	4	3	4	3	6	4	B11-0160	Montana Conservation G.	WY	GF040709	fetus, Horse Creek FG, .	Elk
2	4	5	1	4	3	3	3	5	4	98-0614	(b)(6)	TX	74DRA8471	1998 - TX herd # 2	cattle
2	5	6	2	4	3	3	1	5	4	98-0617	(b)(6)	TX	74DNY4218	1998 - TX herd # 2	cattle
3	5	4	2	3	3	4	3	4	7	09-0295	Anderson, Neil (MFWP)	MT	BR081174	field isolate	elk
3	5	4	2	3	3	5	3	5	7	09-0354	Anderson, Neil	MT	BR081308	field isolate	elk
5	9	4	2	3	3	5	3	4	6	B11-0015	(b)(6) (b)(6)	WY	83VSO4205		Cattle,
5	9	4	2	3	3	5	3	4	6	B11-0038	(b)(6)	WY	83AKC8899 / 10B-.	field isolate	Cattle
5	9	4	2	3	3	5	3	4	6	B11-0038	(b)(6)	WY	83AKC8899 / 10B-.	field isolate	Cattle
5	12	4	2	3	3	5	3	5	6	B11-0037	[redacted]	WY	83VS04205 / 10B-.	field isolate	Cattle
1	2	4	2	3	3	6	3	4	9	05-0018	Edwards, Hank	WY	01W307	field isolate NER 2001	bison
1	2	4	2	3	3	7	3	4	9	05-0019	Edwards, Hank	WY	01W311	field isolate NER 2001	bison
0	2	4	2	3	3	8	3	4	7	B11-0141	WY Game and Fish Dept.	WY	GF101408	bison from NER (adult)	Bison
4	8	4	2	3	3	3	3	4	8	B11-0053	(b)(6)	WY	83VZR4294 #White.	field isolate	Bison

Figure 1. (continued)



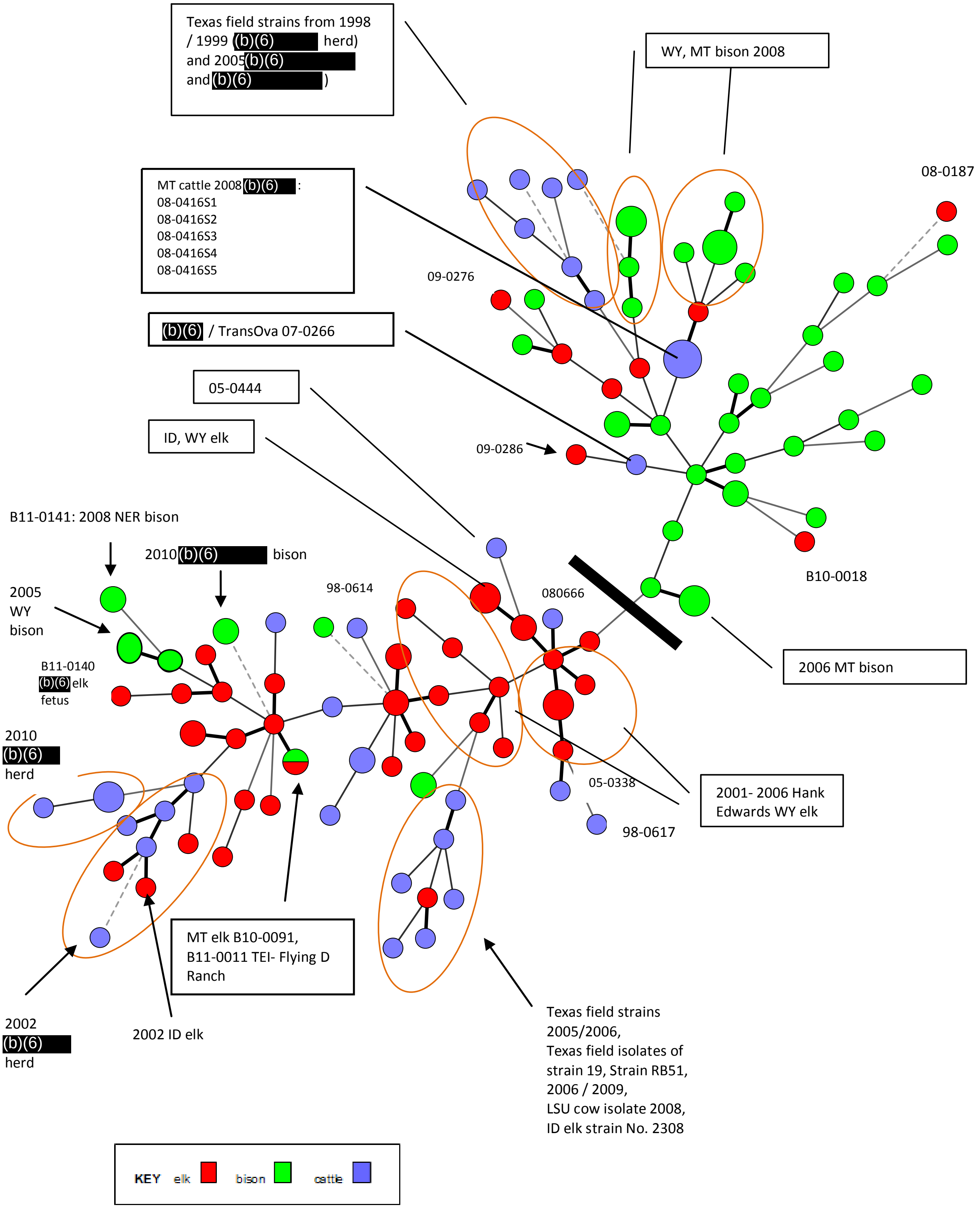
VNTR															
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	Case No.	Owner	State	Animal ID	Epidemiology	Species
0	2	4	2	3	3	8	3	4	7	B11-0141	WY Game and Fish Dept.	WY	GF101408	bison from NER (adult)	Bison
4	8	4	2	3	3	3	3	4	8	B11-0053	(b)(6)	WY	83VZR4294 #White.	field isolate	Bison
4	8	4	2	3	3	3	3	4	8	B11-0053	(b)(6)	WY	83VZR4294 #White.	field isolate	Bison
2	4	4	2	3	3	4	3	4	12	B10-0091	MT FWP	MT	BR090731	field isolate	Elk
2	4	4	2	3	3	4	3	4	12	B11-0011	TEI- Flying D Ranch	MT	9-273/FC 0124	field isolate	Bison
2	4	4	2	3	3	4	3	4	5	09-0443	Neil Anderson	MT	BR080548	field isolate	elk
2	4	4	2	3	3	4	3	4	10	B10-0514	MT FWP	MT	BR090381	field isolate	Elk
2	4	5	2	3	3	4	3	4	10	B11-0140	WY Game and Fish Dept.	WY	07W4970	fetus, Cody, 2007 ?	Elk
2	4	4	2	3	3	4	3	5	5	B11-0143	Montana Conservation G.	WY	00W5798	fetus, Black Butte FG, 2.	Elk
2	4	4	2	3	3	5	3	4	9	05-0908	Fish, Wildlife & Parks	MT	unavail	South Fork Indian Creek	elk
2	4	4	2	3	3	7	3	4	9	B10-0252	MT FWP	MT	BR092578	field isolate	Elk
2	4	4	2	3	3	7	3	4	14	B10-0566	MT FWP	MT	BR090476	field isolate	Elk
2	4	5	2	3	3	5	3	4	11	09-0328	Anderson, Neil (MFWP)	MT	BR081200	field isolate	Elk
2	3	4	2	3	3	5	3	4	10	02-0235	(b)(6)	ID	82VIU1921	2002 ID herd	cattle
2	3	10	2	3	3	5	3	4	10	02-0236	(b)(6)	ID	82ALK6225	2002 ID herd	cattle
2	3	6	2	3	3	5	3	4	10	02-0241	(b)(6)	ID	82VME6918	2002 ID herd	cattle
2	3	13	2	3	3	5	3	4	9	02-0123	Idaho Fish & Game Wildli.	ID	60217	Conant Creek	elk
2	3	7	2	3	3	5	3	4	9	02-0124	Animal Health Labs	ID	60218	Conant Creek	elk
2	3	10	2	3	3	5	3	4	9	02-0237	(b)(6) (b)(6)	ID	82AKN7632	2002 ID herd	cattle
2	5	4	2	3	3	5	3	4	13	B10-0517	MT FWP	MT	BR090384	field isolate	Elk
2	4	6	2	3	3	5	3	5	5	04-0418	Cimpl Packing Co.	SD	83VNY6729	(b)(6) isolate - SDSU .	cattle
0	3	5	2	3	3	4	3	4	5	B11-0147	Montana Conservation G.	WY	06W2080	joint, NER 2006	Elk
7	5	8	2	4	3	8	3	8	6	08-0187	Montana Fish Wildlife & .	MT	#53 / (b) (6) / 12/.	Bozeman	elk
4	5	4	2	4	3	7	3	5	6	05-1285	(b)(6)	TX	74FAA0148 (milk)	2005 - TX herd # 2	cattle
8	2	3	2	4	3	4	3	4	6	05-1002	Yellowstone National Park	WY	3203	surveillance	Bison
8	2	3	2	4	3	4	3	4	6	05-1005	Yellowstone National Park	WY	3221	surveillance	Bison
7	4	2	2	4	3	2	3	5	10	06-0861 A	B. abortus strain 19	TX	74EDJ9879	(b)(6)	Cattle
5	4	2	2	4	3	2	3	5	7	06-0513	(b)(6) .	TX	74FDL6104	field isolate, Calliham, TX	Cattle
6	4	2	2	4	3	2	3	5	6	05-1317	King Ranch Inc.	TX	NL	field isolate	Cattle
6	7	2	2	4	3	2	3	5	6	06-0025	(b)(6)	TX	NL	field isolate	Cattle
6	4	2	2	4	3	2	3	7	4	03-0072	B. abortus strain 2308	ID	yellow 332 (N03-06.	Canyon, ID	elk
5	4	2	2	4	3	2	3	7	4	08-0101	Louisiana State University	LA	1922 / 2007-1	isolate from cow / Elzer .	Cattle
7	4	4	2	4	3	2	3	7	4	09-0269	B. abortus RB51	TX	Sample 1 / 74SQY.	(b)(6)	Cattle
7	3	4	2	4	3	6	3	6	12	08-0404	YNP	MT	8-340 / P08-010	Yellowstone National Park	bison
7	3	4	2	4	3	6	3	6	12	08-0933	Rhyan, Dr. Jack (Nat'l .	WY	81-08	Bison quarantine study	Bison
7	3	4	2	4	3	6	3	6	12	08-1080	USDA/APHIS/VS/BQFS	MT	91-08 / 81APM1605	Bison quarantine study	Bison
7	3	4	2	4	3	6	3	6	12	08-1120	BQFS	MT	54-08 / 81AYE4054	Bison quarantine study	Bison
8	3	4	2	4	3	6	3	6	12	08-1122	BQFS	MT	91-08 / 81APM1605	Bison quarantine study	Bison
7	3	6	2	4	3	6	3	6	11	08-0386	University of Montana	MT	#33 / Bison	Yellowstone National Park	bison
11	3	12	2	4	3	6	3	6	8	08-0934	Rhyan, Dr. Jack (Nat'l .	WY	97-08	Bison quarantine study	Bison
14	3	9	2	4	3	5	3	6	9	08-1119	BQFS	MT	50-08 / 81AYE4071	Bison quarantine study	Bison
14	3	9	2	4	3	5	3	6	9	08-1121	BQFS	MT	6 / 81AYE4053	Bison quarantine study	Bison
9	3	9	2	4	3	5	3	6	9	08-0638	Yellowstone National Park	WY	YNP-82	field isolate	Bison
7	3	9	2	4	3	5	3	6	8	08-0416S1	(b)(6 (b)(6)	MT	1-1340 / #601 / 81.	new herd Montana - 2008	cattle
7	3	9	2	4	3	5	3	6	8	08-0416S2	[redacted]	MT	1-1340 / #601 / 81.	new herd Montana - 2008	cattle
7	3	9	2	4	3	5	3	6	8	08-0416S3	[redacted] (b)(6)	MT	1-1340 / #601 / 81.	new herd Montana - 2008	cattle
7	3	9	2	4	3	5	3	6	8	08-0416S4	[redacted]	MT	1-1340 / #601 / 81.	new herd Montana - 2008	cattle
7	3	9	2	4	3	5	3	6	8	08-0416S5	[redacted] (b)(6)	MT	1-1340 / #601 / 81.	new herd Montana - 2008	cattle
7	3	9	2	4	3	6	3	6	8	B10-0529	MT FWP	MT	BR092820	field isolate	Elk
9	3	8	2	4	3	5	3	6	8	B10-0273	MT FWP	MT	BR092770	field isolate	Elk
8	3	6	2	4	3	5	3	6	6	08-0392	University of Montana	MT	#39 / Bison	Yellowstone National Park	bison
11	3	6	2	4	3	5	3	6	6	08-0869	Yellowstone National Park	WY	YNP-292	field isolate	Bison
8	3	6	2	4	3	5	3	6	13	08-0921	Rhyan, Dr. Jack (Nat'l .	WY	32-08	Bison quarantine study	Bison
5	3	6	2	4	3	5	3	6	10	08-0382	University of Montana	MT	#27 / Bison	Yellowstone National Park	bison

Figure 1. (continued)



VNTR															
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	Case No	Owner	State	Animal ID	Epidemiology	Species
8	3	6	2	4	3	5	3	6	13	08-0921	Rhyan, Dr. Jack (Nat'l .	WY	32-08	Bison quarantine study	Bison
5	3	6	2	4	3	5	3	6	10	08-0382	University of Montana	MT	#27 / Bison	Yellowstone National Park	bison
5	3	6	2	4	3	5	3	6	10	08-1147	Yellowstone National Park	WY	YNP-206	field isolate	Bison
5	3	6	2	4	3	5	3	6	12	08-0876	Yellowstone National Park	WY	YNP-289	field isolate	Bison
5	3	6	2	4	3	5	3	6	9	08-1019	USDA/APHIS/VS/BQFS	MT	20-08 / 81ARG3869	Bison quarantine study	Bison
7	3	6	2	4	3	5	3	6	5	B10-0973	Clarke, P. Ryan-bull biso.	MT	YNP95021	field study	Bison
9	3	7	2	4	3	4	3	6	6	09-0286	Anderson, Neil (MFWP)	MT	BR081161	field isolate	elk
5	3	7	2	4	3	5	3	6	6	07-0266	(b) (6) (later TransO.	MT	81 TSK 4771	new herd - Montana 2007	cattle
10	3	7	2	4	3	4	3	6	12	08-0389	University of Montana	MT	#36 / Bison	Yellowstone National Park	bison
12	3	7	2	4	3	5	3	6	12	08-0966	Yellowstone National Park	WY	YNP- 398	field isolate	Bison
6	3	7	2	4	3	6	3	6	10	B10-0118	MT FWP	MT	BR091887	field isolate	Elk
12	3	3	2	4	3	4	3	6	10	08-0928	Rhyan, Dr. Jack (Nat'l .	WY	4058	Bison quarantine study	Bison
6	3	8	2	4	3	5	3	7	13	08-0390	University of Montana	MT	#37 / Bison	Yellowstone National Park	bison
4	3	8	2	4	3	5	3	7	11	08-0929	Rhyan, Dr. Jack (Nat'l .	WY	45-08	Bison quarantine study	Bison
8	3	9	2	4	3	6	3	7	13	08-1256	Corwin Springs	MT	24-08	surveillance	Bison
6	3	3	2	4	3	5	3	5	5	06-0281	Bison Quarantine Study	MT	07-06/bison	seropositive YNP bison .	bison
6	3	3	2	4	3	5	3	5	5	06-0429	Bison Quarantine Feasibil.	MT	sample 29-06	YNP B. abortus-exposed.	bison
6	3	3	2	4	3	5	3	5	5	06-0887	USDA/APHIS/VS	MT	77-06 (1.5 yo F)	Corwin Springs, Belgrade	bison
7	3	3	2	4	3	5	3	5	5	06-0279	Bison Quarantine Study	MT	14-06/bison	seropositive YNP bison .	bison
9	3	5	2	4	3	5	3	5	9	B10-0584	MT FWP	MT	BR090822	field isolate	Elk
10	3	4	2	4	3	6	3	5	4	08-0863	Yellowstone National Park	WY	YNP-180	field isolate	Bison
10	3	4	2	4	3	6	3	5	4	08-0923	Rhyan, Dr. Jack (Nat'l .	WY	3877	Bison quarantine study	Bison
10	3	4	2	4	3	6	3	5	4	08-1354	Corwin Springs	MT	92-08 / 8-161	field isolate	Bison
9	3	4	2	4	3	6	3	5	4	08-0923	Rhyan, Dr. Jack (Nat'l .	WY	3877	Bison quarantine study	Bison
9	3	4	2	4	3	5	3	5	4	08-1123	BQFS	MT	38-08 / 81ARG3848	Bison quarantine study	Bison
2	6	4	2	4	3	5	3	5	12	05-0444	(b)(6) .	TX	74EXP6760	2005 - TX herd # 1	cattle
8	3	7	2	4	3	4	2	6	8	05-0911	Yellowstone National Par.	MT	81APF6425	surveillance	Bison
9	4	8	2	4	3	5	3	6	11	08-0151	Montana Fish Wildlife & .	MT	#17 / Madden / 08/.	Bozeman	elk
9	4	8	2	4	3	6	3	6	11	08-0410	YNP	MT	8-407-08 / P08-071 .	Mammoth Hot Springs	bison
9	4	5	2	4	3	5	2	6	11	05-1282	APHIS, VS	MT	81 APH 5970 (1 yo.	Corwin Springs	bison
10	4	7	2	4	3	7	3	6	11	09-0276	Anderson, Neil (MFWP)	MT	BR081145	field isolate	Elk
6	6	8	2	4	3	8	3	7	5	05-1132	USDA	MT	81APF6442	reactor	Bison
12	5	8	2	4	3	6	3	7	5	08-1221	Yellowstone National Park	WY	YNP-347	surveillance	Bison
12	6	7	2	4	3	6	3	6	5	09-0553	BQFS- Bison Quarantine.	MT	85-08	Bison quarantine study	Bison
2	3	9	2	3	4	7	1	4	9	02-0239	(b)(6)	ID	AKN7636	2002 ID herd	cattle

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



APHIS

National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388 Fax (515) 337-7315

Brucella Genotyping Final Report - Confidential

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.

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.

James Higgins, MS PhD
Mycobacteria and Brucella Section
NVSL / APHIS / USDA
1920 Dayton Ave.
Ames, IA 50010
515-337-7034 (fax 7315)
James.A.Higgins@aphis.usda.gov

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	(b)(6) [redacted]	ID	82VIU1921	2002 ID herd
2	3	10	2	3	3	5	3	4	10	179599	02-0236	[redacted]	ID	82ALK6225	2002 ID herd
2	3	10	2	3	3	5	3	4	9	179599	02-0237	[redacted]	ID	82AKN7632	2002 ID herd
2	3	9	2	3	4	7	1	4	9	179599	02-0239	[redacted]	ID	AKN7636	2002 ID herd
2	3	6	2	3	3	5	3	4	10	179599	02-0241	[redacted]	ID	82VME6918	2002 ID herd
2	6	3	2	3	3	3	3	4	4	406666	06-0034	(b)(6) [redacted]	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	(b)(6) [redacted]	ID	82APC7447 (W-133)	field isolate
2	3	3	2	3	3	3	3	4	6	09-033482	B10-0173	[redacted])	ID	82VTT9873	3718 milk
5	3	7	2	4	3	5	3	6	6	496198	07-0266	(b) (6) [redacted] (later TransO.	MT	81 TSK 4771	new herd - Montana 2007
7	3	9	2	4	3	5	3	6	8	555145	08-0416	(b)(6) [redacted]	MT	1-1340 / #601 / 81TUG0218	new herd Montana - 2008
2	4	6	2	3	3	5	3	5	5	324739	04-0418	Cimpl Packing Co.	SD	83VNY6729	(b)(6) [redacted] isolate - SDSU .
2	6	4	2	4	3	5	3	5	12	354997	05-0444	(b)(6) [redacted]	TX	74EXP6760	2005 - TX herd # 1
5	3	7	2	3	3	3		5	6	375152	05-1065	[redacted]	TX	fetus C	MX origin ?
4	5	4	2	4	3	7	3	5	6	392810	05-1285	(b)(6) [redacted]	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	(b)(6) [redacted]
7	4	4	2	4	3	2	3	7	4	586532	09-0269	B. abortus RB51	TX	Sample 1 / 74SQY3675	(b)(6) [redacted]
9	3	5	2	3	3	3	1	5	9	20370	98-0559	(b)(6) [redacted]	TX	74EAF0421	1998 - TX herd # 1
12	3	5	2	3	3	3	3	5	7	20370	98-0564	[redacted]	TX	74EAF0453	1998 - TX herd # 1
2	4	5	1	4	3	3	3	5	4	22062	98-0614	(b)(6) [redacted]	TX	74DRA8471	1998 - TX herd # 2
2	5	6	2	4	3	3	1	5	4	22062	98-0617	[redacted]	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	(b)(6) [redacted]	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	(b)(6) [redacted]	TX	NL	field isolate
5	4	2	2	4	3	2	3	5	7	436983	06-0513	(b)(6) [redacted].	TX	NL	field isolate
2	7	3	2	4	3	3	1	5	7	348993	05-0338	(b)(6) [redacted]	WY	83XX 4453	reactors
2	7	3	2	4	3	3	3	5	10	557690	08-0666	(b)(6) [redacted]	WY	83VTH1249/ Y-113/ sample .	seropositive herd 2008

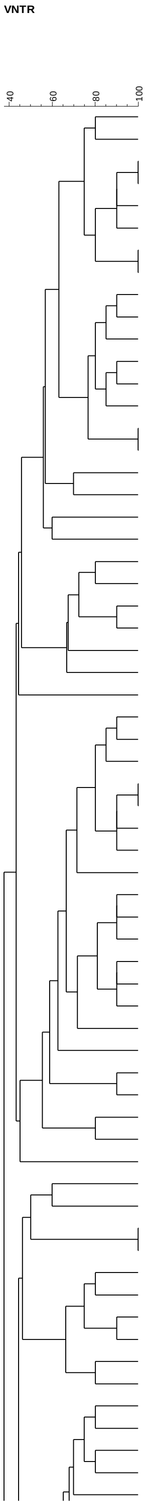
Table 2. List of bison isolates from the NVSL BioNumerics database used in the clustering analysis

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
12	3	3	2	4	3	4	3	6	10	561953	08-0928	Rhyan, Dr. Jack (Nat'l .	WY	4058	Bison quarantine study
4	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

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

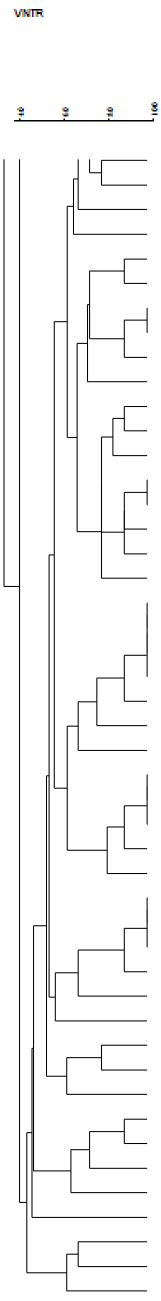
VNTR																
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	NVSL Acc.	Case No.	Owner	State	Animal ID	Epidemiology	
2	7	3	2	4	3	3	1	5	9		00-0669		ID	60237-89	Rainey Creek - Feb 2000	00-0669
2	3	13	2	3	3	5	3	4	9	163343	02-0123	Idaho Fish & Game Wildlife L.	ID	60217	Conant Creek	02-0123
2	3	7	2	3	3	5	3	4	9	163343	02-0124	Animal Health Labs	ID	60218	Conant Creek	02-0124
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	02-0246
6	4	2	2	4	3	2	3	7	4	243781	03-0072	B. abortus strain 2308	ID	yellow 332 (N03-060)	Canyon, ID	03-0072
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	04-0003
2	6	3	2	4	3	3	3	5	7	365387	05-0757	ID Dept of Fish & Game .	ID	4985031105	Rainey Creek - Feb 2005	05-0757
2	4	4	2	3	3	5	3	4	9	370796	05-0908	Fish, Wildlife & Parks	MT	unavail	South Fork Indian Creek	05-0908
9	4	8	2	4	3	5	3	6	11	539969	08-0151	Montana Fish Wildlife & Parks	MT	#17 / Madden / 08/03/19.	Bozeman	08-0151
7	5	8	2	4	3	8	3	8	6	539969	08-0187	Montana Fish Wildlife & Parks	MT	#53 /(b) (6) [redacted] / 12/24/19.	Bozeman	08-0187
10	4	7	2	4	3	7	3	6	11	588409	09-0276	Anderson, Neil (MFWP)	MT	BR081145	field isolate	09-0276
9	3	7	2	4	3	4	3	6	6	588409	09-0286	Anderson, Neil (MFWP)	MT	BR081161	field isolate	09-0286-S1
3	5	4	2	3	3	4	3	4	7	588409	09-0295	Anderson, Neil (MFWP)	MT	BR081174	field isolate	09-0295-S1
2	9	3	2	3	3	3	3	5	4	588409	09-0298	Anderson, Neil (MFWP)	MT	BR081181	field isolate	09-0298-S1
2	4	5	2	3	3	5	3	4	11	589401	09-0328	Anderson, Neil (MFWP)	MT	BR081200	field isolate	09-0328-S-3
3	5	4	2	3	3	5	3	5	7	590672	09-0354	Anderson, Neil	MT	BR081308	field isolate	09-0354-S-1
2	4	4	2	3	3	4	3	4	5	596921	09-0443	Neil Anderson	MT	BR080548	field isolate	09-0443-S1
2	4	4	2	3	3	4	3	4	12	09-032078	B10-0091	MT FWP	MT	BR090731	field isolate	B10-0091-S1
6	3	7	2	4	3	6	3	6	10	09-032080	B10-0118	MT FWP	MT	BR091887	field isolate	B10-0118-S1
2	4	3	2	3	3	3	3	5	4	09-033518	B10-0203	MT FWP	MT	BR092077	field isolate	B10-0203 S-1
2	4	4	2	3	3	7	3	4	9	09-033523	B10-0252	MT FWP	MT	BR092578	field isolate	B10-0252 S-1
9	3	8	2	4	3	5	3	6	8	09-033525	B10-0273	MT FWP	MT	BR092770	field isolate	B10-0273 S-1
2	4	3	2	3	3	3	3	5	4	09-033531	B10-0325	MT FWP	MT	BR092992	field isolate	B10-0325 S-1
2	4	4	2	3	3	4	3	4	10	10-001635	B10-0514	MT FWP	MT	BR090381	field isolate	B10-0514S-1
2	5	4	2	3	3	5	3	4	13	10-001635	B10-0517	MT FWP	MT	BR090384	field isolate	B10-0517S-1
7	3	9	2	4	3	6	3	6	8	10-001635	B10-0529	MT FWP	MT	BR092820	field isolate	B10-0529S-1
2	4	4	2	3	3	7	3	4	14	10-008688	B10-0566	MT FWP	MT	BR090476	field isolate	B10-0566S-1
9	3	5	2	4	3	5	3	5	9	10-008689	B10-0584	MT FWP	MT	BR090822	field isolate	B10-0584S-2
2	8	3	2	3	3	3	3	5	4	339944	05-0014	Edwards, Hank	WY	00W2173	undetermined	05-0014
1	2	4	2	3	3	6	3	4	9	339944	05-0018	Edwards, Hank	WY	01W307	field isolate	05-0018
1	2	4	2	3	3	7	3	4	9	339944	05-0019	Edwards, Hank	WY	01W311	field isolate	05-0019
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.	09-0005
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.	09-0006
2	5	3	2	5	3	4	3	6	4	575556	09-0007	Wyoming Game and Fish Dep.	WY	GF031908	from vaginal implant tran.	09-0007
2	4	3	2	5	3	5	3	5	4	575556	09-0008	Wyoming Game and Fish Dep.	WY	GF031408	from vaginal implant tran.	09-0008

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



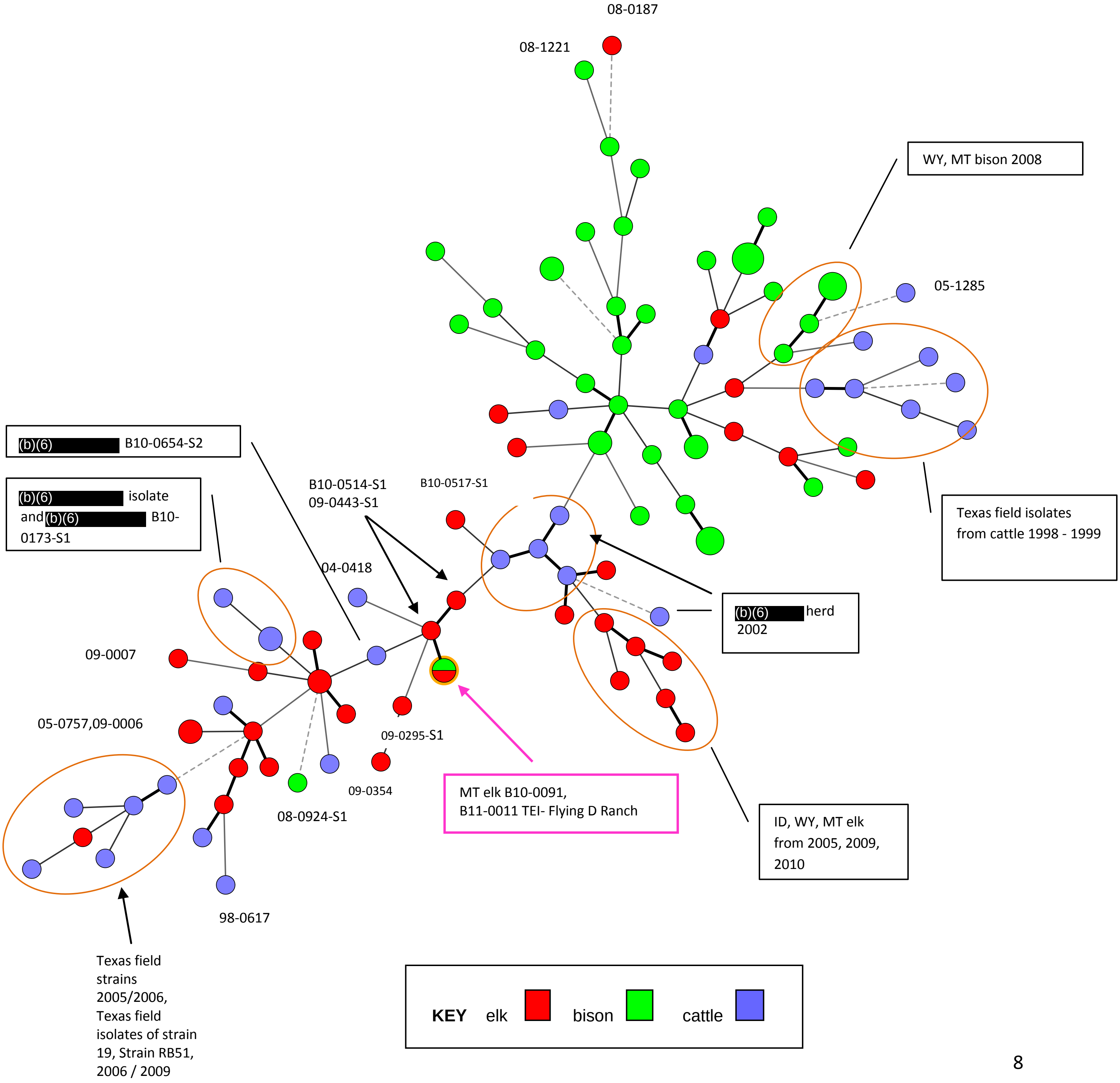
VNTR															
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A		Acc. #	Case #	State	Owner	Epidemiology	Species
2	4	3	2	3	3	3	3	4	5	10-011124	B10-0654	ID	(b)(6)	field isolate	Cattle
2	3	3	2	3	3	3	3	4	6	09-033482	B10-0173	ID	[redacted]	3718 milk	Cattle
2	4	3	2	3	3	3	3	5	4	09-033518	B10-0203	MT	MT FWP	field isolate	Elk
2	4	3	2	3	3	3	3	5	4	09-033531	B10-0325	MT	MT FWP	field isolate	Elk
2	8	3	2	3	3	3	3	5	4	339944	05-0014	WY	Edwards, Hank	undetermined	elk
2	9	3	2	3	3	3	3	5	4	588409	09-0298	MT	Anderson, Neil (MFWP)	field isolate	elk
2	6	3	2	3	3	3	3	4	4	406666	06-0034	ID	(b)(6)	2005 - ID herd	cattle
2	6	3	2	3	3	3	3	4	4	434125	06-0423	ID	Aborted fetus from Brucellosis p.	2005 - ID herd	cattle
2	7	3	2	4	3	3	1	5	9		00-0669	ID		Rainey Creek - Feb 2000	elk
2	7	3	2	4	3	3	3	5	9	575556	09-0005	WY	Wyoming Game and Fish Dept.	elk fetus from Franz feedground	elk
2	7	3	2	4	3	3	1	5	7	348993	05-0338	WY	(b)(6) LTD	reactors	Cattle
2	7	3	2	4	3	3	3	4	8	180373	02-0246	ID	ID. Dept. of Fish & Game Wildlife.	field isolate	elk
2	7	3	2	4	3	3	3	5	8	278724	04-0003	ID	IDFG Wildlife Health Lab-Caldwell	Rainey Creek - Jun 2002	elk
2	7	3	2	4	3	3	3	5	10	557690	08-0666	WY	(b)(6)	seropositive herd 2008	cattle
2	6	3	2	4	3	3	3	5	7	365387	05-0757	ID	ID Dept of Fish & Game Cal.	Rainey Creek - Feb 2005	elk
2	6	3	2	4	3	3	3	5	7	575556	09-0006	WY	Wyoming Game and Fish Dept	elk fetus from (b)(6) z feedground	elk
2	5	3	2	5	3	4	3	6	4	575556	09-0007	WY	Wyoming Game and Fish Dept.	from vaginal implant transmitter recovered f.	elk
2	4	3	2	5	3	5	3	5	4	575556	09-0008	WY	Wyoming Game and Fish Dept.	from vaginal implant transmitter recovered f.	elk
2	4	5	1	4	3	3	3	5	4	22062	98-0614	TX	(b)(6)	1998 - TX herd # 2	cattle
2	5	6	2	4	3	3	1	5	4	22062	98-0617	TX	(b)(6)	1998 - TX herd # 2	cattle
12	3	5	2	3	3	3	3	5	7	20370	98-0564	TX	(b)(6)	1998 - TX herd # 1	cattle
8	3	5	2	3	3	3	3	5	10	31486	99-0978	TX	None Listed	1999 - TX herd # 1	cattle
9	3	5	2	3	3	3	1	5	9	20370	98-0559	TX	(b)(6)	1998 - TX herd # 1	cattle
8	3	5	2	3	3	3	1	5	9	31486	99-0983	TX	None Listed	1999 - TX herd # 1	cattle
11	3	6	2	3	3	3	1	5	7	20370	98-0563	TX	(b)(6)	1998 - TX herd # 1	cattle
5	3	7	2	3	3	3		5	6	375152	05-1065	TX	(b)(6)	MX origin ?	cattle
12	5	8	2	3	6	3	3	5	4	561953	08-0924	WY	Rhyan, Dr. Jack (Nat'l WL Res Ct.	Bison quarantine study	Bison
2	4	4	2	3	3	5	3	4	9	370796	05-0908	MT	Fish, Wildlife & Parks	South Fork Indian Creek	elk
2	4	4	2	3	3	7	3	4	9	09-033523	B10-0252	MT	MT FWP	field isolate	Elk
2	4	4	2	3	3	7	3	4	14	10-008688	B10-0566	MT	MT FWP	field isolate	Elk
2	4	4	2	3	3	4	3	4	12	09-032078	B10-0091	MT	MT FWP	field isolate	Elk
2	4	4	2	3	3	4	3	4	12	10-053081	B11-0011	MT	TEI- Flying D Ranch	field isolate	Bison
2	4	4	2	3	3	4	3	4	5	596921	09-0443	MT	Neil Anderson	field isolate	elk
2	4	4	2	3	3	4	3	4	10	10-001635	B10-0514	MT	MT FWP	field isolate	Elk
2	4	5	2	3	3	5	3	4	11	589401	09-0328	MT	Anderson, Neil (MFWP)	field isolate	Elk
2	3	4	2	3	3	5	3	4	10	179599	02-0235	ID	(b)(6)	2002 ID herd	cattle
2	3	10	2	3	3	5	3	4	10	179599	02-0236	ID	(b)(6)	2002 ID herd	cattle
2	3	6	2	3	3	5	3	4	10	179599	02-0241	ID	(b)(6)	2002 ID herd	cattle
2	3	13	2	3	3	5	3	4	9	163343	02-0123	ID	Idaho Fish & Game Wildlife Lab	Conant Creek	elk
2	3	7	2	3	3	5	3	4	9	163343	02-0124	ID	Animal Health Labs	Conant Creek	elk
2	3	10	2	3	3	5	3	4	9	179599	02-0237	ID	(b)(6)	2002 ID herd	cattle
2	5	4	2	3	3	5	3	4	13	10-001635	B10-0517	MT	MT FWP	field isolate	Elk
2	4	6	2	3	3	5	3	5	5	324739	04-0418	SD	Cimpl Packing Co.	(b)(6) isolate - SDSU possible x-contam .	cattle
1	2	4	2	3	3	6	3	4	9	339944	05-0018	WY	Edwards, Hank	field isolate	elk
1	2	4	2	3	3	7	3	4	9	339944	05-0019	WY	Edwards, Hank	field isolate	elk
3	5	4	2	3	3	4	3	4	7	588409	09-0295	MT	Anderson, Neil (MFWP)	field isolate	elk
3	5	4	2	3	3	5	3	5	7	590672	09-0354	MT	Anderson, Neil	field isolate	elk
2	3	9	2	3	4	7	1	4	9	179599	02-0239	ID	(b)(6)	2002 ID herd	cattle
7	5	8	2	4	3	8	3	8	6	539969	08-0187	MT	Montana Fish Wildlife & Parks	Bozeman	elk
4	5	4	2	4	3	7	3	5	6	392810	05-1285	TX	(b)(6)	2005 - TX herd # 2	cattle
8	2	3	2	4	3	4	3	4	6	372442	05-1002	WY	Yellowstone National Park	surveillance	Bison
8	2	3	2	4	3	4	3	4	6	372442	05-1005	WY	Yellowstone National Park	surveillance	Bison
7	4	2	2	4	3	2	3	5	10	451292	06-0861 A	TX	B. abortus strain 19	(b)(6)	Cattle
5	4	2	2	4	3	2	3	5	7	436983	06-0513	TX	(b)(6)	field isolate, Calliham, TX	Cattle
6	4	2	2	4	3	2	3	5	6	396213	05-1317	TX	King Ranch Inc.	field isolate	Cattle
6	7	2	2	4	3	2	3	5	6	405615	06-0025	TX	(b)(6)	field isolate	Cattle
6	4	2	2	4	3	2	3	7	4	243781	03-0072	ID	B. abortus strain 2308	Canyon, ID	elk
7	4	4	2	4	3	2	3	7	4	586532	09-0269	TX	B. abortus RB51	(b)(6)	Cattle
9	3	7	2	4	3	4	3	6	6	588409	09-0286	MT	Anderson, Neil (MFWP)	field isolate	elk
5	3	7	2	4	3	5	3	6	6	496198	07-0266	MT	(b) (6) (later TransOva)	new herd - Montana 2007	cattle
10	3	7	2	4	3	4	3	6	12	553670	08-0389	MT	University of Montana	Yellowstone National Park	bison
12	3	7	2	4	3	5	3	6	12	562880	08-0966	WY	Yellowstone National Park	field isolate	Bison
6	3	7	2	4	3	6	3	6	10	09-032080	B10-0118	MT	MT FWP	field isolate	Elk

Figure 1. (continued)



VNTR															
Hoof 1	Hoof3	Hoof 4	Hoof8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	Acc. #	Case #	State	Owner	Epidemiology	Species
10	3	7	2	4	3	4	3	6	12						
12	3	7	2	4	3	5	3	6	12	562880	08-0966	WY	Yellowstone National Park	field isolate	Bison
6	3	7	2	4	3	6	3	6	10	09-032080	B10-0118	MT	MT FWP	field isolate	Elk
12	3	3	2	4	3	4	3	6	10	561953	08-0928	WY	Rhyan, Dr. Jack (Nat'l WL Res Ct.	Bison quarantine study	Bison
7	3	9	2	4	3	6	3	6	8	10-001635	B10-0529	MT	MT FWP	field isolate	Elk
7	3	9	2	4	3	5	3	6	8	555145	08-0416	MT	(b)(6)	new herd Montana - 2008	cattle
14	3	9	2	4	3	5	3	6	9	563912	08-1119	MT	BQFS	Bison quarantine study	Bison
14	3	9	2	4	3	5	3	6	9	563912	08-1121	MT	BQFS	Bison quarantine study	Bison
9	3	9	2	4	3	5	3	6	9	557001	08-0638	WY	Yellowstone National Park	field isolate	Bison
9	3	8	2	4	3	5	3	6	8	09-033525	B10-0273	MT	MT FWP	field isolate	Elk
8	3	6	2	4	3	5	3	6	6	553674	08-0392	MT	University of Montana	Yellowstone National Park	bison
11	3	6	2	4	3	5	3	6	6	560760	08-0869	WY	Yellowstone National Park	field isolate	Bison
8	3	6	2	4	3	5	3	6	13	561953	08-0921	WY	Rhyan, Dr. Jack (Nat'l WL Res Ct.	Bison quarantine study	Bison
5	3	6	2	4	3	5	3	6	10	553656	08-0382	MT	University of Montana	Yellowstone National Park	bison
5	3	6	2	4	3	5	3	6	10	563918	08-1147	WY	Yellowstone National Park	field isolate	Bison
5	3	6	2	4	3	5	3	6	12	560761	08-0876	WY	Yellowstone National Park	field isolate	Bison
5	3	6	2	4	3	5	3	6	9	563110	08-1019	MT	USDA/APHIS/VS/BQFS	Bison quarantine study	Bison
7	3	6	2	4	3	5	3	6	5	10-023707	B10-0973	MT	Clarke, P. Ryan-bull bison study	field study	Bison
7	3	4	2	4	3	6	3	6	12	553707	08-0404	MT	YNP	Yellowstone National Park	bison
7	3	4	2	4	3	6	3	6	12	561953	08-0933	WY	Rhyan, Dr. Jack (Nat'l WL Res Ct.	Bison quarantine study	Bison
7	3	4	2	4	3	6	3	6	12	563110	08-1080	MT	USDA/APHIS/VS/BQFS	Bison quarantine study	Bison
7	3	4	2	4	3	6	3	6	12	563912	08-1120	MT	BQFS	Bison quarantine study	Bison
8	3	4	2	4	3	6	3	6	12	563912	08-1122	MT	BQFS	Bison quarantine study	Bison
7	3	6	2	4	3	6	3	6	11	553663	08-0386	MT	University of Montana	Yellowstone National Park	bison
11	3	12	2	4	3	6	3	6	8	561953	08-0934	WY	Rhyan, Dr. Jack (Nat'l WL Res Ct.	Bison quarantine study	Bison
10	3	4	2	4	3	6	3	5	4	560173	08-0863	WY	Yellowstone National Park	field isolate	Bison
10	3	4	2	4	3	6	3	5	4	561953	08-0923	WY	Rhyan, Dr. Jack (Nat'l WL Res Ct.	Bison quarantine study	Bison
10	3	4	2	4	3	6	3	5	4	571693	08-1354	MT	Corwin Springs	field isolate	Bison
9	3	4	2	4	3	6	3	5	4	561953	08-0923	WY	Rhyan, Dr. Jack (Nat'l WL Res Ct.	Bison quarantine study	Bison
9	3	4	2	4	3	5	3	5	4	563912	08-1123	MT	BQFS	Bison quarantine study	Bison
6	3	3	2	4	3	5	3	5	5	426555	06-0281	MT	Bison Quarantine Study	seropositive YNP bison calf euthanized at B.	bison
6	3	3	2	4	3	5	3	5	5	436720	06-0429	MT	Bison Quarantine Feasibility Stu.	YNP B. abortus-exposed bison calf as part .	bison
6	3	3	2	4	3	5	3	5	5	453922	06-0887	MT	USDA/APHIS/VS	Corwin Springs, Belgrade	bison
7	3	3	2	4	3	5	3	5	5	426558	06-0279	MT	Bison Quarantine Study	seropositive YNP bison calf euthanized at B.	bison
9	3	5	2	4	3	5	3	5	9	10-008689	B10-0584	MT	MT FWP	field isolate	Elk
2	6	4	2	4	3	5	3	5	12	354997	05-0444	TX	(b)(6)	2005 - TX herd # 1	cattle
6	3	8	2	4	3	5	3	7	13	553671	08-0390	MT	University of Montana	Yellowstone National Park	bison
4	3	8	2	4	3	5	3	7	11	561953	08-0929	WY	Rhyan, Dr. Jack (Nat'l WL Res Ct.	Bison quarantine study	Bison
8	3	9	2	4	3	6	3	7	13	567648	08-1256	MT	Corwin Springs	surveillance	Bison
9	4	8	2	4	3	5	3	6	11	539969	08-0151	MT	Montana Fish Wildlife & Parks	Bozeman	elk
9	4	8	2	4	3	6	3	6	11	554884	08-0410	MT	YNP	Mammoth Hot Springs	bison
9	4	5	2	4	3	5	2	6	11	391747	05-1282	MT	APHIS, VS	Corwin Springs	bison
10	4	7	2	4	3	7	3	6	11	588409	09-0276	MT	Anderson, Neil (MFWP)	field isolate	Elk
8	3	7	2	4	3	4	2	6	8	371106	05-0911	MT	Yellowstone National Park Wes.	surveillance	Bison
6	6	8	2	4	3	8	3	7	5	378788	05-1132	MT	USDA	reactor	Bison
12	5	8	2	4	3	6	3	7	5	567486	08-1221	WY	Yellowstone National Park	surveillance	Bison
12	6	7	2	4	3	6	3	6	5	602350	09-0553	MT	BQFS- Bison Quarantine Feasibil.	Bison quarantine study	Bison

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



WY, MT bison 2008

Texas field isolates from cattle 1998 - 1999

(b)(6) [redacted] B10-0654-S2

(b)(6) [redacted] isolate and (b)(6) [redacted] B10-0173-S1

B10-0514-S1
09-0443-S1

(b)(6) [redacted] herd 2002

MT elk B10-0091,
B11-0011 TEI- Flying D Ranch

ID, WY, MT elk from 2005, 2009, 2010

Texas field strains 2005/2006, Texas field isolates of strain 19, Strain RB51, 2006 / 2009

APHIS

National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388 Fax (515) 337-7315

Brucella Genotyping Final Report - Confidential

March 25, 2011

This is a genotyping report on a *Brucella abortus* biovar 1 isolate (NVSL Accession 11-012117, case No. B11-0280) forwarded to the Mycobacteria and Brucella Section, NVSL, by the Montana Department of Livestock Diagnostic Laboratory Division. The isolate was cultured from an aborted bison calf owned by Turner Flying D Ranch, Gallatin Gateway, MT.

The isolate was received at the NVSL on March 10, 2011, identified as strain RB51 by standard biochemical tests, and during the week of March 20 genotyped using a variable number tandem repeat (VNTR; also referred to as MLVA) analysis of 10 individual loci within the *Brucella* genome.

Results:

One other isolate in the NVSL database provided an exact match to the VNTR profile (5-4-2-2-4-1-2-2-6-3) of the *B. abortus* isolate recovered from the Flying D Ranch bison calf: that of a *B. abortus* biovar 1 isolate (NVSL case No. 08-0101, animal ID 1922 / 2007-1) cultured from tissues received at the NVSL in late December 2007, from a 3 - 4 year-old cow from Louisiana State University. The cow was pastured in proximity to containment facility housing cattle that were receiving *B. abortus* strain 2308 as part of a scientific study.

Other isolates that did not exactly match the bison calf strain, but could be considered 'nearest neighbors' based on a degree of similarity in VNTR profile, include a *B. abortus* strain 2308 isolated in 2003 from an elk from Canyon, Idaho (Case No. 03-0072), which differs by only one tandem repeat for locus 'Hoof 1'. Another RB-51 isolate (Case No. 09-0269), obtained in 2009 from a bovine owned by the (b)(6) Estate, Damon , Texas, differs at two loci, 'Hoof 1' and 'Hoof 4'.

A *B. abortus* isolate obtained in November 2010 from another Flying D Ranch bison, animal ID 9-273/FC 0124, had a VNTR profile (2-4-4-2-3-3-4-3-4-12) markedly different from the isolate recovered from this calf.

Table 1 (below) provides detailed epidemiologic information on the isolates displaying closest similarity to the Flying D Ranch bison calf isolate (outlined in the red box).

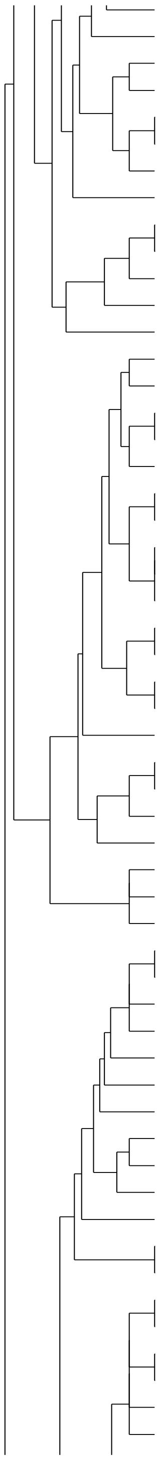
Figure 1 shows the phenogram generated by a UPGMA (unweighted pair-group method with arithmetic mean) analysis of the VNTR profiles of 356 *B. abortus* strains of cattle, elk, and bison in the NVSL database. The Flying D Ranch bison isolate is indicated by the red box; horizontal red lines are used to demarcate important clusters or groupings. [Note that this is one page of a seven-page phenogram, and those sections / pages of the phenogram with limited relevance to the Flying D Ranch bison isolate have not been included).

James Higgins, MS PhD
Mycobacteria and Brucella Section
NVSL / APHIS / USDA
1920 Dayton Ave.
Ames, IA 50010
515-337-7034 (fax 7315)
James.A.Higgins@aphis.usda.gov

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

Case No.	Owner	State	Animal ID	Epidemiology	Host	Strain info
08-0423	USDA/APHIS/VS	MT	3893 / 81AMG3893	Belgrade, MT	bison	B. abortus bv. 1
08-0390	University of Montana	MT	#37 / Bison	Yellowstone National Park	bison	B. abortus bv. 1
08-0644	Yellowstone National Park	WY	YNP-65	surveillance	Bison	B. abortus bv. 1
08-0988	Yellowstone National Park	WY	YNP- 368	surveillance	Bison	B. abortus bv. 1
08-0405	YNP	MT	8-344 / P08- / Bison	Mammoth Hot Springs	bison	B. abortus bv. 1
08-1112	Yellowstone National Park	WY	YNP-350	surveillance	Bison	B. abortus bv. 1
08-1256	Corwin Springs	MT	24-08	surveillance	Bison	B. abortus bv. 1
08-1161	Yellowstone National Park	WY	YNP-352	surveillance	Bison	B. abortus bv. 1
08-0832	Yellowstone National Park	WY	YNP-162	surveillance	Bison	B. abortus bv. 1
08-0830	Yellowstone National Park	WY	YNP-150	surveillance	Bison	B. abortus bv. 1
06-0287	(b)(6)	TX	NL		Cattle	B. abortus Strain 19
06-0763	(b)(6)	TX	NL		Cattle	B. abortus Strain 19
05-0012	(b)(6)	TX	NL		Cattle	B. abortus Strain 19
05-1317	King Ranch Inc.	TX	NL	field isolate	Cattle	B. abortus Strain 19
06-0025	(b)(6)	TX	NL	field isolate	Cattle	B. abortus Strain 19
						B. abortus Strain 19
Strain 19	NVSL ctrl			Isolate used as biochem		B. abortus Strain 19
06-0513	(b)(6) .	TX	74FDL6104	field isolate, Calliham, TX	Cattle	B. abortus Strain 19
B10-1032	(b)(6)	TX	74HK0600	isolate 1 from young cow	Cattle	B. abortus Strain 19
B11-0194	(b)(6)	TX	74FGM8662	field isolate	Cattle, Do.	B. abortus Strain 19
06-0861 A	B. abortus strain 19	TX	74EDJ9879	(b)(6)	Cattle	B. abortus strain 19
07-0503	(b)(6)	TX	Sample 1 / 74FGD4607		Cattle	B. abortus Strain 19
06-0537	(b)(6)	TX	NL		Cattle	B. abortus Strain 19
06-0861 B	(b)(6)	TX	74EDJ9879	(b)(6)	Cattle	B. abortus strain 19
07-0062	(b)(6)	TX	Sample 1 74SVW6276		Cattle	B. abortus Strain 19
08-0101	Louisiana State University	LA	1922 / 2007-1	str 2308 challenge study ?	Cattle	B. abortus bv. 1, Strain 23.
B11-0280	Turner Flying D Ranch	MT	aborted bison calf	field isolate	Bison, dom.	B. abortus RB51
03-0072	B. abortus strain 2308	ID	yellow 332 (N03-060)	Canyon, ID	elk	B. abortus bv. 1, strain 23.
09-0269	B. abortus RB51	TX	Sample 1 / 74SQY3675	(b)(6)	Cattle	B. abortus RB51
B11-0202	(b)(6)	TX	74FUT7066	field isolate	Cattle	B. abortus bv. 1
B11-0204	(b)(6)	TX	74FUT7069 / 74PK0204 .	field isolate	Cattle, Do.	B. abortus bv. 1
B11-0206	(b)(6)	TX	74EWR4840 / X49925, .	field isolate	Cattle	B. abortus bv. 1
B10-0091	MT FWP	MT	BR090731	field isolate	Elk	B. abortus bv. 1
B11-0011	TEI- Flying D Ranch	MT	9-273/FC 0124	field isolate	Bison	B. abortus bv. 1
09-0443	Neil Anderson	MT	BR080548	field isolate	elk	B. abortus bv. 1
B10-0514	MT FWP	MT	BR090381	field isolate	Elk	B. abortus bv. 1
B11-0140	WY Game and Fish Dept. / Hank.	WY	07W4970	fetus, Cody, 2007 ?	Elk	B. abortus bv. 1
B11-0143	Montana Conservation Genetics .	WY	00W5798	fetus, Black Butte FG, 2000	Elk	B. abortus bv. 1, bv 4 ?
B11-0264	Edwards, Hank / WY Game and F.	WY	82 Retro	HA 67	Elk	B. abortus bv. 1
05-0908	Fish, Wildlife & Parks	MT	unavail	South Fork Indian Creek	elk	B. abortus bv. 1
B10-0252	MT FWP	MT	BR092578	field isolate	Elk	B. abortus bv. 1
B10-0566	MT FWP	MT	BR090476	field isolate	Elk	B. abortus bv. 1
09-0328	Anderson, Neil (MFWP)	MT	BR081200		elk	B. abortus bv. 1
B11-0275	Edwards, Hank / WY Game and F.	WY	10-1167 Iliac	HA 84	Elk	B. abortus bv. 1
B11-0276	Edwards, Hank / WY Game and F.	WY	10-1167 Supra-mam	HA 84	Elk	B. abortus bv. 1
B10-0203	MT FWP	MT	BR092077	field isolate	Elk	B. abortus bv. 1
B10-0325	MT FWP	MT	BR092992	field isolate	Elk	B. abortus bv. 1
09-0298	Anderson, Neil (MFWP)	MT	BR081181	field isolate	elk	B. abortus bv. 1
B11-0142	Montana Conservation Genetics .	WY	NER-1999	1999 isolate from NER	Elk	B. abortus bv. 1, bv 4 ?
05-0014	Edwards, Hank	WY	00W2173	elk from NER, Febr 2000	elk	B. abortus bv. 4
B11-0261	Edwards, Hank / WY Game and F.	WY	10-1133 Iliac	NER	Bison	B. abortus bv. 1
B11-0252	Edwards, Hank / WY Game and F.	WY	10-1038 Retro	NER	Bison	B. abortus bv. 1
B11-0258	Edwards, Hank / WY Game and F.	WY	10-1089 Iliac	NER	Bison	B. abortus bv. 1
B11-0262	Edwards, Hank / WY Game and F.	WY	10-1162 Iliac	NER	Bison	B. abortus bv. 1

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



VNTR														
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	Case No.	Owner	State	Animal ID	Epidemiology
6	3	8	2	4	1	5	2	6	12	08-0390	University of Montana	MT	#37 / Bison	Yellowstone National Park
6	4	8	2	4	1	5	2	6	13	08-0644	Yellowstone National Park	WY	YNP-65	surveillance
7	3	8	2	4	1	5	2	6	11	08-0996	Yellowstone National Park	WY	YNP- 377	surveillance
4	3	8	2	4	1	5	2	6	11	08-1229	Yellowstone National Park	WY	YNP-327	surveillance
4	3	8	2	4	1	5	2	6	10	08-0929	Rhyan, Dr. Jack (Nat'l WL Res Ctr)	WY	45-08	Bison quarantine study
4	3	8	2	4	1	5	2	6	10	08-1229	Yellowstone National Park	WY	YNP-327	
5	3	8	2	4	1	5	2	6	10	08-0423	USDA/APHIS/VS	MT	3893 / 81AMG3893	Belgrade, MT
10	3	8	2	4	1	7	2	6	8	08-0988	Yellowstone National Park	WY	YNP- 368	surveillance
12	5	8	2	4	1	6	2	6	4	08-0924	Rhyan, Dr. Jack (Nat'l Wildlife Rese.	MT	3892	Bison quarantine study (.
12	5	8	2	4	1	6	2	6	4	08-1221	Yellowstone National Park	WY	YNP-347	surveillance
13	5	8	2	4	1	6	2	6	4	08-1111	Yellowstone National Park	WY	YNP-346	surveillance
7	5	8	2	4	1	7	2	6	4	08-0445	Yellowstone National Park	WY	YNP-37	surveillance
7	5	8	2	4	1	8	2	7	5	08-0187	Montana Fish Wildlife & Parks	MT	#53 /(b) (6) / 12/24.	Bozeman
4	4	2	2	4	1	2	2	4	5	06-0287	(b)(6)	TX	NL	
9	4	2	2	4	1	2	2	4	5	06-0763	(b)(6)	TX	NL	
6	4	2	2	4	1	2	2	4	5	05-0012	(b)(6)	TX	NL	
6	4	2	2	4	1	2	2	4	5	05-1317	King Ranch Inc.	TX	NL	field isolate
6	7	2	2	4	1	2	2	4	5	06-0025	(b)(6)	TX	NL	field isolate
5	4	2	2	4	1	2	2	4	5					
5	4	2	2	4	1	2	2	4	5	Strain 19	NVSL ctrl			Isolate used as biochem
5	4	2	2	4	1	2	2	4	6	06-0513	(b)(6)	TX	74FDL6104	field isolate, Calliham, TX
5	4	2	2	4	1	2	2	4	6	B10-1032	[redacted]	TX	74HK0600	isolate 1 from young cow
5	4	2	2	4	1	2	2	4	6	B11-0194	(b)(6)	TX	74FGM8662	field isolate
7	4	2	2	4	1	2	2	4	9	06-0861 A	B. abortus strain 19	TX	74EDJ9879	(b)(6)
7	4	2	2	4	1	2	2	4	9	07-0503	(b)(6)	TX	Sample 1 / 74FGD46.	
8	4	2	2	4	1	2	2	4	9	06-0537	(b)(6)	TX	NL	
8	4	2	2	4	1	2	2	4	9	06-0861 B	(b)(6)	TX	74EDJ9879	(b)(6)
6	4	2	2	4	1	2	2	5	7	07-0062	(b)(6)	TX	Sample 1 74SVW62.	
5	4	2	2	4	1	2	2	6	3	08-0101	Louisiana State University	LA	1922 / 2007-1	isolate from cow / Elzer .
5	4	2	2	4	1	2	2	6	3	B11-0280	Turner Flying D Ranch	MT	aborted bison calf	field isolate
6	4	2	2	4	1	2	2	6	3	03-0072	B. abortus strain 2308	ID	yellow 332 (N03-060)	Canyon, ID
7	4	4	2	4	1	2	2	6	3	09-0269	B. abortus RB51	TX	Sample 1 / 74SQY36.	(b)(6)
8	5	2	2	5	1	2	2	4	7	B11-0202	(b)(6)	TX	74FUT7066	field isolate
8	5	2	2	5	1	2	2	4	6	B11-0204	(b)(6)	TX	74FUT7069 / 74PK0.	field isolate
8	5	2	2	5	1	2	2	4	4	B11-0206	(b)(6)	TX	74EWR4840 / X499.	field isolate
2	4	4	2	3	1	4	2	3	11	B10-0091	MT FWP	MT	BR090731	field isolate
2	4	4	2	3	1	4	2	3	11	B11-0011	TEI- Flying D Ranch	MT	9-273/FC 0124	field isolate
2	4	4	2	3	1	4	2	3	4	09-0443	Neil Anderson	MT	BR080548	field isolate
2	4	4	2	3	1	4	2	3	9	B10-0514	MT FWP	MT	BR090381	field isolate
2	4	5	2	3	1	4	2	3	9	B11-0140	WY Game and Fish Dept. / Hank E.	WY	07W4970	fetus, Cody, 2007 ?
2	4	4	2	3	1	4	2	4	4	B11-0143	Montana Conservation Genetics Lab	WY	00W5798	fetus, Black Butte FG, 2.
2	4	6	2	3	1	4	2	3	5	B11-0264	Edwards, Hank / WY Game and Fis.	WY	82 Retro	HA 67
2	4	4	2	3	1	5	2	3	8	05-0908	Fish, Wildlife & Parks	MT	unavail	South Fork Indian Creek
2	4	4	2	3	1	7	2	3	8	B10-0252	MT FWP	MT	BR092578	field isolate
2	4	4	2	3	1	7	2	3	13	B10-0566	MT FWP	MT	BR090476	field isolate
2	4	5	2	3	1	5	2	3	10	09-0328	Anderson, Neil (MFWP)	MT	BR081200	
2	4	4	2	3	1	3	2	4	6	B11-0275	Edwards, Hank / WY Game and Fis.	WY	10-1167 Iliac	HA 84
2	4	4	2	3	1	3	2	4	6	B11-0276	Edwards, Hank / WY Game and Fis.	WY	10-1167 Supra-mam	HA 84
2	4	3	2	3	1	3	2	4	3	B10-0203	MT FWP	MT	BR092077	field isolate
2	4	3	2	3	1	3	2	4	3	B10-0325	MT FWP	MT	BR092992	field isolate
2	9	3	2	3	1	3	2	4	3	09-0298	Anderson, Neil (MFWP)	MT	BR081181	field isolate
2	9	3	2	3	1	3	2	4	3	B11-0142	Montana Conservation Genetics Lab	WY	NER-1999	1999 isolate from NER
2	8	3	2	3	1	3	2	4	3	05-0014	Edwards, Hank	WY	00W2173	elk from NER, Febr 2000
2	3	3	2	3	1	3	2	4	3	B11-0261	Edwards, Hank / WY Game and Fis.	WY	10-1133 Iliac	NER

National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388   Fax (515) 337-7315

Brucella Genotyping Final Report - Confidential

March 18, 2011

This is a genotyping report for NVSL Accession No. 11-011567, a set of 15 elk and 15 bison isolates of *Brucella abortus* provided to the NVSL on March 9, 2011, by Hank Edwards of the Wyoming Fish and Game Department. The bison were collected from the National Elk Refuge (NER), and the elk from selected Hunting Areas (HA), during the Winter of 2010-2011. Mike McDole had requested genotyping of the isolates as part of the investigation of the *B. abortus* strains involved in infections in cattle in Park County in the Meeteetse area (b)(6) herd) and the Cody area (the (b)(6) herd). Also of interest are *B. abortus* strains isolated in November 2010 from a two year-old bison from the (b)(6) in Meetseetse.

The isolates were subjected to VNTR (Variable Number Tandem Repeat) analysis (also referred to as MLVA) during the week of March 13, 2011.

The analysis included 356 *B. abortus* strains of cattle, elk, and bison origin in the NVSL BioNumerics database for which different VNTR (Variable Number Tandem Repeat) analysis (also referred to as MLVA) profiles were available. The VNTR analysis targeted 10 individual loci, or 'hoofprints', within the *B. abortus* genome.

Results:

Table 1 (below) provides detailed information on Hank's isolates and the relevant Park County strains of *B. abortus*.

Because of the large number of isolates (n = 356) used in the genotyping analysis, I have provided two sets of Figures (below). The first set, Figures 1A and 1B, is designed to provide a comprehensive overview of the relationships among all the VNTR profiles from all *B. abortus* isolates in the NVSL BioNumerics database using a Minimum Spanning Tree (MST) analysis. The spheres in the MST 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.

The new Edwards elk and bison isolates segregated into the left-hand portion of the MST (Figure 1A). A number of Hank's *B. abortus* elk strains clustered with the (b)(6) cattle isolates and the (b)(6) bison isolate (clade No. **1** in Figure 1A). Other elk and bison isolates from Hank's Winter 2011 collection display considerably less similarity with the Park County *B. abortus* isolates, and form their own independent cluster (clade No. **4** in Figure 1A).

Figure 2 is an excerpt from a much larger (i.e., 7 pages) phenogram generated from a UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) analysis of the VNTR data, and focuses on the new isolates from Hank that are 'nearest neighbors' to the Park County cattle and bison isolates. Not unexpectedly, none of Hank's elk and bison isolates exactly matched the Park County *B. abortus* VNTR profiles, but they do constitute the most genetically similar isolates among all those present in our database, supplanting older (2009) isolates provided by Neil Anderson from Montana elk.

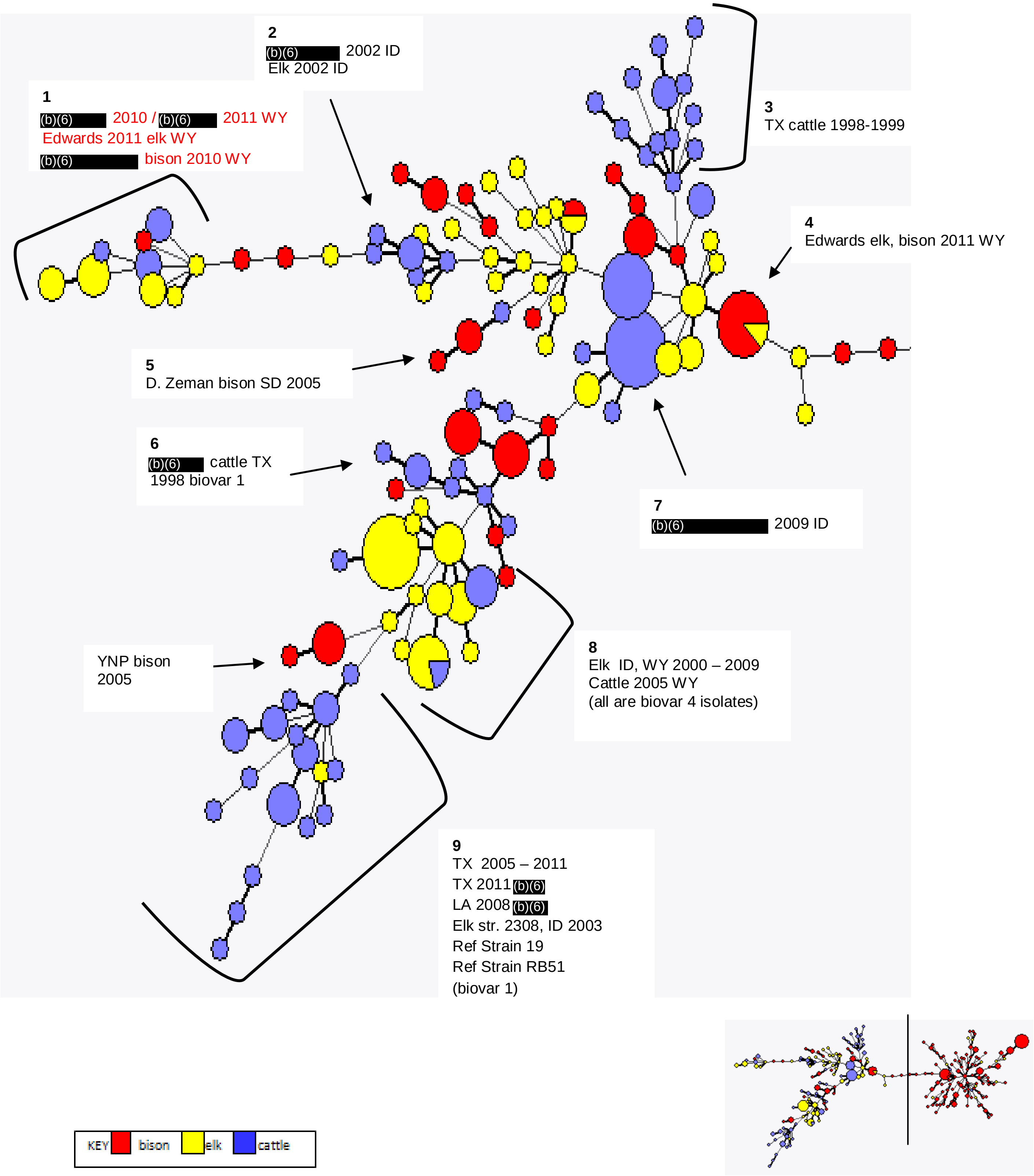
While some degree of caution should be employed in using VNTR data as a standalone measure for investigating relationships between brucellosis outbreaks, the data suggest shared epidemiologic links among *B. abortus* detected in these elk, and cattle in the Park County region of Wyoming.

James Higgins, MS, PhD
Mycobacteria and Brucella Section
USDA/APHIS/NVSL
1920 Dayton Ave
Ames, IA 50010
515-337-7034 (fax 7315)

Table 1. List of *B. abortus* isolates from H. Edwards collected in Winter 2011, and other relevant Park County, WY isolates used in the UPGMA dendrogram assembly (Figure 2)

Acc. No.	Case No.	Owner	State	Animal ID	Epidemiology	Host	Brucella strain
10-054053	B11-0015	(b)(6) (b)(6)	WY	83VSO4205		Cattle, Simmental	B. abortus bv. 1
10-054515	B11-0037	(b)(6)	WY	83VS04205 / 10B-11758	field isolate	Cattle	B. abortus bv. 1
field isolate	B11-0038	(b)(6)	WY	83AKC8899 / 10B-11757	field isolate	Cattle	B. abortus bv. 1
10-054515	B11-0038	[redacted]	WY	83AKC8899 / 10B-11757	field isolate	Cattle	B. abortus bv. 1
10-057783	B11-0053	(b)(6)	WY	83VZR4294 #White 80	field isolate	Bison	B. abortus bv. 1
10-057783	B11-0053	(b)(6)	WY	83VZR4294 #White 80	field isolate	Bison	B. abortus bv. 1
10-057783	B11-0053	(b)(6)	WY	83VZR4294 #White 80	field isolate	Bison	B. abortus bv. 1
11-007611	B11-0208	(b)(6)	WY	81TUK5466 / 81BC2563	field isolate	Cattle, Angus	B. abortus bv. 1
11-008075	B11-0210	(b)(6) (b)(6)	WY	81TUK5466 / 81BC2563.	field isolate	Cattle, Black Angus	B. abortus bv. 1
11-011567	B11-0248	Edwards, Hank / WY Game and Fish Dept.	WY	10-1012 Retro	NER	Bison	B. abortus bv. 1
11-011567	B11-0249	Edwards, Hank / WY Game and Fish Dept.	WY	10-1019 Retro	NER	Bison	B. abortus bv. 1
11-011567	B11-0250	Edwards, Hank / WY Game and Fish Dept.	WY	10-1025 Supra-mam	NER	Bison	B. abortus bv. 1
11-011567	B11-0251	Edwards, Hank / WY Game and Fish Dept.	WY	10-1030 Iliac	NER	Bison	B. abortus bv. 1
11-011567	B11-0252	Edwards, Hank / WY Game and Fish Dept.	WY	10-1038 Retro	NER	Bison	B. abortus bv. 1
11-011567	B11-0253	Edwards, Hank / WY Game and Fish Dept.	WY	10-1058 Retro	NER	Bison	B. abortus bv. 1
11-011567	B11-0254	Edwards, Hank / WY Game and Fish Dept.	WY	10-1060 Iliac	NER	Bison	B. abortus bv. 1
11-011567	B11-0255	Edwards, Hank / WY Game and Fish Dept.	WY	10-1074 Supra-mam	NER	Bison	B. abortus bv. 1
11-011567	B11-0256	Edwards, Hank / WY Game and Fish Dept.	WY	10-1075 Supra-ing	NER	Bison	B. abortus bv. 1
11-011567	B11-0257	Edwards, Hank / WY Game and Fish Dept.	WY	10-1086 Hide	NER	Bison	B. abortus bv. 1
11-011567	B11-0258	Edwards, Hank / WY Game and Fish Dept.	WY	10-1089 Iliac	NER	Bison	B. abortus bv. 1
11-011567	B11-0259	Edwards, Hank / WY Game and Fish Dept.	WY	10-1125 Iliac-Retro	NER	Bison	B. abortus bv. 1
11-011567	B11-0260	Edwards, Hank / WY Game and Fish Dept.	WY	10-1126 Retro	NER	Bison	B. abortus bv. 1
11-011567	B11-0261	Edwards, Hank / WY Game and Fish Dept.	WY	10-1133 Iliac	NER	Bison	B. abortus bv. 1
11-011567	B11-0262	Edwards, Hank / WY Game and Fish Dept.	WY	10-1162 Iliac	NER	Bison	B. abortus bv. 1
11-011567	B11-0263	Edwards, Hank / WY Game and Fish Dept.	WY	26 Retro	Hunt Area (HA) 62	Elk	B. abortus bv. 1
11-011567	B11-0264	Edwards, Hank / WY Game and Fish Dept.	WY	82 Retro	HA 67	Elk	B. abortus bv. 1
11-011567	B11-0265	Edwards, Hank / WY Game and Fish Dept.	WY	07-3128 Iliac	HA 61	Elk	B. abortus bv. 1
11-011567	B11-0266	Edwards, Hank / WY Game and Fish Dept.	WY	07-3128 Retro	HA 61	Elk	B. abortus bv. 1
11-011567	B11-0267	Edwards, Hank / WY Game and Fish Dept.	WY	07-3128 Supra-mam	HA 61	Elk	B. abortus bv. 1
11-011567	B11-0268	Edwards, Hank / WY Game and Fish Dept.	WY	09-5135 Iliac	HA 63	Elk	B. abortus bv. 1
11-011567	B11-0269	Edwards, Hank / WY Game and Fish Dept.	WY	09-5135 Supra-mam	HA 63	Elk	B. abortus bv. 1
11-011567	B11-0270	Edwards, Hank / WY Game and Fish Dept.	WY	09-5139 Supra-mam	HA 61	Elk	B. abortus bv. 1
11-011567	B11-0271	Edwards, Hank / WY Game and Fish Dept.	WY	10-0439 Retro	HA 61	Elk	B. abortus bv. 1
11-011567	B11-0272	Edwards, Hank / WY Game and Fish Dept.	WY	10-0439 Supra-mam	HA 61	Elk	B. abortus bv. 1
11-011567	B11-0273	Edwards, Hank / WY Game and Fish Dept.	WY	10-0449 Iliac	HA 61	Elk	B. abortus bv. 1
11-011567	B11-0274	Edwards, Hank / WY Game and Fish Dept.	WY	10-0449 Supra-mam	HA 61	Elk	B. abortus bv. 1
11-011567	B11-0275	Edwards, Hank / WY Game and Fish Dept.	WY	10-1167 Iliac	HA 84	Elk	B. abortus bv. 1
11-011567	B11-0276	Edwards, Hank / WY Game and Fish Dept.	WY	10-1167 Supra-mam	HA 84	Elk	B. abortus bv. 1
11-011567	B11-0277	Edwards, Hank / WY Game and Fish Dept.	WY	10-3598 Supra-mam	HA 61	Elk	B. abortus bv. 1

Figure 1A. Minimum Spanning Tree (MST) of *B. abortus* isolates from elk, bison, and cattle from the NVSL database. **This is the left-side portion of a larger diagram (inset, lower right)**



1
(b)(6) 2010 / (b)(6) 2011 WY
Edwards 2011 elk WY
(b)(6) bison 2010 WY

2
(b)(6) 2002 ID
Elk 2002 ID

3
TX cattle 1998-1999

4
Edwards elk, bison 2011 WY

5
D. Zeman bison SD 2005

6
(b)(6) cattle TX
1998 biovar 1

7
(b)(6) 2009 ID

YNP bison
2005

8
Elk ID, WY 2000 – 2009
Cattle 2005 WY
(all are biovar 4 isolates)

9
TX 2005 – 2011
TX 2011 (b)(6)
LA 2008 (b)(6)
Elk str. 2308, ID 2003
Ref Strain 19
Ref Strain RB51
(biovar 1)

Figure 1B. Minimum Spanning Tree (MST) of *B. abortus* isolates from elk, bison, and cattle from the NVSL database. This is the right-side portion of a larger diagram (inset, lower right)

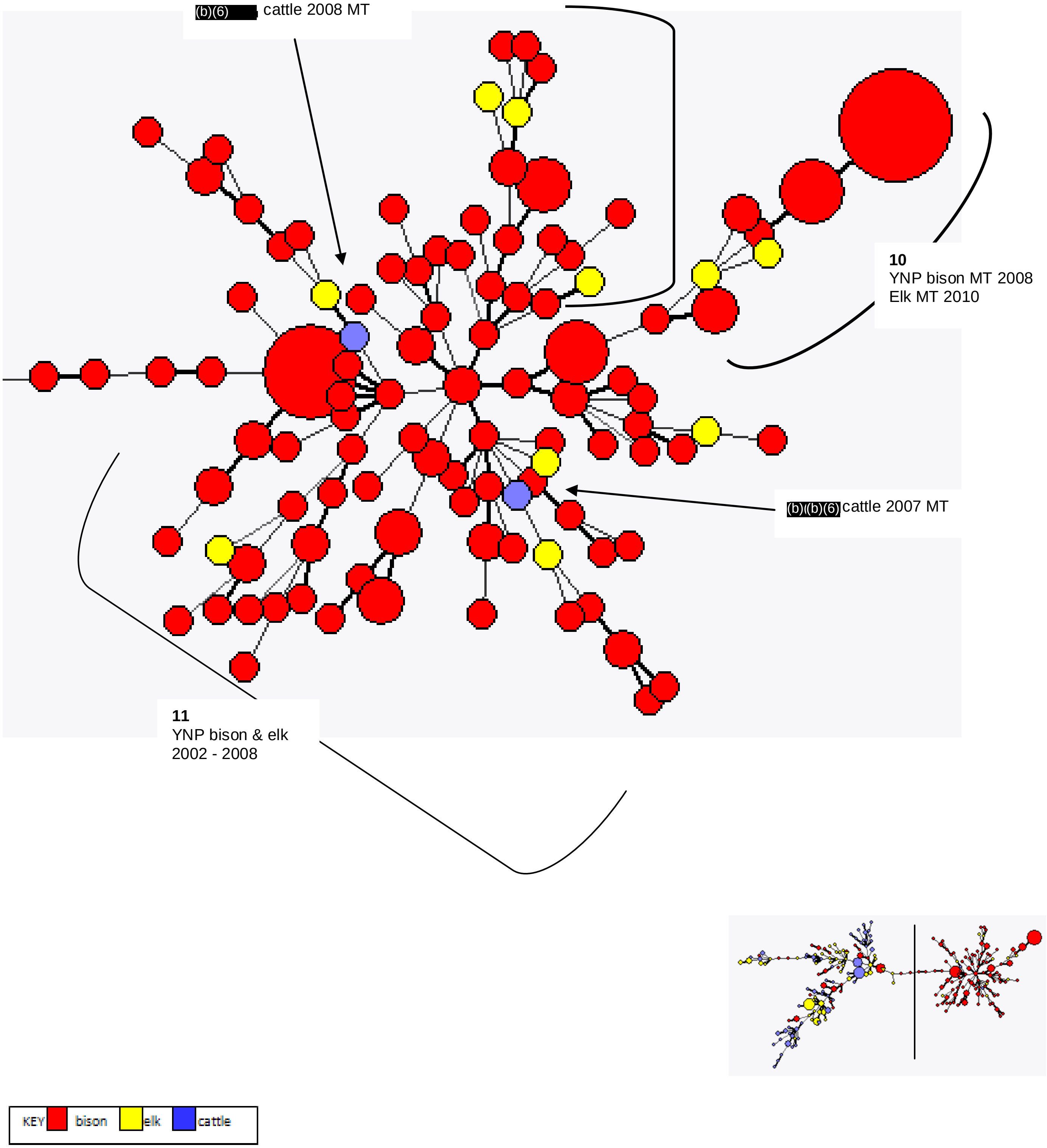
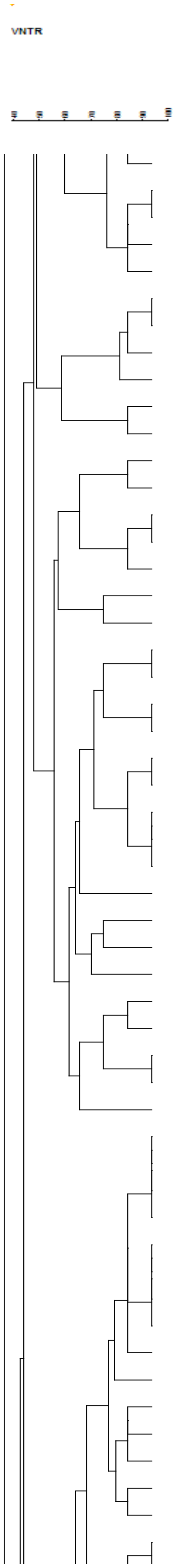


Figure 2. UPGMA clustering dendrogram of *B. abortus* isolates displaying 'nearest neighbor' status with the H. Edwards Winter 2011 isolates and relevant Park County, WY isolates



VNTR														
Hoof1	Hoof3	Hoof4	Hoof8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	Case No.	Owner	State	Animal ID	Host
2	3	9	2	3	1	5	2	3	8	02-0239	(b)(6) [redacted]	ID	AKN7636	cattle
2	3	10	2	3	1	5	2	3	9	02-0236	(b)(6) [redacted]	ID	82ALK6225	cattle
2	3	10	2	3	1	5	2	3	9	02-0242	(b)(6) [redacted]	ID	82ALK223	cattle
2	3	4	2	3	1	5	2	3	9	02-0235	(b)(6) (b)(6 [redacted]	ID	82VIU1921	cattle
2	3	6	2	3	1	5	2	3	9	02-0241	(b)(6) (b)(6 [redacted]	ID	82VME6918	cattle
1	4	6	2	3	1	5	2	4	4	05-0002	Zeman, David Brook.	SD	None Listed in Access	Bison
1	4	6	2	3	1	5	2	4	4	05-0005	Zeman, David	SD	None Listed in Access	Bison
2	4	6	2	3	1	5	2	4	4	04-0418	Cimpl Packing Co.	SD	83VNY6729	cattle
1	4	6	2	3	1	5	0	4	4	05-0001	Zeman, David Brook.	SD	None Listed in Access	Bison
2	3	7	2	3	1	8	2	4	4	05-0004	Zeman, David	SD	None Listed in Access	Bison
1	3	7	2	3	1	8	2	4	4	05-0007	Zeman, David	SD	None Listed in Access	Bison
1	2	4	2	3	1	6	2	3	8	05-0018	Edwards, Hank	WY	01W307	bison
1	2	4	2	3	1	7	2	3	8	05-0019	Edwards, Hank	WY	01W311	bison
0	2	4	2	3	1	8	2	3	6	B11-0141	WY Game and Fish De.	WY	GF101408	Bison
0	2	4	2	3	1	8	2	3	6	B11-0248	Edwards, Hank / WY G.	WY	10-1012 Retro	Bison
0	2	4	2	3	1	9	2	3	6	B11-0257	Edwards, Hank / WY G.	WY	10-1086 Hide	Bison
3	5	4	2	3	1	4	2	3	6	09-0295	Anderson, Neil (MFWP)	MT	BR081174	elk
3	5	4	2	3	1	5	2	4	6	09-0354	Anderson, Neil	MT	BR081308	elk
3	7	4	2	3	1	5	2	3	5	B11-0208	(b)(6) [redacted] y(b)(6) [redacted] (.	WY	81TUK5466 / 81BC2563	Cattle, Ang
3	7	4	2	3	1	5	2	3	5	B11-0210	(b)(6) [redacted] / Quarter.	WY	81TUK5466 / 81BC2563 .	Cattle, Bla
5	9	4	2	3	1	5	2	3	5	B11-0015	(b)(6) [redacted]	WY	83VSO4205	Cattle, Sim
5	9	4	2	3	1	5	2	3	5	B11-0038	(b)(6) [redacted]	WY	83AKC8899 / 10B-11757	Cattle
4	10	4	2	3	1	5	2	3	4	B11-0273	Edwards, Hank / WY G.	WY	10-0449 Iliac	Elk
4	10	4	2	3	1	5	2	3	4	B11-0274	Edwards, Hank / WY G.	WY	10-0449 Supra-mam	Elk
4	10	4	2	3	1	5	2	3	5	B11-0265	Edwards, Hank / WY G.	WY	07-3128 Iliac	Elk
4	10	4	2	3	1	5	2	3	5	B11-0266	Edwards, Hank / WY G.	WY	07-3128 Retro	Elk
4	10	4	2	3	1	5	2	3	5	B11-0267	Edwards, Hank / WY G.	WY	07-3128 Supra-mam	Elk
5	12	4	2	3	1	5	2	4	5	B11-0037	(b)(6) [redacted]	WY	83VS04205 / 10B-11758	Cattle
2	5	4	2	3	1	5	2	3	12	B10-0517	MT FWP	MT	BR090384	Elk
0	5	4	2	3	1	5	2	3	7	B11-0249	Edwards, Hank / WY G.	WY	10-1019 Retro	Bison
0	5	4	2	3	1	3	2	3	5	B11-0256	Edwards, Hank / WY G.	WY	10-1075 Supra-ing	Bison
6	7	4	2	3	1	3	2	3	5	B11-0270	Edwards, Hank / WY G.	WY	09-5139 Supra-mam	Elk
5	7	4	2	3	1	3	2	3	5	B11-0277	Edwards, Hank / WY G.	WY	10-3598 Supra-mam	Elk
4	7	5	2	3	1	3	2	3	5	B11-0271	Edwards, Hank / WY G.	WY	10-0439 Retro	Elk
4	7	5	2	3	1	3	2	3	5	B11-0272	Edwards, Hank / WY G.	WY	10-0439 Supra-mam	Elk
4	8	4	2	3	1	3	2	3	7	B11-0053	(b)(6) [redacted]	WY	83VZR4294 #White 80	Bison
2	8	4	2	4	1	3	2	4	3	08-0955	Yellowstone National Pa.	WY	YNP- 396	Bison
2	8	4	2	4	1	3	2	4	3	08-1103	Yellowstone National Pa.	MT	YNP-236	Bison
2	8	4	2	4	1	3	2	4	3	08-1165	Yellowstone National Pa.	WY	YNP-332	Bison
2	8	4	2	4	1	3	2	4	3	08-1180	Yellowstone National Pa.	WY	YNP-373	Bison
2	7	4	2	4	1	3	2	4	3	08-0440	Yellowstone National Pa.	WY	YNP-14	Bison
2	7	4	2	4	1	3	2	4	3	08-0859	Yellowstone National Pa.	WY	YNP-165	Bison
2	7	4	2	4	1	3	2	4	3	08-0927	Rhyan, Dr. Jack (Nat'l .	MT	4046	Bison
2	7	4	2	4	1	3	2	4	3	08-1106	Yellowstone National Pa.	MT	YNP-365	Bison
2	6	4	2	4	1	3	2	4	3	08-1106	Yellowstone National Pa.	MT	YNP-365	Bison
2	8	5	2	4	1	3	2	4	3	08-1118	Yellowstone National Pa.	WY	YNP-356	Bison
2	3	6	2	4	1	3	2	4	3	98-0612	(b)(6) [redacted]	TX	74DSF4190	cattle
2	6	6	2	4	1	3	2	4	3	98-0614	[redacted] ((b)(6)	TX	74DRA8471	cattle
2	5	6	2	4	1	3	2	4	3	98-0618	[redacted]	TX	74DUV1204	cattle
2	7	5	2	4	1	3	2	4	3	08-0986	Yellowstone National Pa.	WY	YNP- 387	Bison
2	7	6	2	4	1	3	2	4	3	98-0615	(b)(6) (b)(6) [redacted]	TX	74DUV9998	cattle
2	5	6	2	4	1	3	0	4	3	98-0613	[redacted] (b)(6)	TX	74DNA2041	cattle

National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388 Fax (515) 337-7315

Brucella Genotyping Report - Confidential

June 7, 2011

This is a genotyping report for five isolates of *Brucella abortus* obtained from tissues, blood, and semen from four bison from Montana:

Accession No. 11-018123, Case No. B11-0442, isolates S1 and S2, animal ID 'P11-016', young cow / abortion event, collected by Rick Wallen, submitted by the Montana Department of Livestock, received at NVSL April 12, 2011

Accession No. 11-019422, Case No. B11-0469, animal ID 'P11-017', submitted by the Montana Department of Livestock, received at NVSL April 19, 2011

Accession No. 11-020278, Case No. B11-0473, animal ID 'FJ0099', owner Turner – Flying D Ranch, submitted by the Montana Department of Livestock, received at NVSL on April 22, 2011

Accession No. 11-020659, Case No. B11-0480, animal ID 'YNP950043', field isolate from West Yellowstone, submitted by Pat Clarke, received at NVSL on April 26, 2011

The analysis included all 376 field isolates of *B. abortus* in the NVSL genotyping database, as well as 4 reference strains (e.g., strain 19, strain RB51, biovar 4, and biovar 2). Genotyping was performed using VNTR (Variable Number Tandem Repeat) analysis (also referred to as MLVA) of 10 individual loci, or 'hoofprints', within the *B. abortus* genome.

<u>Results:</u>

As with previous genotyping reports, a 'global' minimum spanning tree (MST) diagram was generated using the VNTR data and is provided in Figure 1 (below) to indicate the placement of the recent Montana bison isolates among other isolates in the NVSL database. Note that the Montana bison isolates are located in Figures 1A and 1B, and are labeled by red font.

Not unexpectedly, the Montana bison isolates cluster with other *B. abortus* biovar 1 isolates recovered from bison and elk in the Greater Yellowstone area.

Along with a MST analysis, a UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) analysis was conducted on all isolates. Because the resulting dendrogram is 8 pages long, those segments containing the Montana bison isolates are excerpted and presented in Figure 2A – 2D.

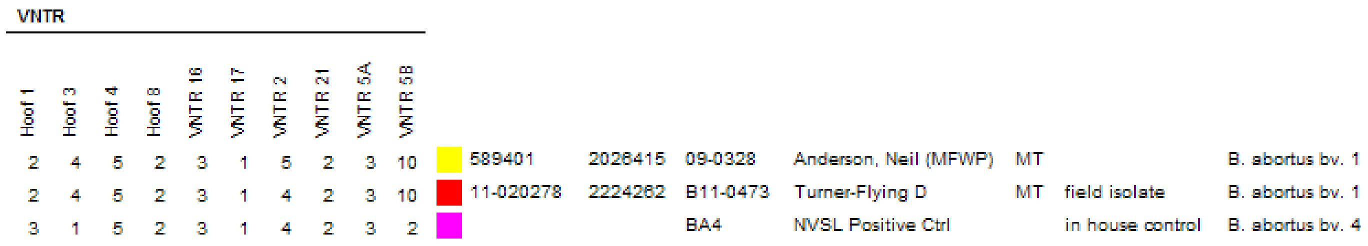
While none of the five Montana bison *B. abortus* showed an exact match with any other isolates in the database, they did show a high degree of similarity to VNTR patterns observed in other *B. abortus* isolates from GYA cattle, bison and elk.

Figure 2A provides the UPGMA excerpt for Case No. B11-0442, isolates S1 and S2; these form their own cluster or clade, with some degree of relatedness to other Montana bison isolates in the NVSL database.

Interestingly, case No. B11-0480 most closely clusters with a *B. abortus* 2007 Montana cattle isolate, Case No. 07-0266, from an animal owned by (b) (6) (later Trans Ova), differing at only one VNTR locus ('HOOF 1') by three repeats (Figure 2B). This is a rather close kinship, by *Brucella* genotyping standards.

The April 2011 Turner / Flying D Ranch isolate, Case No. B11-0473, also shows considerable similarity to an existing isolate, in this case No. 09-0328, a *B. abortus* obtained from a Montana elk (provided by Neil Anderson); the difference here is in the VNTR 2 locus by one repeat (Figure 2C). This also represents close kinship, in terms of *Brucella* spp. VNTR genotyping.

Interestingly, although isolates B11-0473 and 09-0328 are both biovar 1, in the MST these isolates cluster with a *B. abortus* biovar 4 control strain (BA4) (Figure 1A). Based on analysis of the VNTR profile for these three isolates, this clustering may be explained by the sharing of repeats for 5 of the 10 examined loci, rather than invoking circulation of biovar 4 among this particular cluster of animals:



VNTR																	
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B								
2	4	5	2	3	1	5	2	3	10	🟨	589401	2026415	09-0328	Anderson, Neil (MFWP)	MT		B. abortus bv. 1
2	4	5	2	3	1	4	2	3	10	🟥	11-020278	2224262	B11-0473	Turner-Flying D	MT	field isolate	B. abortus bv. 1
3	1	5	2	3	1	4	2	3	2	🟪			BA4	NVSL Positive Ctrl		in house control	B. abortus bv. 4

Note that another *B. abortus* isolate from a Turner / Flying D bison, Case No. B11-0011, obtained from tissues submitted by the Montana Department of Livestock Diagnostic Lab Division from animal ID 9-273/FC 0124 (received at the NVSL on October 19, 2010) is present in this particular excerpt of the UPGMA tree, but differs at more than one VNTR locus (Figure 2C).

[Yet another Flying D isolate, obtained in March 2011 from an aborted calf and typed as strain RB51 based on biochemical and PCR analyses, has a VNTR profile (5-4-2-2-4-1-2-2-6-3) that is markedly different from the bison isolates analyzed here, and is not contained in the excerpted UPGMA trees.]

Figure 2D provides the UPGMA excerpt for Case No. 11-0469; this bison isolate represents a standalone branch in the tree, with relatedness to *B. abortus* isolates from Montana elk and bison.

In conclusion, these bison isolates show genetic similarity to other *B. abortus* previously recovered from Montana cattle, elk, and bison. Isolate No. B11-0480, from a bison located in West Yellowstone, now represents the closest match to the (b) (6) / (b)(6) herd (later Trans Ova) cattle *B. abortus* isolate among all wildlife isolates in the NVSL database.

James Higgins, MS, PhD
Mycobacteria and Brucella Section
USDA/APHIS/NVSL
1920 Dayton Ave
Ames, IA 50010
515-337-7034 (fax 7315)

Figure 1A. Left-side portion of a minimum spanning tree (MST) analysis of the VNTR-10 profiles for all isolates of *Brucella abortus* included in this study. Color code: yellow = elk, red = bison, blue = cattle, pink = *B. abortus* reference strains, mauve = feral hog, dark green = reindeer. Smaller spheres contain one isolate, while larger spheres contain multiple, closely related isolates. Noteworthy clusters are identified by number. Labels denote state and year of origin; host animal species are provided in cluster descriptions when multiple species occupy a cluster. Inset at lower right depicts the entire MST, with the vertical bar dividing the tree into the left- and right-side portions depicted in Figures 1A and 1B, respectively. Total network length = 391, cophenetic correlation = 63%

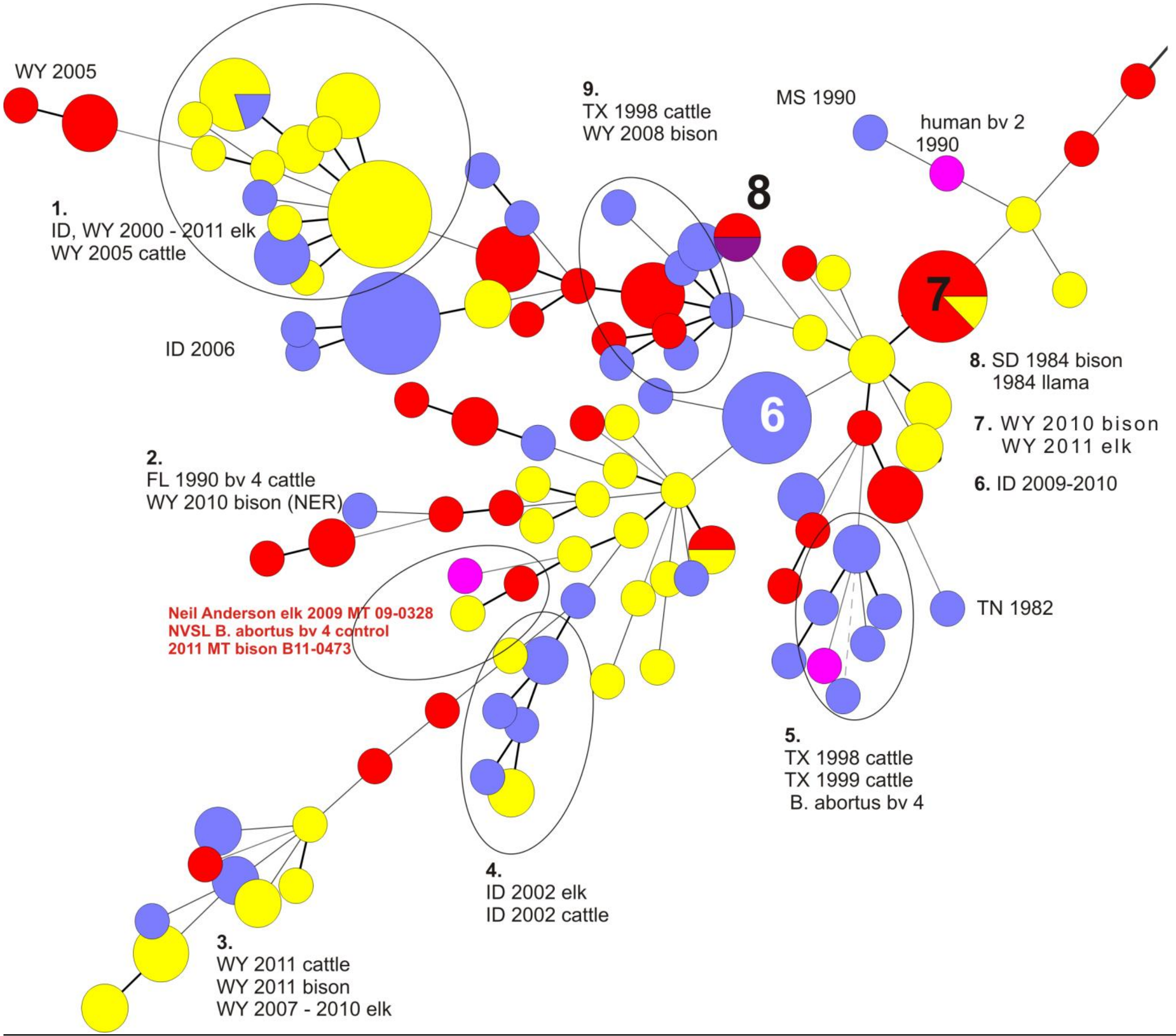


Figure 1B. Right-side portion of a minimum spanning tree (MST) analysis of the VNTR-10 profiles for all isolates of *Brucella abortus* included in this study. Color code: yellow = elk, red = bison, blue = cattle, pink = *B. abortus* reference strains, mauve = feral hog, dark green = reindeer. Smaller spheres contain one isolate, while larger spheres contain multiple, closely related isolates. Noteworthy clusters are identified by number. Labels denote state and year of origin; host animal species are provided in cluster descriptions when multiple species occupy a cluster. Inset at upper right depicts the entire MST, with the vertical bar dividing the tree into the left- and right-side portions depicted in Figures 1A and 1B, respectively. Total network length = 391, cophenetic correlation = 63%

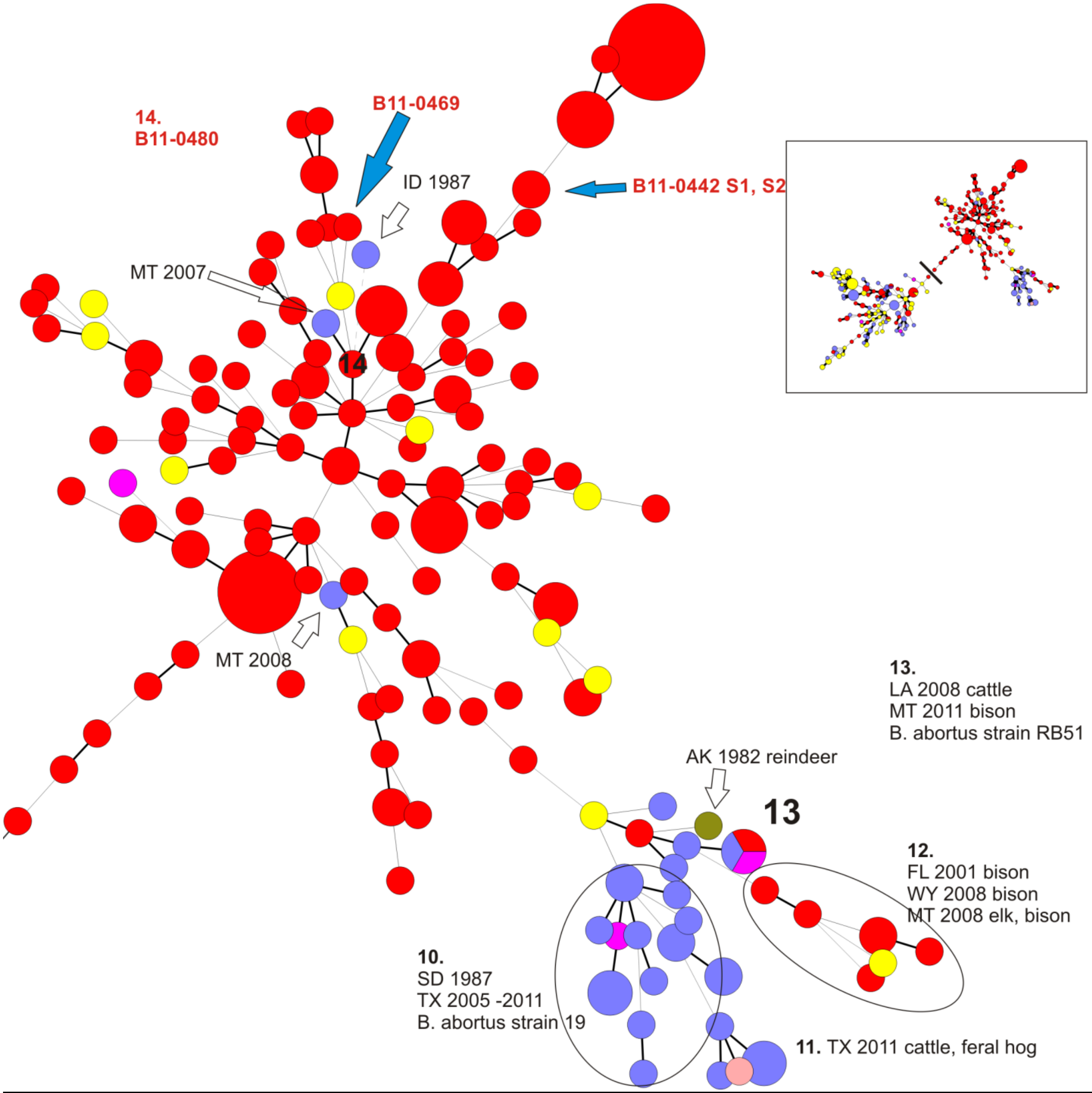
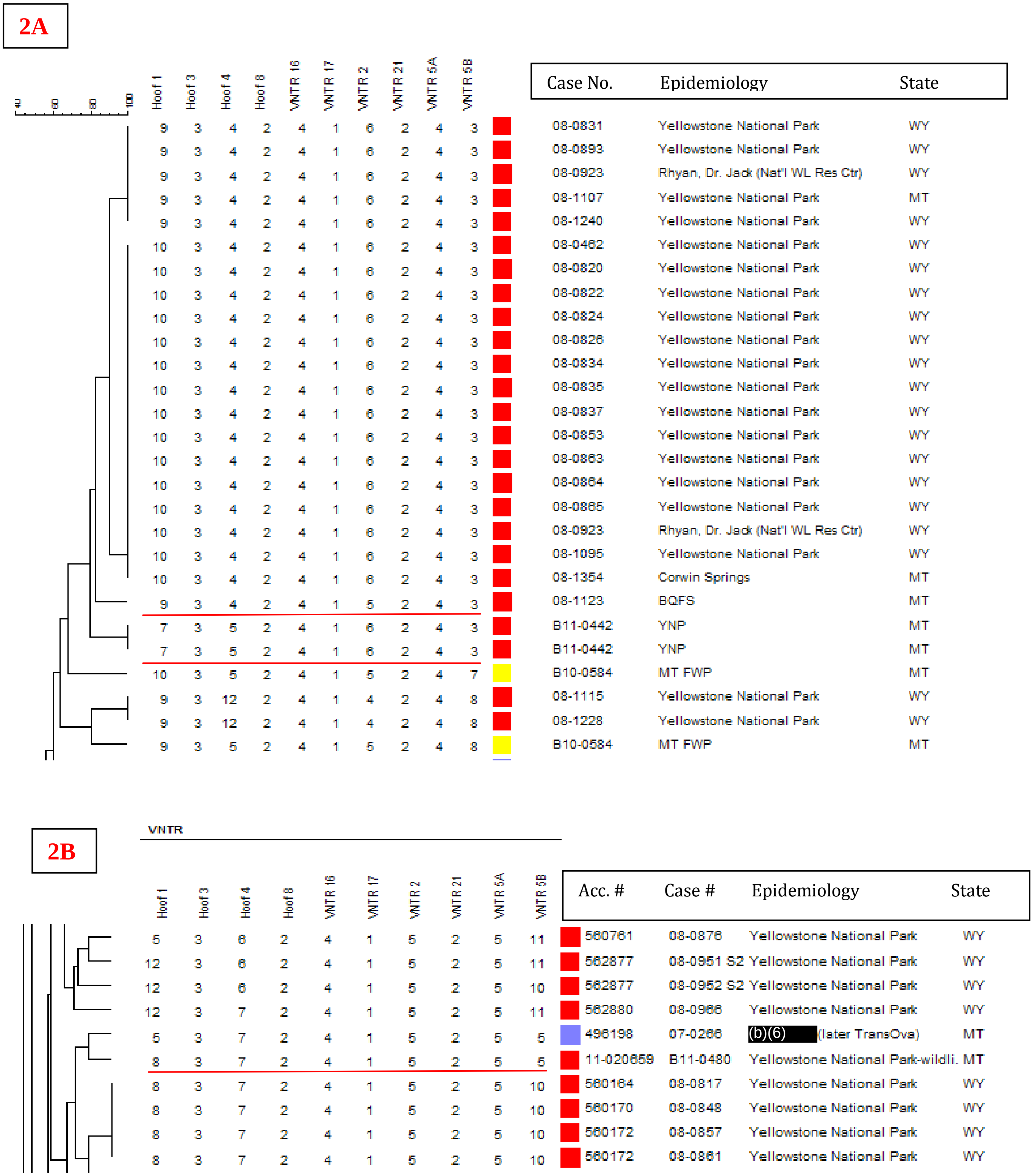


Figure 2. Selected excerpts (panels 2A – 2D) of a global UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) analysis dendrogram showing the locations of the five Montana bison isolates (demarcated by horizontal red lines). Note that both isolates S1 and S2 from case No. B11-0442 are present in excerpt 2A. Color code: red, bison; yellow, elk; blue, cattle



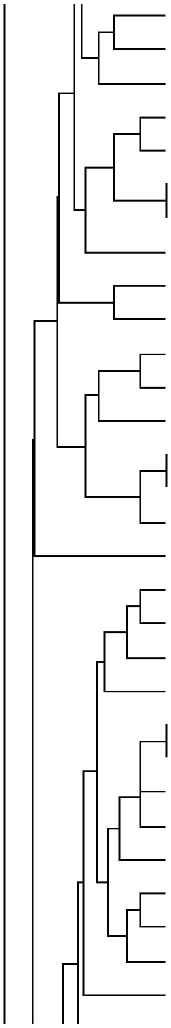
2A

Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B		Case No.	Epidemiology	State
9	3	4	2	4	1	6	2	4	3	🟥	08-0831	Yellowstone National Park	WY
9	3	4	2	4	1	6	2	4	3	🟥	08-0893	Yellowstone National Park	WY
9	3	4	2	4	1	6	2	4	3	🟥	08-0923	Rhyan, Dr. Jack (Nat'l WL Res Ctr)	WY
9	3	4	2	4	1	6	2	4	3	🟥	08-1107	Yellowstone National Park	MT
9	3	4	2	4	1	6	2	4	3	🟥	08-1240	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0462	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0820	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0822	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0824	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0826	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0834	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0835	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0837	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0853	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0863	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0864	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0865	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-0923	Rhyan, Dr. Jack (Nat'l WL Res Ctr)	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-1095	Yellowstone National Park	WY
10	3	4	2	4	1	6	2	4	3	🟥	08-1354	Corwin Springs	MT
9	3	4	2	4	1	5	2	4	3	🟥	08-1123	BQFS	MT
7	3	5	2	4	1	6	2	4	3	🟥	B11-0442	YNP	MT
7	3	5	2	4	1	6	2	4	3	🟥	B11-0442	YNP	MT
10	3	5	2	4	1	5	2	4	7	🟨	B10-0584	MT FWP	MT
9	3	12	2	4	1	4	2	4	8	🟥	08-1115	Yellowstone National Park	WY
9	3	12	2	4	1	4	2	4	8	🟥	08-1228	Yellowstone National Park	WY
9	3	5	2	4	1	5	2	4	8	🟨	B10-0584	MT FWP	MT

2B

Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B		Acc. #	Case #	Epidemiology	State
5	3	6	2	4	1	5	2	5	11	🟥	560761	08-0876	Yellowstone National Park	WY
12	3	6	2	4	1	5	2	5	11	🟥	562877	08-0951 S2	Yellowstone National Park	WY
12	3	6	2	4	1	5	2	5	10	🟥	562877	08-0952 S2	Yellowstone National Park	WY
12	3	7	2	4	1	5	2	5	11	🟥	562880	08-0966	Yellowstone National Park	WY
5	3	7	2	4	1	5	2	5	5	🟦	496198	07-0266	(b)(6) (later TransOva)	MT
8	3	7	2	4	1	5	2	5	5	🟥	11-020659	B11-0480	Yellowstone National Park-wildli.	MT
8	3	7	2	4	1	5	2	5	10	🟥	560164	08-0817	Yellowstone National Park	WY
8	3	7	2	4	1	5	2	5	10	🟥	560170	08-0848	Yellowstone National Park	WY
8	3	7	2	4	1	5	2	5	10	🟥	560172	08-0857	Yellowstone National Park	WY
8	3	7	2	4	1	5	2	5	10	🟥	560172	08-0861	Yellowstone National Park	WY

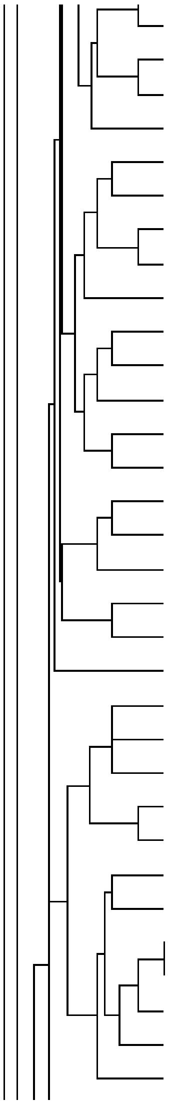
2C



	VNTR												
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B		Case #	Epidemiology	State
2	5	4	2	3	1	5	2	3	12	🟨	B10-0517	MT FWP	MT
0	5	4	2	3	1	5	2	3	7	🟥	B11-0249	Edwards, Hank / WY Game and Fish De.	WY
0	5	4	2	3	1	3	2	3	5	🟥	B11-0256	Edwards, Hank / WY Game and Fish De.	WY
6	7	4	2	3	1	3	2	3	5	🟨	B11-0270	Edwards, Hank / WY Game and Fish De.	WY
5	7	4	2	3	1	3	2	3	5	🟨	B11-0277	Edwards, Hank / WY Game and Fish De.	WY
4	7	5	2	3	1	3	2	3	5	🟨	B11-0271	Edwards, Hank / WY Game and Fish De.	WY
4	7	5	2	3	1	3	2	3	5	🟨	B11-0272	Edwards, Hank / WY Game and Fish De.	WY
4	8	4	2	3	1	3	2	3	7	🟥	B11-0053	(b)(6)	WY
3	5	4	2	3	1	4	2	3	6	🟨	09-0295	Anderson, Neil (MFWP)	MT
3	5	4	2	3	1	5	2	4	6	🟨	09-0354	Anderson, Neil	MT
1	2	4	2	3	1	6	2	3	8	🟥	05-0018	Edwards, Hank	WY
1	2	4	2	3	1	7	2	3	8	🟥	05-0019	Edwards, Hank	WY
5	2	4	2	3	1	6	2	3	2	🟦			FL
0	2	4	2	3	1	8	2	3	6	🟥	B11-0141	WY Game and Fish Dept. / Hank Edwar.	WY
0	2	4	2	3	1	8	2	3	6	🟥	B11-0248	Edwards, Hank / WY Game and Fish De.	WY
0	2	4	2	3	1	9	2	3	6	🟥	B11-0257	Edwards, Hank / WY Game and Fish De.	WY
8	3	4	2	3	1	6	2	7	4	🟪			FL
2	4	5	2	3	1	5	2	3	10	🟨	09-0328	Anderson, Neil (MFWP)	MT
2	4	5	2	3	1	4	2	3	10	🟥	B11-0473	Turner-Flying D	MT
2	4	5	2	3	1	4	2	3	9	🟨	B11-0140	WY Game and Fish Dept. / Hank Edwar.	WY
2	4	6	2	3	1	4	2	3	5	🟨	B11-0264	Edwards, Hank / WY Game and Fish De.	WY
2	4	4	2	3	1	4	2	3	11	🟨	B10-0091	MT FWP	MT
2	4	4	2	3	1	4	2	3	11	🟥	B11-0011	TEI- Flying D Ranch	MT
2	4	4	2	3	1	4	2	3	4	🟨	09-0443	Neil Anderson	MT
2	4	4	2	3	1	4	2	3	9	🟨	B10-0514	MT FWP	MT
2	4	4	2	3	1	4	2	4	4	🟨	B11-0143	Montana Conservation Genetics Lab	WY
2	4	4	2	3	1	5	2	3	8	🟨	05-0908	Fish, Wildlife & Parks	MT
2	4	4	2	3	1	7	2	3	8	🟨	B10-0252	MT FWP	MT
2	4	4	2	3	1	7	2	3	13	🟨	B10-0566	MT FWP	MT
2	4	4	2	3	2	5	2	3	4	🟦			OK

2D

VNTR



Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B		Case #	Epidemiology	State
9	3	8	2	4	1	5	2	5	7	🟨	B10-0273	MT FWP	MT
7	3	9	2	4	1	5	2	5	7	🟦	08-0416S1	(b)(6)	MT
7	3	9	2	4	1	6	2	5	7	🟨	B10-0529	MT FWP	MT
11	3	12	2	4	1	6	2	5	7	🟥	08-0934	Rhyan, Dr. Jack (Nat'l WL Res Ctr)	WY
8	3	5	2	4	1	4	2	5	8	🟥	08-0656	Yellowstone National Park	WY
8	3	8	2	4	1	4	2	5	5	🟨	09-0286	Anderson, Neil (MFWP)	MT
8	3	6	2	4	1	4	2	5	7	🟥	08-0930	Rhyan, Dr. Jack (Nat'l Wildlife Research.	MT
7	3	6	2	4	1	4	2	5	7	🟥	08-0930	Rhyan, Dr. Jack (Nat'l Wildlife Research.	MT
12	3	3	2	4	1	4	2	5	9	🟥	08-0928	Rhyan, Dr. Jack (Nat'l WL Res Ctr)	WY
10	3	7	2	4	1	4	2	5	11	🟥	08-0389	University of Montana	MT
9	3	7	2	4	1	4	2	5	5	🟨	09-0286	Anderson, Neil (MFWP)	MT
9	3	7	2	4	1	4	2	3	10	🟥	B11-0469	YNP	MT
8	3	7	2	4	1	4	1	5	7	🟥	05-0911	Yellowstone National Park West Yello.	MT
9	3	7	2	4	1	4	1	5	6	🟥	05-1008	Yellowstone National Park	WY
7	3	7	2	4	1	7	2	5	12	🟥	02-0137	MT. Dept. of Livestock	MT
13	3	7	2	4	1	7	2	5	10	🟥			MT
10	4	7	2	4	1	7	2	5	10	🟨	09-0276	Anderson, Neil (MFWP)	MT
8	3	9	2	4	1	7	2	5	11	🟥	06-0402	Yellowstone Natioinal Park: Rick Wallen	WY
8	3	5	2	4	1	7	2	6	11	🟥	08-1182	Yellowstone National Park	WY
7	3	8	2	4	2	5	2	5	9	🟥	05-0822	Yellowstone National Park	WY
7	4	6	2	4	1	4	2	5	9	🟥	05-1001	Yellowstone National Park	WY
10	4	6	2	4	1	7	2	5	9	🟥	08-0849	Yellowstone National Park	WY
10	4	6	2	4	1	4	2	5	3	🟥	08-1247	Yellowstone National Park	WY
9	4	6	2	4	1	7	2	5	11	🟥	08-1232	Yellowstone National Park	WY
9	4	6	2	4	1	6	2	5	11	🟥	08-1232	Yellowstone National Park	WY
14	4	6	2	4	1	5	2	5	10	🟥	08-0393	University of Montana	MT
4	4	11	2	4	1	5	2	5	10	🟥	08-0843	Yellowstone National Park	WY
9	4	7	2	4	1	5	2	5	10	🟥	08-0973	Yellowstone National Park	WY
9	4	7	2	4	1	5	2	5	10	🟥	08-1160	Yellowstone National Park	WY
9	4	8	2	4	1	5	2	5	10	🟨	08-0151	Montana Fish Wildlife & Parks	MT
9	4	8	2	4	1	6	2	5	10	🟥	08-0410	YNP	MT
9	4	5	2	4	1	5	1	5	10	🟥	05-1282	APHIS, VS	MT

National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388   Fax (515) 337-7315

Brucella Genotyping Final Report - Confidential

March 7, 2011

This is a genotyping report for NVSL Accession No. 11-007611, two isolates of *Brucella abortus* obtained from tissues and milk from a cow (animal ID 81TUK5466) owned by (b)(6) LLC of Cody, Wyoming. The samples were received at the NVSL on February 15, 2011 and cultures subjected to VNTR analysis during the week of February 27, 2011.

The analysis included 326 *B. abortus* strains of cattle, elk, and bison origin in the NVSL BioNumerics database for which different VNTR (Variable Number Tandem Repeat) analysis (also referred to as MLVA) profiles were available. The VNTR analysis targeted 10 individual loci, or ‘hoofprints’, within the *B. abortus* genome.

<u>Results:</u>

Results indicate that the two *B. abortus* isolates from the (b)(6) cow have the same VNTR profile (3-7-4-2-3-1-5-2-3-5); this profile is not shared with any other strains in the NVSL database. The ‘nearest neighbors’ to these isolates are those recovered in October and November 2010 from cattle owned by (b)(6) / Worland Livestock Auction, Meeteetse, Wyoming.

More distantly related to the (b)(6) clade are two 2009 Montana elk isolates from Neil Anderson, Case Nos. 09-0295 and 09-0354. While the genotyping analysis on its own does not definitively link the (b)(6) isolates with those acquired from the (b)(6) herd, it does suggest the possibility of shared epidemiologic links between these two groups of animals.

Table 1 (below) provides detailed information on the isolates demonstrated to have the closest association with the *B. abortus* strains recovered from the (b)(6) animal.

Because of the large number of isolates (n = 326) used in the genotyping analysis, I have provided two sets of Figures (below). The first set, Figures 1A and 1B, is designed to provide a comprehensive overview of the relationships among all the VNTR profiles from all *B. abortus* isolates in the NVSL BioNumerics database using a Minimum Spanning Tree (MST) analysis. The clade containing the (b)(6) cattle isolates is clade number **12** in Figure 1B. The spheres in the MST 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.

Figure 2 is an excerpt from a much larger (i.e., 7 pages) diagram, and focuses on the (b)(6) samples and their immediate neighbors. Figure 2 depicts the VNTR profiles generated from the ‘nearest neighbor’ strains used for the comparison, and a phenogram (the far left side of the Figure) generated via UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) to show general relatedness of the strains included in the comparison. The (b)(6) isolates are indicated by the red outline, and horizontal red lines are used to demarcate the VNTR profiles for these isolates.

You may notice that the tandem repeat values for the isolates in Figure 2 may have changed from the values provided in previous genotyping reports; for example, the (b)(6) genotyping report emailed out on December 3, 2010. In that report, the (b)(6) cattle isolate with Case No. B11-0015 had the profile 5-9-4-2-3-**3**-5-**3**-**4**-**6**; it was changed to 5-9-4-2-3-**1**-5-**2**-**3**-**5** in February 2011, after we sequenced the tandem repeat regions from a number of *B. abortus* isolates as part of a QA / QC check on the accuracy of our repeat calls.

Since the corrections made as a result of our sequencing analysis all involved adding or subtracting tandem repeat values by the same increment for all isolates in our database, the distance relationships between isolates provided in previous MST and UPGMA diagrams in previous genotyping reports remain valid. However, we are proceeding with more in-depth QA / QC assays on VNTR data for older isolates and we may need to implement further corrections to these isolates. Accordingly, the positioning of *B. abortus* isolates from 2002 – 2008 (the year of culture is indicated by Case No.) in the Figures should be interpreted with some caution, as their placement may be altered in forthcoming analyses.

James Higgins, MS, PhD
Mycobacteria and Brucella Section
USDA/APHIS/NVSL
1920 Dayton Ave
Ames, IA 50010
515-337-7034 (fax 7315)

Table 1. List of isolates used in the UPGMA dendrogram assembly (Figure 2)

Acc. No.	Case No.	Owner	State	Animal ID	Epidemiology	Host	Bacterial strain
565739	08-1161	Yellowstone National Park	WY	YNP-352	surveillance	Bison	B. abortus bv. 1
560167	08-0832	Yellowstone National Park	WY	YNP-162	surveillance	Bison	B. abortus bv. 1
560166	08-0830	Yellowstone National Park	WY	YNP-150	surveillance	Bison	B. abortus bv. 1
428657	06-0287	(b)(6) .	TX	NL		Cattle	B. abortus Strain 19
449219	06-0763	(b)(6)	TX	NL		Cattle	B. abortus Strain 19
338938	05-0012	[redacted], (b)(6)	TX	NL		Cattle	B. abortus Strain 19
396213	05-1317	King Ranch Inc.	TX	NL	field isolate	Cattle	B. abortus Strain 19
405615	06-0025	(b)(6)	TX	NL	field isolate	Cattle	B. abortus Strain 19
							B. abortus Strain 19
	Strain 19	NVSL ctrl			Isolate used as biochem		B. abortus Strain 19
436983	06-0513	(b)(6) .	TX	74FDL6104	field isolate, Calliham, TX	Cattle	B. abortus Strain 19
10-029872	B10-1032	(b)(6) (b)(6)	TX	74HK0600	isolate 1 from young cow	Cattle	B. abortus Strain 19
11-000244	B11-0194	[redacted]	TX	74FGM8662	field isolate	Cattle, Do.	B. abortus Strain 19
451292	06-0861 A	B. abortus strain 19	TX	74EDJ9879	(b)(6) r (b)(6)	Cattle	B. abortus strain 19
512872	07-0503	(b)(6) (b)(6)	TX	Sample 1 / 74FGD4607		Cattle	B. abortus Strain 19
441658	06-0537	(b)(6)	TX	NL		Cattle	B. abortus Strain 19
451292	06-0861 B	(b)(6)	TX	74EDJ9879	(b)(6) (b)(6)	Cattle	B. abortus strain 19
471330	07-0062	[redacted]	TX	Sample 1 74SVW6276		Cattle	B. abortus Strain 19
243781	03-0072	B. abortus strain 2308	ID	yellow 332 (N03-060)	Canyon, ID	elk	B. abortus bv. 1, strai
530609	08-0101	Louisiana State University	LA	1922 / 2007-1	isolate from cow / Elzer ou.	Cattle	B. abortus bv. 1, Stra
586532	09-0269	B. abortus RB51	TX	Sample 1 / 74SQY3675	(b)(6)	Cattle	B. abortus RB51
11-003420	B11-0202	(b)(6)	TX	74FUT7066	field isolate	Cattle	B. abortus bv. 1
11-004685	B11-0204	[redacted] (b)(6)	TX	74FUT7069 / 74PK0204 /.	field isolate	Cattle, Do.	B. abortus bv. 1
11-006383	B11-0206	(b)(6)	TX	74EWR4840 / X49925, Ve.	field isolate	Cattle	B. abortus bv. 1
339944	05-0018	[redacted]	WY	01W307	field isolate NER 2001	bison	B. abortus bv. 1
339944	05-0019	[redacted], (b)(6)	WY	01W311	field isolate NER 2001	bison	B. abortus bv. 1
10-061623	B11-0141	WY Game and Fish Dept. / Han.	WY	GF101408	bison from NER (adult)	Bison	B. abortus bv. 1
10-057783	B11-0053	(b)(6) (b)(6)	WY	83VZR4294 #White 80	field isolate	Bison	B. abortus bv. 1
11-007611	B11-0208	[redacted] .	WY	81TUK5466 / 81BC2563	field isolate	Cattle, Ang.	B. abortus bv. 1
11-008075	B11-0210	(b)(6) / (b)(6)	WY	81TUK5466 / 81BC2563 .	field isolate	Cattle, Blac.	B. abortus bv. 1
10-054053	B11-0015	(b)(6)	WY	83VSO4205		Cattle, Si.	B. abortus bv. 1
field isolate	B11-0038	[redacted] (b)(6)	WY	83AKC8899 / 10B-11757	field isolate	Cattle	B. abortus bv. 1
10-054515	B11-0037	[redacted]	WY	83VS04205 / 10B-11758	field isolate	Cattle	B. abortus bv. 1
588409	09-0295	Anderson, Neil (MFWP)	MT	BR081174	field isolate	elk	B. abortus bv. 1
590672	09-0354	Anderson, Neil	MT	BR081308	field isolate	elk	B. abortus bv. 1
09-032078	B10-0091	MT FWP	MT	BR090731	field isolate	Elk	B. abortus bv. 1
10-053081	B11-0011	TEI- Flying D Ranch	MT	9-273/FC 0124	field isolate	Bison	B. abortus bv. 1
596921	09-0443	Neil Anderson	MT	BR080548	field isolate	elk	B. abortus bv. 1
10-001635	B10-0514	MT FWP	MT	BR090381	field isolate	Elk	B. abortus bv. 1
10-061623	B11-0140	WY Game and Fish Dept. / Han.	WY	07W4970	fetus, Cody, 2007 ?	Elk	B. abortus bv. 1
10-061994	B11-0143	Montana Conservation Genetics.	WY	00W5798	fetus, Black Butte FG, 20.	Elk	B. abortus bv. 1, bv 4
370796	05-0908	Fish, Wildlife & Parks	MT	unavail	South Fork Indian Creek	elk	B. abortus bv. 1
09-033523	B10-0252	MT FWP	MT	BR092578	field isolate	Elk	B. abortus bv. 1
10-008688	B10-0566	MT FWP	MT	BR090476	field isolate	Elk	B. abortus bv. 1
589401	09-0328	Anderson, Neil (MFWP)	MT	BR081200		elk	B. abortus bv. 1
163343	02-0123	Idaho Fish & Game Wildlife Lab	ID	60217	Conant Creek	elk	B. abortus bv. 1
163343	02-0124	Animal Health Labs	ID	60218	Conant Creek	elk	B. abortus bv. 1
179599	02-0237	(b)(6)	ID	82AKN7632	2002 ID herd	cattle	B. abortus bv. 1
179599	02-0239	(b)(6)	ID	AKN7636	2002 ID herd	cattle	B. abortus bv. 1
179599	02-0236	(b)(6)	ID	82ALK6225	2002 ID herd	cattle	B. abortus bv. 1
179599	02-0242	(b)(6)	ID	82ALK223	2002 ID herd	cattle	B. abortus bv. 1
179599	02-0235	(b)(6)	ID	82VIU1921	2002 ID herd	cattle	B. abortus bv. 1
179599	02-0241	(b)(6)	ID	82VME6918	2002 ID herd	cattle	B. abortus bv. 1
10-001635	B10-0517	MT FWP	MT	BR090384	field isolate	Elk	B. abortus bv. 1
560760	08-0870	Yellowstone National Park	WY	YNP-298	surveillance	Bison	B. abortus bv. 1
10-061994	B11-0147	Montana Conservation Genetics.	WY	06W2080	joint, NER 2006	Elk	B. abortus bv. 1, bv 4
09-033518	B10-0203	MT FWP	MT	BR092077	field isolate	Elk	B. abortus bv. 1
09-033531	B10-0325	MT FWP	MT	BR092992	field isolate	Elk	B. abortus bv. 1

Figure 1A. Minimum Spanning Tree (MST) of *B. abortus* isolates from elk, bison, and cattle from the NVSL database. **This is the left-side portion of a larger diagram (inset, lower right)**

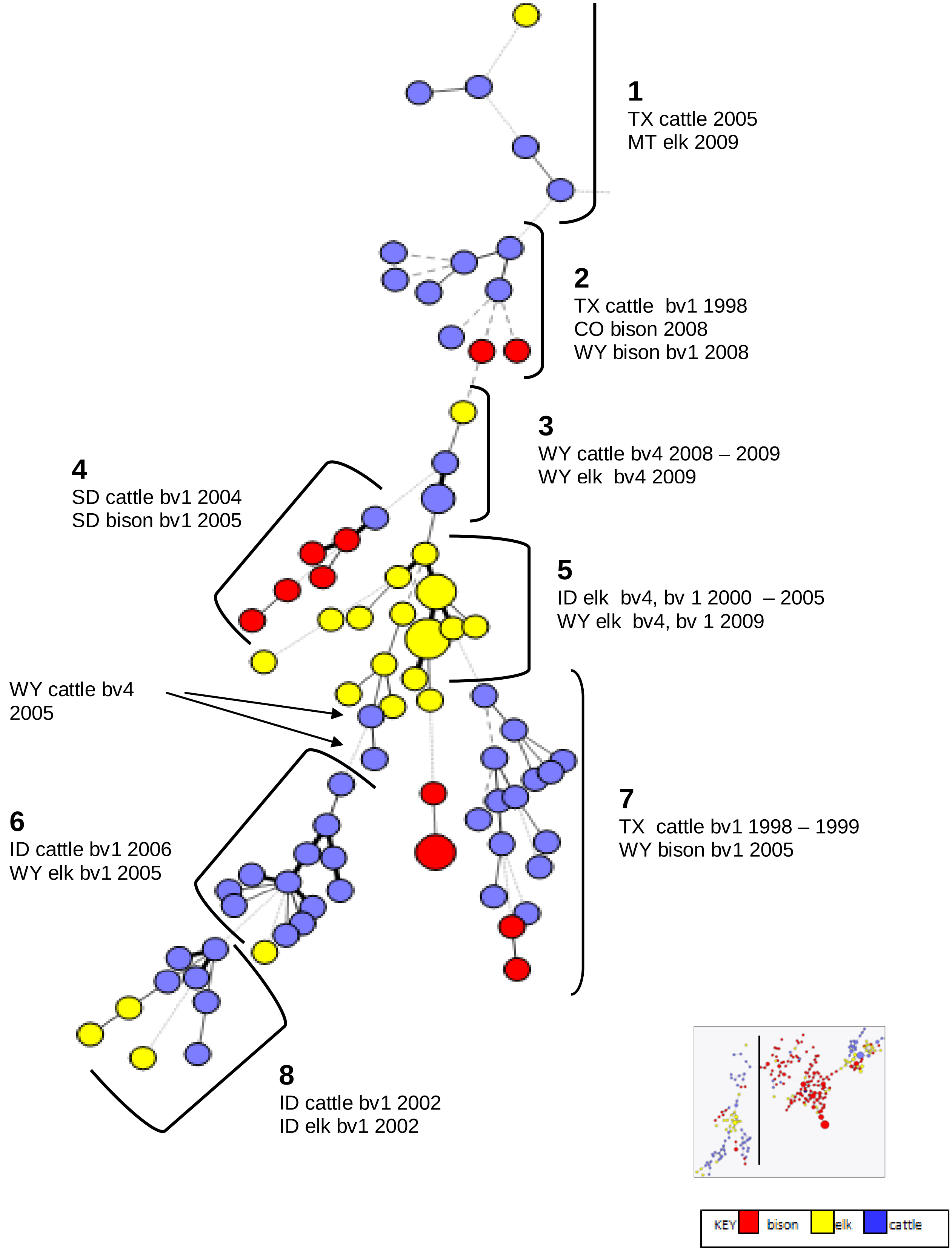


Figure 1B. Minimum Spanning Tree (MST) of *B. abortus* isolates from elk, bison, and cattle from the NVSL database. **This is the right-side portion of a larger diagram (inset, lower right)**

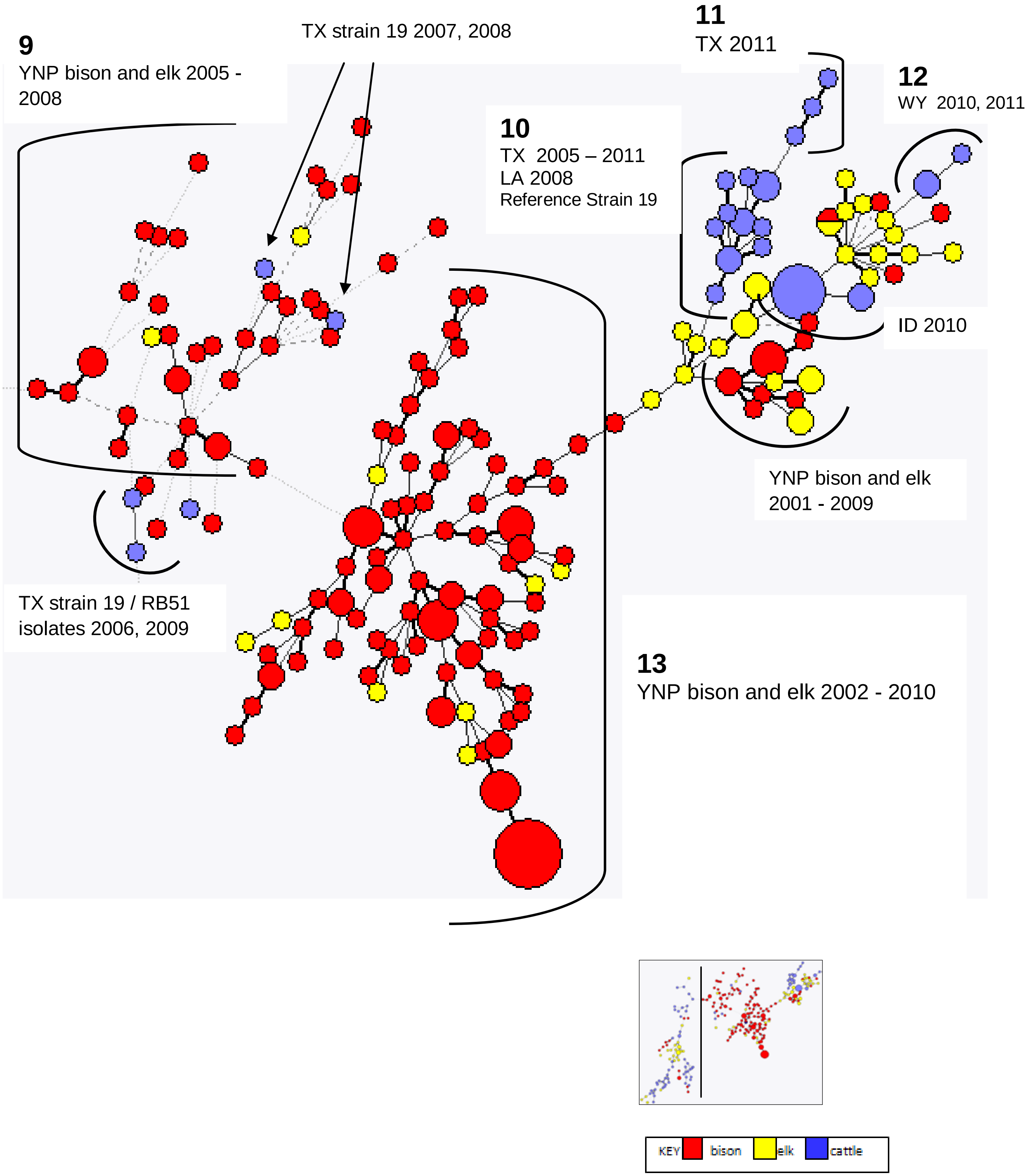
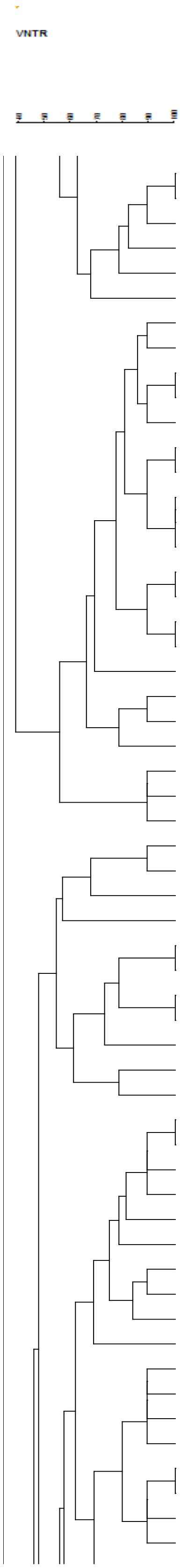


Figure 2. UPGMA clustering dendrogram of *B. abortus* isolates displaying 'nearest neighbor' status with the (b)(6) isolates



VNTR														
Hoof1	Hoof3	Hoof4	Hoof8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	Case No.	Owner	State	Animal ID	Host
8	3	8	2	4	1	6	2	6	12	08-0405	YNP	MT	8-344 / P08- / Bison	bison
8	3	8	2	4	1	6	2	6	12	08-1112	Yellowstone National Park	WY	YNP-350	Bison
8	3	9	2	4	1	6	2	6	12	08-1256	Corwin Springs	MT	24-08	Bison
7	3	9	2	4	1	6	2	6	12	08-1161	Yellowstone National Park	WY	YNP-352	Bison
10	3	5	2	4	1	6	2	6	12	08-0832	Yellowstone National Park	WY	YNP-162	Bison
8	3	8	2	4	1	8	2	6	14	08-0830	Yellowstone National Park	WY	YNP-150	Bison
4	4	2	2	4	1	2	2	4	5	06-0287	(b)(6)(6)	TX	NL	Cattle
9	4	2	2	4	1	2	2	4	5	06-0763	(b)(6) (b)(6)	TX	NL	Cattle
6	4	2	2	4	1	2	2	4	5	05-0012	[redacted]	TX	NL	Cattle
6	4	2	2	4	1	2	2	4	5	05-1317	King Ranch Inc.	TX	NL	Cattle
6	7	2	2	4	1	2	2	4	5	06-0025	(b)(6)	TX	NL	Cattle
5	4	2	2	4	1	2	2	4	5					B. abortus Strain 19
5	4	2	2	4	1	2	2	4	5	Strain 19	NVSL ctrl			
5	4	2	2	4	1	2	2	4	6	06-0513	(b)(6)	TX	74FDL6104	Cattle
5	4	2	2	4	1	2	2	4	6	B10-1032	(b)(6) (b)(6)	TX	74HK0600	Cattle
5	4	2	2	4	1	2	2	4	6	B11-0194	[redacted]	TX	74FGM8662	Cattle, Domest
7	4	2	2	4	1	2	2	4	9	06-0861.	B. abortus strain 19	TX	74EDJ9879	Cattle
7	4	2	2	4	1	2	2	4	9	07-0503	(b)(6)	TX	Sample 1 / 74FGD4607	Cattle
8	4	2	2	4	1	2	2	4	9	06-0537	(b)(6)	TX	NL	Cattle
8	4	2	2	4	1	2	2	4	9	06-0861.	(b)(6) (b)(6)	TX	74EDJ9879	Cattle
6	4	2	2	4	1	2	2	5	7	07-0062	[redacted]	TX	Sample 1 74SVW6276	Cattle
6	4	2	2	4	1	2	2	6	3	03-0072	B. abortus strain 2308	ID	yellow 332 (N03-060)	elk
5	4	2	2	4	1	2	2	6	3	08-0101	Louisiana State University	LA	1922 / 2007-1	Cattle
7	4	4	2	4	1	2	2	6	3	09-0269	B. abortus RB51	TX	Sample 1 / 74SQY3675	Cattle
8	5	2	2	5	1	2	2	4	7	B11-0202	(b)(6)	TX	74FUT7066	Cattle
8	5	2	2	5	1	2	2	4	6	B11-0204	(b)(6)	TX	74FUT7069 / 74PK0204 /.	Cattle, Domestic
8	5	2	2	5	1	2	2	4	4	B11-0206	[redacted] (b)(6)	TX	74EWR4840 / X49925, Ve.	Cattle
1	2	4	2	3	1	6	2	3	8	05-0018	Edwards, Hank	WY	01W307	bison
1	2	4	2	3	1	7	2	3	8	05-0019	Edwards, Hank	WY	01W311	bison
0	2	4	2	3	1	8	2	3	6	B11-0141	WY Game and Fish Dept. .	WY	GF101408	Bison
4	8	4	2	3	1	3	2	3	7	B11-0053	(b)(6)	WY	83VZR4294 #White 80	Bison
3	7	4	2	3	1	5	2	3	5	B11-0208	[redacted] (b)(6) (Mar.	WY	81TUK5466 / 81BC2563	Cattle, Angus
3	7	4	2	3	1	5	2	3	5	B11-0210	(b)(6) Ci.	WY	81TUK5466 / 81BC2563 .	Cattle, Black An
5	9	4	2	3	1	5	2	3	5	B11-0015	(b)(6)	WY	83VSO4205	Cattle, Simment
5	9	4	2	3	1	5	2	3	5	B11-0038	[redacted] (b)(6)	WY	83AKC8899 / 10B-11757	Cattle
5	12	4	2	3	1	5	2	4	5	B11-0037	[redacted]	WY	83VS04205 / 10B-11758	Cattle
3	5	4	2	3	1	4	2	3	6	09-0295	Anderson, Neil (MFWP)	MT	BR081174	elk
3	5	4	2	3	1	5	2	4	6	09-0354	Anderson, Neil	MT	BR081308	elk
2	4	4	2	3	1	4	2	3	11	B10-0091	MT FWP	MT	BR090731	Elk
2	4	4	2	3	1	4	2	3	11	B11-0011	TEI- Flying D Ranch	MT	9-273/FC 0124	Bison
2	4	4	2	3	1	4	2	3	4	09-0443	Neil Anderson	MT	BR080548	elk
2	4	4	2	3	1	4	2	3	9	B10-0514	MT FWP	MT	BR090381	Elk
2	4	5	2	3	1	4	2	3	9	B11-0140	WY Game and Fish Dept. .	WY	07W4970	Elk
2	4	4	2	3	1	4	2	4	4	B11-0143	Montana Conservation Ge.	WY	00W5798	Elk
2	4	4	2	3	1	5	2	3	8	05-0908	Fish, Wildlife & Parks	MT	unavail	elk
2	4	4	2	3	1	7	2	3	8	B10-0252	MT FWP	MT	BR092578	Elk
2	4	4	2	3	1	7	2	3	13	B10-0566	MT FWP	MT	BR090476	Elk
2	4	5	2	3	1	5	2	3	10	09-0328	Anderson, Neil (MFWP)	MT	BR081200	elk
2	3	13	2	3	1	5	2	3	8	02-0123	Idaho Fish & Game Wildlif.	ID	60217	elk
2	3	7	2	3	1	5	2	3	8	02-0124	Animal Health Labs	ID	60218	elk
2	3	10	2	3	1	5	2	3	8	02-0237	(b)(6)	ID	82AKN7632	cattle
2	3	9	2	3	1	5	2	3	8	02-0239	(b)(6)	ID	AKN7636	cattle
2	3	10	2	3	1	5	2	3	9	02-0236	(b)(6)	ID	82ALK6225	cattle
2	3	10	2	3	1	5	2	3	9	02-0242	(b)(6)	ID	82ALK223	cattle
2	3	4	2	3	1	5	2	3	9	02-0235	(b)(6)	ID	82VIU1921	cattle
2	3	6	2	3	1	5	2	3	9	02-0241	(b)(6)	ID	82VME6918	cattle

National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388   Fax (515) 337-7315

Brucella Genotyping Final Report - Confidential

November 16, 2011

This is a genotyping report for the following two isolates of *Brucella abortus* recovered from the (b)(6) (b)(6) herd in Meeteetse, Park County, Wyoming, in October, 2011:

Accession 11-049870, (b)(6) case No. B12-0002, animal ID 83VZZ7726, 13 month-old female Angus. Tissues collected on Sept. 29, received at the NVSL on Oct. 4.

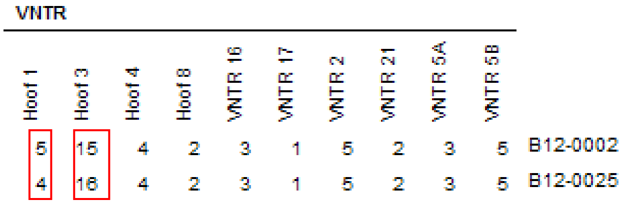
Accession 11-053717, (b)(6), case No. B12-0025, animal ID 83VZZ8288 white tag 772, 13 month-old female Angus. Tissues collected on Oct. 19, received at the NVSL on Oct. 21.

[According to VS personnel, the owner of (b)(6), has indicated that he observed some of his younger cattle licking or sniffing an elk calf.]

The genotyping analysis included 372 *B. abortus* strains of cattle, elk, and bison origin in the NVSL BioNumerics database for which different VNTR (Variable Number Tandem Repeat) analysis (also referred to as MLVA) profiles were available. The VNTR analysis targeted 10 individual loci, or 'hoofprints', within the *B. abortus* genome.

Results:

Interestingly, the isolates from the two (b)(6) Angus not only possessed different VNTR profiles, mediated by differences at two loci (boxed areas below), but they each possessed an unusually large number of tandem repeats for locus HOOF 3:



VNTR

Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	
5	15	4	2	3	1	5	2	3	5	B12-0002
4	16	4	2	3	1	5	2	3	5	B12-0025

The previous 'record' for the largest number of tandem repeats for HOOF 3 that we have observed is 12, associated with a 2010 *B. abortus* isolate from a bovine owned by (b)(6) of Meeteetse, WY. Indeed, one of the hallmarks of some of the *B. abortus* strains recovered from Meeteetse cattle and elk is the presence of ≥ 10 repeats for HOOF 3.

A minimum spanning tree (MST) analysis of the VNTR profiles is provided in Figure 1 (below). Not surprisingly, the (b)(6) isolates segregated into a cluster occupied by other livestock and wildlife isolates associated with the Meeteetse area from 2007 – 2010.

As confirmation of the genetic relatedness indicated by the MST analysis, a UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) analysis of the VNTR profiles was performed, and generated the dendrogram shown in Figure 2 (below). As with the MST analysis, the two (b)(6) isolates clustered with isolates of Meeteetse origin.

Within the clade of Meeteetse isolates, there is a rather large disparity in the branch placement of the two (b)(6) (b)(6) isolates, despite the fact that they differ at only 2 of their 10 VNTR loci; this disparity also was observed in the MST analysis. Without additional, in-depth analyses of the genetic makeup of the two isolates, the significance of this branching assignment is unclear, but may reflect reduced weighting by the MST and UPGMA algorithms to alterations in tandem repeat numbers associated with the HOOF 3 locus, as opposed to the HOOF 1 locus.

The isolate (B11-0053) recovered in November 2010 from a bison owned by (b)(6), also located in Meeteetse / Park County, displayed limited genetic similarity to the elk and cattle strains, and thus constitutes the out-group in the ‘Meeteetse clade’ comparison (Figure 2).

In conclusion, the genotyping data suggest that the lineage of *B. abortus* responsible for the infections in the (b)(6) Angus cows has been circulating in elk and cattle in the Meeteetse / Park County area since ~ 2007.

James Higgins, MS, PhD
Mycobacteria and Brucella Section
USDA/APHIS/NVSL
1920 Dayton Ave
Ames, IA 50010
515-337-7034 (fax 7315)

Figure 1. Minimum Spanning Tree (MST) of *B. abortus* isolates from elk, bison, and cattle from the NVSL database. **This is the left-side portion of a larger diagram (inset, lower right).** (b)(6) / (b)(6) **isolates are present in the bottom left portion of the tree; text color indicates the species of host animal**

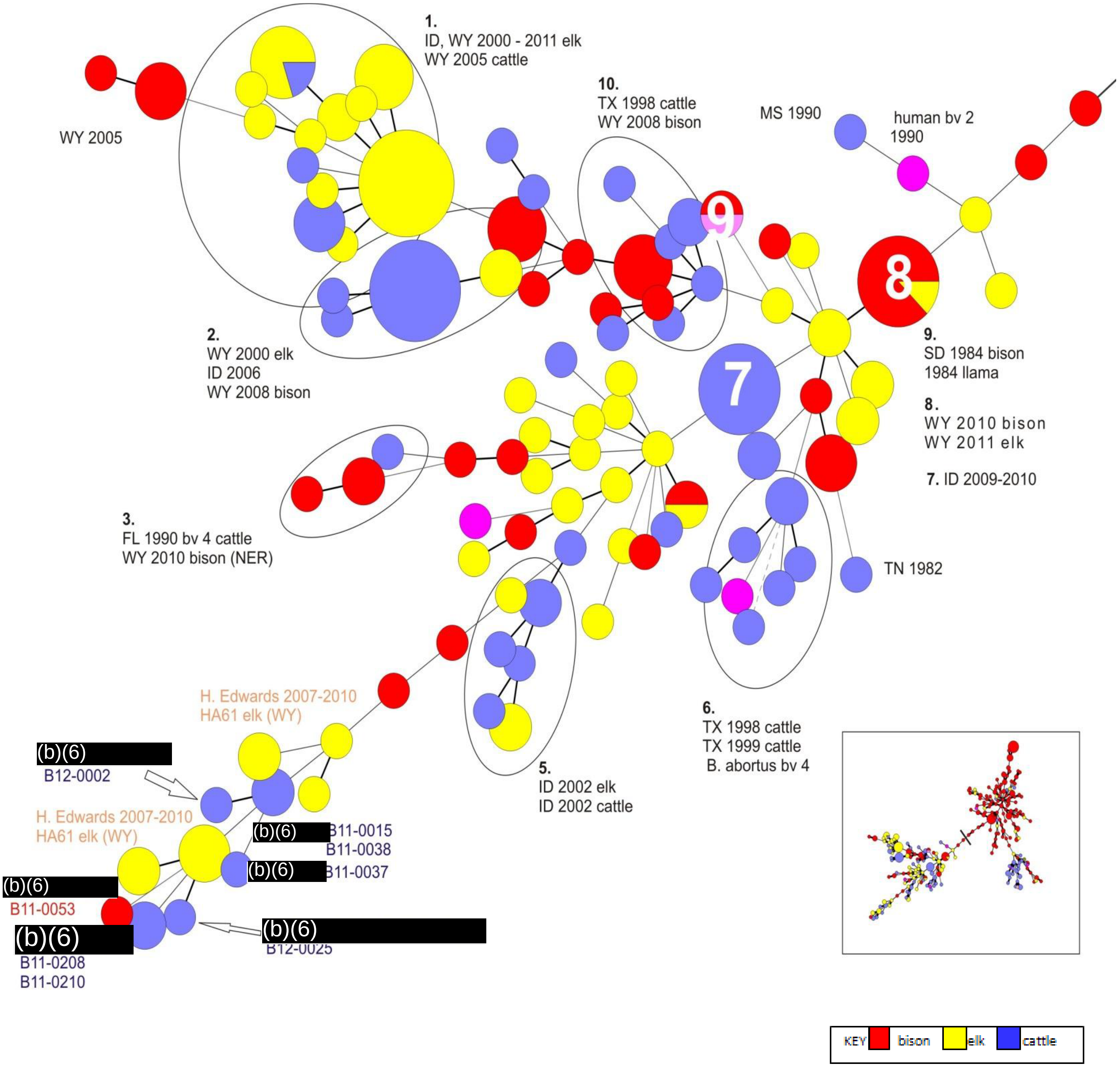
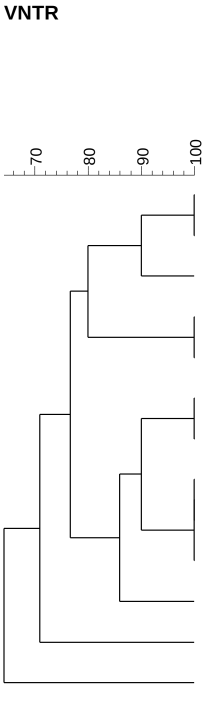


Figure 2. UPGMA clustering dendrogram of *B. abortus* isolates displaying 'nearest neighbor' status with the (b)(6) herd isolates (outlined in red boxes). The host animal species is coded by color: blue, cattle; yellow, elk; and red, bison.



VNTR															
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B		Case No.	Owner	State	Animal ID	
5	9	4	2	3	1	5	2	3	5	🟦	B11-0015-S1	(b)(6)	WY	83VSO4205	B. abort
5	9	4	2	3	1	5	2	3	5	🟦	B11-0038-S1	(b)(6)	WY	83AKC8899 / 10B-1.	B. abort
5	15	4	2	3	1	5	2	3	5	🟦	B12-0002-S1	(b)(6) Co.	WY	83V227726	B. abort
3	7	4	2	3	1	5	2	3	5	🟦	B11-0208	(b)(6)	WY	81TUK5466 / 81BC2.	B. abort
3	7	4	2	3	1	5	2	3	5	🟦	B11-0210	(b)(6) (b)(6)	WY	81TUK5466 / 81BC2.	B. abort
4	10	4	2	3	1	5	2	3	4	🟨	B11-0273	Edwards, Hank / WY Game and Fis.	WY	10-0449 Iliac	B. abort
4	10	4	2	3	1	5	2	3	4	🟨	B11-0274	Edwards, Hank / WY Game and Fis.	WY	10-0449 Supra-mam	B. abort
4	10	4	2	3	1	5	2	3	5	🟨	B11-0265	Edwards, Hank / WY Game and Fis.	WY	07-3128 Iliac	B. abort
4	10	4	2	3	1	5	2	3	5	🟨	B11-0266	Edwards, Hank / WY Game and Fis.	WY	07-3128 Retro	B. abort
4	10	4	2	3	1	5	2	3	5	🟨	B11-0267	Edwards, Hank / WY Game and Fis.	WY	07-3128 Supra-mam	B. abort
4	16	4	2	3	1	5	2	3	5	🟦	B12-0025-S1	(b)(6)	WY	83VZZ8288, White T.	B. abort
5	12	4	2	3	1	5	2	4	5	🟦	B11-0037-S1	(b)(6)	WY	83VS04205 / 10B-1.	B. abort
4	8	4	2	3	1	3	2	3	7	🟥	B11-0053-S1	(b)(6)	WY	83VZR4294 #White .	B. abort

APHIS

National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388 Fax (515) 337-7315

Brucella Genotyping Final Report - Confidential

September 28, 2011

This is a genotyping report for a *Brucella abortus* biovar 1 isolate (Accession No. 11-045811, NVSL case No. B11-0604) obtained from mammary secretions from an Angus female, 18 months of age, animal ID No. 3309, owner listed as (b)(6), Livingston, Park County, MT. The infected animal was detected through a DSA program testing.

The secretions were collected by the Montana Department of Livestock Diagnostic Lab Division on September 13, 2011, and received at the NVSL on September 14. Genotyping of the resultant isolate, using VNTR (variable number tandem repeat; also referred to as MLVA) analysis of 10 individual loci within the *Brucella* genome, was performed the week of September 25.

Also included in this report is a genotyping analysis of two isolates of *B. abortus* biovar 1 recovered from tissue from a GYA (MT) female bison, > 5 years of age, animal ID P11-017, Accession No. 11-019422, case No. B11-0469, collected by Rick Wallen on April 13, 2011, with samples received at the NVSL on April 19.

Results: The (b)(6) Angus isolate has a unique VNTR profile (6-3-9-2-4-1-5-2-5-5) not shared with any other *B. abortus* isolate among the > 370 in the NVSL BioNumerics database. Use of a UPGMA (unweighted pair-group method with arithmetic mean) analysis of the VNTR profiles indicates that the 'nearest neighbors' to the (b)(6) isolate are a cattle isolate from the (b)(6) (MT, 2008) herd, with the profile 7-3-9-2-4-1-5-2-5-7; and a 2010 Montana elk isolate (case No. B10-0529) with a VNTR profile of 7-3-9-2-4-1-6-2-5-7 (Figure 1).

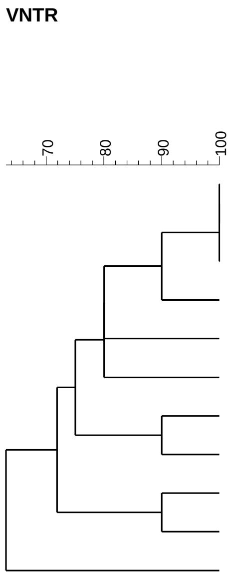
Less closely related to the (b)(6) isolate are other biovar 1 *B. abortus* strains from elk and bison located within the GYA during 2005 - 2008.

Figure 2 (below) provides the UPGMA analysis of the isolate of *B. abortus* biovar 1 recovered from tissue from a YNP (MT) female bison, > 5 years of age, animal ID P11-017, case Nos. B11-0469-S1, collected by Rick Wallen on April 13, 2011. This isolate's VNTR profile is not shared by any other *B. abortus* isolate in the NVSL genotyping database. The 'nearest neighbor' to this April 2011 isolate is a strain of *B. abortus* obtained from tissues of a Montana elk, provided to the NVSL by Neil Anderson in 2009 (case No. 09-0286, Figure 2).

Figure 3 (below) provides a 'global ' view of a genotype comparison as a minimum spanning tree (MST) analysis of the all GYA –related *B. abortus* isolates in the NVSL database. Because the MST calculations rely on a different algorithm as compared to that used for UPGMA, the spatial placement (and relatedness) of the isolates is different, with the (b)(6) Montana cattle isolate from 2008 being located at a greater remove from the (b)(6) herd isolate than is apparent in the UPGMA dendrogram. Nearest neighbors to the (b)(6) isolate in the MST are a 2008 isolate from a Wyoming bison, animal ID YNP-227, and a 2009 isolate from a Montana elk collected by Neil Anderson. In my opinion, while the MST is useful for making general conclusions about the relatedness of all the GYA *B. abortus* strains, identification of the isolates most closely related to the (b)(6) Angus isolate is best inferred from the UPGMA dendrogram (i.e., Figure 1).

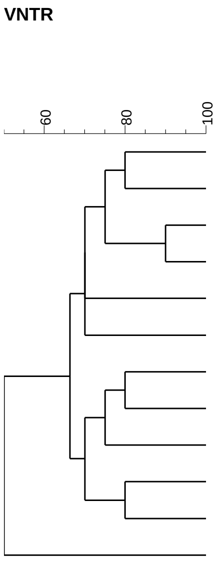
James Higgins, MS PhD
Mycobacteria and Brucella Section
NVSL / APHIS / USDA
1920 Dayton Ave.
Ames, IA 50010
515-337-7034 (fax 7315)
James.A.Higgins@aphis.usda.gov

Figure 1. Dendrogram constructed from a UPGMA analysis of the VNTR profiles for the (b)(6) isolate (case No. B11-0604) and 'nearest neighbors' in the NVSL *Brucella abortus* genotyping database. The (b)(6) isolate is outlined in the red box. Color blocks code for host species: red, bison; yellow, elk; blue, cattle.



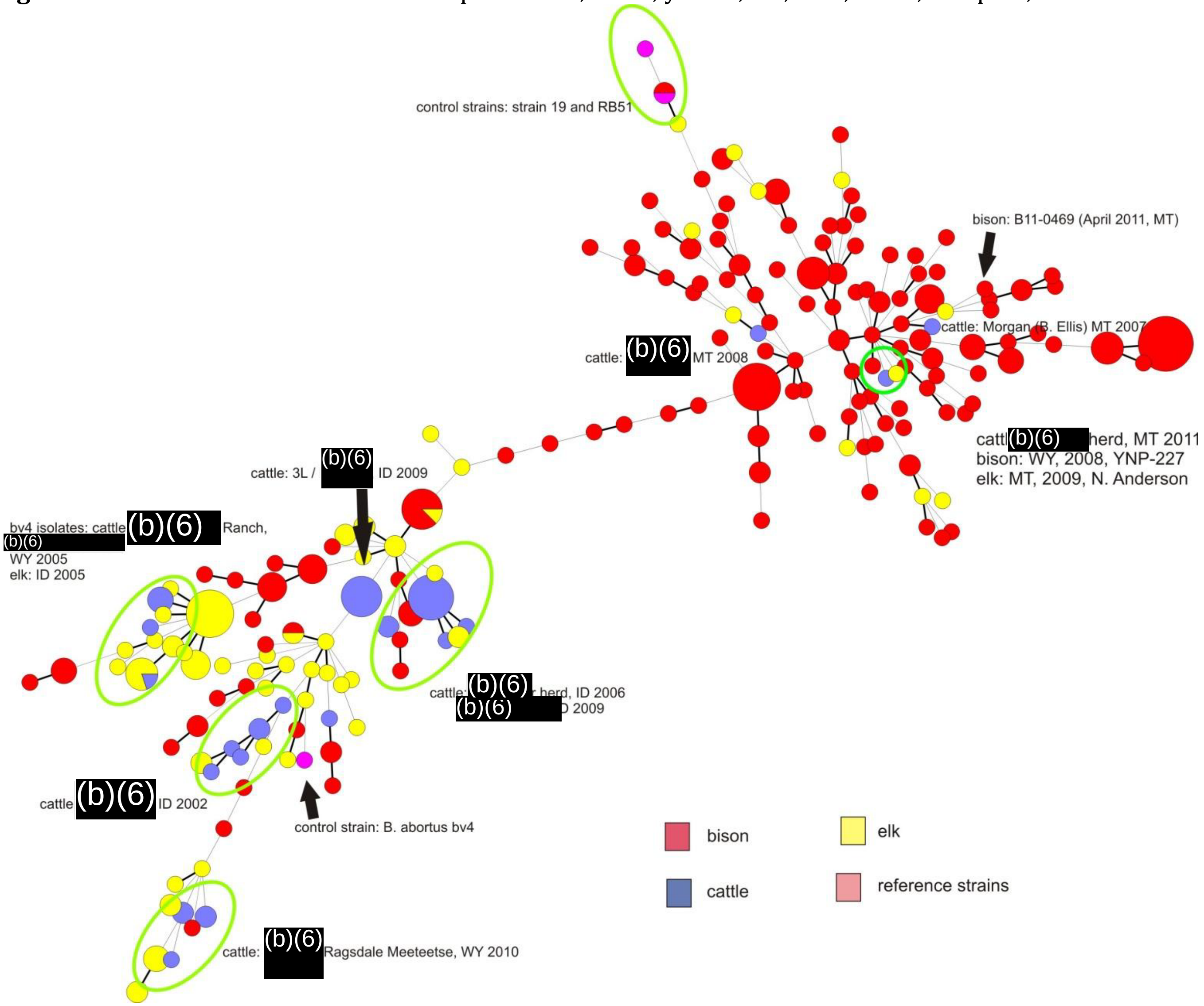
VNTR														
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B		Case No.	State	Animal ID	Owner
14	3	9	2	4	1	5	2	5	8	🟥	08-1108	WY	YNP-355	Yellowstone National Park
14	3	9	2	4	1	5	2	5	8	🟥	08-1119	MT	50-08 / 81AYE4071	BQFS
14	3	9	2	4	1	5	2	5	8	🟥	08-1121	MT	6 / 81AYE4053	BQFS
9	3	9	2	4	1	5	2	5	8	🟥	08-0638	WY	YNP-82	Yellowstone National Park
11	3	9	2	4	1	5	2	5	6	🟥	05-0999	WY	unavail	Yellowstone National Park
6	3	9	2	4	1	5	2	5	5	🟦	B11-0604	MT	#3309 / Angus	(b)(6)
7	3	9	2	4	1	5	2	5	7	🟦	08-0416S1	MT	1-1340 / #601 / 81.	(b)(6)
7	3	9	2	4	1	6	2	5	7	🟨	B10-0529	MT	BR092820	MT FWP
12	3	8	2	4	1	5	2	5	7	🟥	08-0647	WY	YNP-118	Yellowstone National Park
9	3	8	2	4	1	5	2	5	7	🟨	B10-0273	MT	BR092770	MT FWP
8	3	8	2	4	1	4	2	5	5	🟨	09-0286	MT	BR081161	Anderson, Neil (MFWP)

Figure 2. Dendrogram constructed from a UPGMA analysis of the VNTR profiles for the *B. abortus* biovar 1 isolate recovered in April, 2011 from a MT bison (case No. B11-0469-S1) and 'nearest neighbors' in the NVSL *Brucella abortus* genotyping database. The April, 2011 bison isolate is outlined in the red box. Color blocks code for host species: red, bison; yellow, elk.



VNTR														
Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B		Case No.	Host	Owner	State
8	3	5	2	4	1	4	2	5	8	🟥	08-0656	Bison	Yellowstone National Park	WY
8	3	8	2	4	1	4	2	5	5	🟨	09-0286-S2	elk	Anderson, Neil (MFWP)	MT
8	3	6	2	4	1	4	2	5	7	🟥	08-0930-S1	Bison	Rhyan, Dr. Jack (Nat'l Wildlife Research Cnt.	MT
7	3	6	2	4	1	4	2	5	7	🟥	08-0930-S2	Bison	Rhyan, Dr. Jack (Nat'l Wildlife Research Cnt.	MT
11	3	4	2	4	1	4	2	5	6	🟥	B11-0488-S1	Bison	YNP	WY
12	3	3	2	4	1	4	2	5	9	🟥	08-0928-S2	Bison	Rhyan, Dr. Jack (Nat'l WL Res Ctr)	MT
10	3	7	2	4	1	4	2	5	11	🟥	08-0389	Bison	University of Montana	MT
9	3	7	2	4	1	4	2	5	5	🟨	09-0286-S1	elk	Anderson, Neil (MFWP)	MT
9	3	7	2	4	1	4	2	3	10	🟥	B11-0469-S1	Bison	YNP	MT
8	3	7	2	4	1	4	1	5	7	🟥	05-0911	Bison	Yellowstone National Park West Yellowsto.	MT
9	3	7	2	4	1	4	1	5	6	🟥	05-1008	Bison	Yellowstone National Park	WY
7	3	8	2	4	2	5	2	5	9	🟥	05-0822	Bison	Yellowstone National Park	WY

Figure 3. Minimum spanning tree analysis of the VNTR profiles for the *B. abortus* biovar 1 isolate recovered from the(b)(6) herd Angus cow (case No. B11-0604), the *B. abortus* biovar 1 isolate recovered in April, 2011 from a MT bison (case No. B11-0469), and all GYA-related isolates in the NVSL genotyping database. Color blocks code for host species: red, bison; yellow, elk; blue, cattle; and pink, reference strains.



National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388   Fax (515) 337-7315

Brucella Preliminary Genotyping Report - Confidential

December 2, 2010

This preliminary genotyping analysis was performed on a set of Wyoming cattle-and bison- derived *Brucella abortus* milk and tissue isolates (below); note that two isolates were typed from each of three animals, for a total of six isolates.

Animal ID 83VS04205, (aka 10B-11758), 10 year-old female crossbred Simmentel, NVSL Acc. No. 10-54053, Case No. B11-0015, owner (b)(6) (Worland Livestock Auction), Meeteetse (Park County, received at the NVSL on October 26, 2010

Animal ID 83VS04205 (additional isolate), NVSL Acc. No. 10-054515, Case No. B11-0037, owner (b)(6) (b)(6), received at the NVSL on October 28, 2010

Animal ID 83AKC8899 (aka 10B-11757), 10 year-old female crossbred Angus, NVSL Acc. No. 10-054515, Case No. B11-0038, owner (b)(6), received at the NVSL on October 28, 2010

Animal ID 83AKC8899 (additional isolate), NVSL Acc. No. 10-054515, Case No. B11-0038, owner (b)(6) (b)(6) received at the NVSL on October 28, 2010

Animal ID 83VZR4294 #White 80, 2 year-old female bison, NVSL Acc. No. 10-057783, case No. B11-0053, owner (b)(6) Meeteetse (Park County) WY, received at the NVSL on November 12, 2010

Animal ID 83VZR4294 #White 80 (additional isolate), NVSL Acc. No. 10-057783, case No. B11-0053, owner (b)(6) received at the NVSL on November 12, 2010

These *B. abortus* isolates were genotyped using VNTR (variable number tandem repeat; also referred to as MLVA) analysis of 10 individual loci within the *Brucella* genome.

Results:

The two isolates from (b)(6) cow No. AKC8899 each possessed the same VNTR profile (5-9-4-2-3-3-5-3-4-6). For (b)(6) cow No. 83VSO4205, one isolate shared this profile, while the other isolate displayed a profile marked by the presence of different repeats at two loci (5-12-4-2-3-3-5-3-5-6). [Differences in VNTR profile among *B. abortus* isolates obtained from the same animal are not unusual].

For the (b)(6) bison isolates, both possessed the same VNTR profile (4-8-4-2-3-3-3-3-4-8).

None of the other entries in the NVSL database shared the VNTR profiles observed for the (b)(6) and (b)(6) isolates.

Figure 1 (below) shows the clustering of these and other isolates from the NVSL database generated by a UPGMA (unweighted pair-group method with arithmetic mean) analysis of the VNTR profiles. A total of 37 cattle, 36 elk, and 45 bison isolates from the database were incorporated into this analysis. The (b)(6) cattle and (b)(6) bison isolates are indicated in the Figure by the light blue highlighting. Horizontal red lines are used to demarcate the clusters containing these isolates.

The 'nearest neighbors' to the (b)(6) cattle *B. abortus* strains are two isolates (case Nos. 09-0295 and 09-0354) recovered from Montana elk, submitted to the NVSL in 2009 by Neil Anderson.

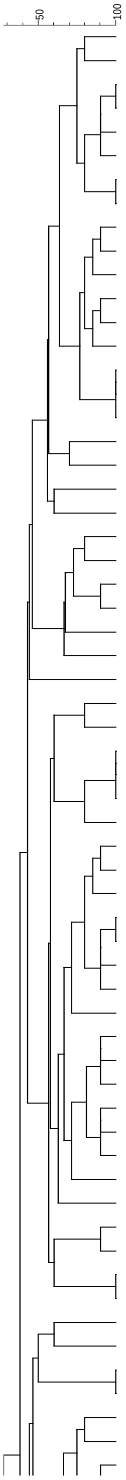
For the (b)(6) bison isolates, the 'nearest neighbors' were two isolates from Wyoming elk (case Nos. 05-0018 and 05-0019) submitted to the NVSL in 2005 by Hank Edwards. (Note that additional Hank Edwards elk isolates submitted to the NVSL in 2009 are Case Nos. 09-0004 to 09-0008, owner listed as 'Wyoming Game and Fish').

As can be seen in Figure 1, the larger clade containing the (b)(6) cattle and (b)(6) bison isolates feature field isolates recovered from elk, bison, and cattle from Montana, Idaho, and South Dakota. Interestingly, the majority of current field isolates from Wyoming animals in the NVSL database do not segregate into this clade.

James Higgins, MS PhD
Mycobacteria and Brucella Section
NVSL / APHIS / USDA
1920 Dayton Ave.
Ames, IA 50010
515-337-7034 (fax 7315)
James.A.Higgins@aphis.usda.gov

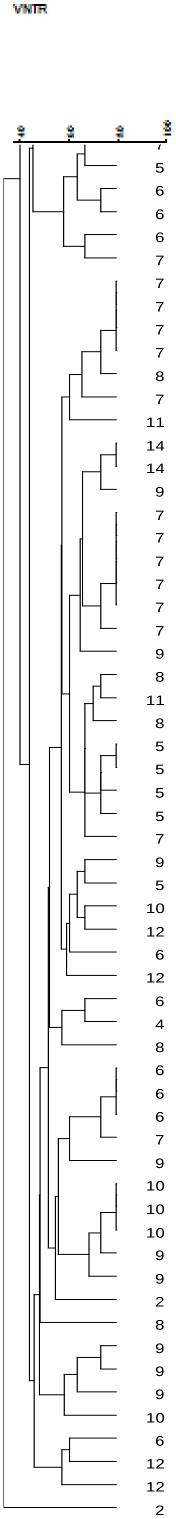
Figure 1. UPGMA clustering dendrogram of *B. abortus* isolates from elk, bison, and cattle isolates from the NVSL database

VNTR **VNTR**



Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	Case No.	Owner	State	Animal ID	Epidemiology	Species
2	4	3	2	3	3	3	3	4	5	B10-0654	3 L Land & Cattle	ID	82APC7447 (W-133)	field isolate	Cattle
2	3	3	2	3	3	3	3	4	6	B10-0173	(b)(6),(b)(6)(6) [redacted]	ID	82VTT9873	3718 milk	Cattle
2	4	3	2	3	3	3	3	5	4	B10-0203	MT FWP	MT	BR092077	field isolate	Elk
2	4	3	2	3	3	3	3	5	4	B10-0325	MT FWP	MT	BR092992	field isolate	Elk
2	8	3	2	3	3	3	3	5	4	05-0014	Edwards, Hank	WY	00W2173	undetermined	elk
2	9	3	2	3	3	3	3	5	4	09-0298	Anderson, Neil (MFWP)	MT	BR081181	field isolate	elk
2	6	3	2	3	3	3	3	4	4	06-0034	(b)(6) [redacted]	ID	409	2005 - ID herd	cattle
2	6	3	2	3	3	3	3	4	4	06-0423	Aborted fetus from Bruce.	ID	409 fetus	2005 - ID herd	cattle
2	7	3	2	4	3	3	1	5	9	00-0669		ID	60237-89	Rainey Creek - Feb 2000	elk
2	7	3	2	4	3	3	3	5	9	09-0005	Wyoming Game and Fi.	WY	GF042208	elk fetus from Franz feedground	elk
2	7	3	2	4	3	3	1	5	7	05-0338	(b)(6) [redacted] D	WY	83XX 4453	reactors	Cattle
2	7	3	2	4	3	3	3	4	8	02-0246	ID. Dept. of Fish & Gam.	ID	7217 fetus # 2 215	field isolate	elk
2	7	3	2	4	3	3	3	5	8	04-0003	IDFG Wildlife Health Lab.	ID	ER03-177 / 2233-160	Rainey Creek - Jun 2002	elk
2	7	3	2	4	3	3	3	5	10	08-0666	(b)(6) [redacted]	WY	83VTH1249/ Y-113/ sa.	seropositive herd 2008	cattle
2	6	3	2	4	3	3	3	5	7	05-0757	ID Dept of Fish & Game .	ID	4985031105	Rainey Creek - Feb 2005	elk
2	6	3	2	4	3	3	3	5	7	09-0006	Wyoming Game and Fi.	WY	GF042908	elk fetus from Franz feedground	elk
2	6	3	2	4	3	3	3	5	7	09-0004	Wyoming Game and Fi.	WY	GF042708	elk fetus from Franz feedground	elk
2	5	3	2	5	3	4	3	6	4	09-0007	Wyoming Game and Fi.	WY	GF031908	from vaginal implant transmitter re.	elk
2	4	3	2	5	3	5	3	5	4	09-0008	Wyoming Game and Fi.	WY	GF031408	from vaginal implant transmitter re.	elk
2	4	5	1	4	3	3	3	5	4	98-0614	(b)(6) [redacted]	TX	74DRA8471	1998 - TX herd # 2	cattle
2	5	6	2	4	3	3	1	5	4	98-0617	[redacted] (b)(6) [redacted] h	TX	74DNY4218	1998 - TX herd # 2	cattle
12	3	5	2	3	3	3	3	5	7	98-0564	(b)(6) [redacted]	TX	74EAF0453	1998 - TX herd # 1	cattle
8	3	5	2	3	3	3	3	5	10	99-0978	None Listed	TX	None Listed	1999 - TX herd # 1	cattle
9	3	5	2	3	3	3	1	5	9	98-0559	(b)(6) [redacted]	TX	74EAF0421	1998 - TX herd # 1	cattle
8	3	5	2	3	3	3	1	5	9	99-0983	None Listed	TX	None Listed	1999 - TX herd # 1	cattle
11	3	6	2	3	3	3	1	5	7	98-0563	(b)(6) (b)(6) [redacted]	TX	74EAF0470	1998 - TX herd # 1	cattle
5	3	7	2	3	3	3		5	6	05-1065	[redacted]	TX	fetus C	MX origin ?	cattle
12	5	8	2	3	6	3	3	5	4	08-0924	Rhyan, Dr. Jack (Nat'l .	WY	3892	Bison quarantine study	Bison
3	5	4	2	3	3	4	3	4	7	09-0295	Anderson, Neil (MFWP)	MT	BR081174	field isolate	elk
3	5	4	2	3	3	5	3	5	7	09-0354	Anderson, Neil	MT	BR081308	field isolate	elk
5	9	4	2	3	3	5	3	4	6	B11-0015	(b)(6) [redacted] (b)(6)	WY	83VSO4205		Cattle, Simmental
5	9	4	2	3	3	5	3	4	6	B11-0038	[redacted] (b)(6)	WY	83AKC8899 / 10B-117.	field isolate	Cattle
5	9	4	2	3	3	5	3	4	6	B11-0038	[redacted] (b)(6)	WY	83AKC8899 / 10B-117.	field isolate	Cattle
5	12	4	2	3	3	5	3	5	6	B11-0037	[redacted]	WY	83VS04205 / 10B-11758	field isolate	Cattle
2	4	4	2	3	3	5	3	4	9	05-0908	Fish, Wildlife & Parks	MT	unavail	South Fork Indian Creek	elk
2	4	4	2	3	3	7	3	4	9	B10-0252	MT FWP	MT	BR092578	field isolate	Elk
2	4	4	2	3	3	7	3	4	14	B10-0566	MT FWP	MT	BR090476	field isolate	Elk
2	4	4	2	3	3	4	3	4	12	B10-0091	MT FWP	MT	BR090731	field isolate	Elk
2	4	4	2	3	3	4	3	4	12	B11-0011	TEI- Flying D Ranch	MT	9-273/FC 0124	field isolate	Bison
2	4	4	2	3	3	4	3	4	5	09-0443	Neil Anderson	MT	BR080548	field isolate	elk
2	4	4	2	3	3	4	3	4	10	B10-0514	MT FWP	MT	BR090381	field isolate	Elk
2	4	5	2	3	3	5	3	4	11	09-0328	Anderson, Neil (MFWP)	MT	BR081200	field isolate	Elk
2	3	4	2	3	3	5	3	4	10	02-0235	(b)(6) [redacted]	ID	82VIU1921	2002 ID herd	cattle
2	3	10	2	3	3	5	3	4	10	02-0236	(b)(6) [redacted]	ID	82ALK6225	2002 ID herd	cattle
2	3	6	2	3	3	5	3	4	10	02-0241	(b)(6) [redacted]	ID	82VME6918	2002 ID herd	cattle
2	3	13	2	3	3	5	3	4	9	02-0123	Idaho Fish & Game Wild.	ID	60217	Conant Creek	elk
2	3	7	2	3	3	5	3	4	9	02-0124	Animal Health Labs	ID	60218	Conant Creek	elk
2	3	10	2	3	3	5	3	4	9	02-0237	(b)(6) [redacted]	ID	82AKN7632	2002 ID herd	cattle
2	5	4	2	3	3	5	3	4	13	B10-0517	MT FWP	MT	BR090384	field isolate	Elk
2	4	6	2	3	3	5	3	5	5	04-0418	Cimpl Packing Co.	SD	83VNY6729	(b)(6) [redacted] isolate - SDSU possible x.	cattle
1	2	4	2	3	3	6	3	4	9	05-0018	Edwards, Hank	WY	01W307	field isolate	elk
1	2	4	2	3	3	7	3	4	9	05-0019	Edwards, Hank	WY	01W311	field isolate	elk
4	8	4	2	3	3	3	3	4	8	B11-0053	(b)(6) (b)(6) [redacted]	WY	83VZR4294 #White 80	field isolate	Bison
4	8	4	2	3	3	3	3	4	8	B11-0053	[redacted]	WY	83VZR4294 #White 80	field isolate	Bison
7	5	8	2	4	3	8	3	8	6	08-0187	Montana Fish Wildlife &.	MT	#53 (b) (6) [redacted] / 12/24/1.	Bozeman	elk
4	5	4	2	4	3	7	3	5	6	05-1285	(b)(6) [redacted]	TX	74FAA0148 (milk)	2005 - TX herd # 2	cattle
8	2	3	2	4	3	4	3	4	6	05-1002	Yellowstone National Pa.	WY	3203	surveillance	Bison
8	2	3	2	4	3	4	3	4	6	05-1005	Yellowstone National Pa.	WY	3221	surveillance	Bison
7	4	2	2	4	3	2	3	5	10	06-0861 A	B. abortus strain 19	TX	74EDJ9879	(b)(6) [redacted] r (b)(6) [redacted]	Cattle
5	4	2	2	4	3	2	3	5	7	06-0513	(b)(6) [redacted] .	TX	74FDL6104	field isolate, Calliham, TX	Cattle
6	4	2	2	4	3	2	3	5	6	05-1317	King Ranch Inc.	TX	NL	field isolate	Cattle

Figure 1. (continued)



VNTR

	Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	Case No.	Owner	State	Animal ID	Epidemiology	Species
7	4	2	2	4	3	2	3	5	10						
5	4	2	2	4	3	2	3	5	7	06-0513	(b)(6) [redacted] .	TX	74FDL6104	field isolate, Calliham, TX	Cattle
6	4	2	2	4	3	2	3	5	6	05-1317	King Ranch Inc.	TX	NL	field isolate	Cattle
6	7	2	2	4	3	2	3	5	6	06-0025	(b)(6) [redacted]	TX	NL	field isolate	Cattle
6	4	2	2	4	3	2	3	7	4	03-0072	B. abortus strain 2308	ID	yellow 332 (N03-060)	Canyon, ID	elk
7	4	4	2	4	3	2	3	7	4	09-0269	B. abortus RB51	TX	Sample 1 / 74SQY3675	(b)(6) [redacted]	Cattle
7	3	4	2	4	3	6	3	6	12	08-0404	YNP	MT	8-340 / P08-010	Yellowstone National Park	bison
7	3	4	2	4	3	6	3	6	12	08-0933	Rhyan, Dr. Jack (Nat'l .	WY	81-08	Bison quarantine study	Bison
7	3	4	2	4	3	6	3	6	12	08-1080	USDA/APHIS/VS/BQFS	MT	91-08 / 81APM1605	Bison quarantine study	Bison
7	3	4	2	4	3	6	3	6	12	08-1120	BQFS	MT	54-08 / 81AYE4054	Bison quarantine study	Bison
8	3	4	2	4	3	6	3	6	12	08-1122	BQFS	MT	91-08 / 81APM1605	Bison quarantine study	Bison
7	3	6	2	4	3	6	3	6	11	08-0386	University of Montana	MT	#33 / Bison	Yellowstone National Park	bison
11	3	12	2	4	3	6	3	6	8	08-0934	Rhyan, Dr. Jack (Nat'l .	WY	97-08	Bison quarantine study	Bison
14	3	9	2	4	3	5	3	6	9	08-1119	BQFS	MT	50-08 / 81AYE4071	Bison quarantine study	Bison
14	3	9	2	4	3	5	3	6	9	08-1121	BQFS	MT	6 / 81AYE4053	Bison quarantine study	Bison
9	3	9	2	4	3	5	3	6	9	08-0638	Yellowstone National Pa.	WY	YNP-82	field isolate	Bison
7	3	9	2	4	3	5	3	6	8	08-0416S1	(b)(6) [redacted]	MT	1-1340 / #601 / 81TUG.	new herd Montana - 2008	cattle
7	3	9	2	4	3	5	3	6	8	08-0416S2	[redacted]	MT	1-1340 / #601 / 81TUG.	new herd Montana - 2008	cattle
7	3	9	2	4	3	5	3	6	8	08-041	s	MT	1-1340 / #601 / 81TUG.	new herd Montana - 2008	cattle
7	3	9	2	4	3	5	3	6	8	08-041	s	MT	1-1340 / #601 / 81TUG.	new herd Montana - 2008	cattle
7	3	9	2	4	3	5	3	6	8	08-0416S5	(b)(6) [redacted]	MT	1-1340 / #601 / 81TUG.	new herd Montana - 2008	cattle
7	3	9	2	4	3	6	3	6	8	B10-0529	MT FWP	MT	BR092820	field isolate	Elk
9	3	8	2	4	3	5	3	6	8	B10-0273	MT FWP	MT	BR092770	field isolate	Elk
8	3	6	2	4	3	5	3	6	6	08-0392	University of Montana	MT	#39 / Bison	Yellowstone National Park	bison
11	3	6	2	4	3	5	3	6	6	08-0869	Yellowstone National Pa.	WY	YNP-292	field isolate	Bison
8	3	6	2	4	3	5	3	6	13	08-0921	Rhyan, Dr. Jack (Nat'l .	WY	32-08	Bison quarantine study	Bison
5	3	6	2	4	3	5	3	6	10	08-0382	University of Montana	MT	#27 / Bison	Yellowstone National Park	bison
5	3	6	2	4	3	5	3	6	10	08-1147	Yellowstone National Pa.	WY	YNP-206	field isolate	Bison
5	3	6	2	4	3	5	3	6	12	08-0876	Yellowstone National Pa.	WY	YNP-289	field isolate	Bison
5	3	6	2	4	3	5	3	6	9	08-1019	USDA/APHIS/VS/BQFS	MT	20-08 / 81ARG3869	Bison quarantine study	Bison
7	3	6	2	4	3	5	3	6	5	B10-0973	Clarke, P. Ryan-bull bis.	MT	YNP95021	field study	Bison
9	3	7	2	4	3	4	3	6	6	09-0286	Anderson, Neil (MFWP)	MT	BR081161	field isolate	elk
5	3	7	2	4	3	5	3	6	6	07-0266	(b) (6) [redacted] (later TransO.	MT	81 TSK 4771	new herd - Montana 2007	cattle
10	3	7	2	4	3	4	3	6	12	08-0389	University of Montana	MT	#36 / Bison	Yellowstone National Park	bison
12	3	7	2	4	3	5	3	6	12	08-0966	Yellowstone National Pa.	WY	YNP- 398	field isolate	Bison
6	3	7	2	4	3	6	3	6	10	B10-0118	MT FWP	MT	BR091887	field isolate	Elk
12	3	3	2	4	3	4	3	6	10	08-0928	Rhyan, Dr. Jack (Nat'l .	WY	4058	Bison quarantine study	Bison
6	3	8	2	4	3	5	3	7	13	08-0390	University of Montana	MT	#37 / Bison	Yellowstone National Park	bison
4	3	8	2	4	3	5	3	7	11	08-0929	Rhyan, Dr. Jack (Nat'l .	WY	45-08	Bison quarantine study	Bison
8	3	9	2	4	3	6	3	7	13	08-1256	Corwin Springs	MT	24-08	surveillance	Bison
6	3	3	2	4	3	5	3	5	5	06-0281	Bison Quarantine Study	MT	07-06/bison	seropositive YNP bison calf euthan.	bison
6	3	3	2	4	3	5	3	5	5	06-0429	Bison Quarantine Feasib.	MT	sample 29-06	YNP B. abortus-exposed bison cal.	bison
6	3	3	2	4	3	5	3	5	5	06-0887	USDA/APHIS/VS	MT	77-06 (1.5 yo F)	Corwin Springs, Belgrade	bison
7	3	3	2	4	3	5	3	5	5	06-0279	Bison Quarantine Study	MT	14-06/bison	seropositive YNP bison calf euthan.	bison
9	3	5	2	4	3	5	3	5	9	B10-0584	MT FWP	MT	BR090822	field isolate	Elk
10	3	4	2	4	3	6	3	5	4	08-0863	Yellowstone National Pa.	WY	YNP-180	field isolate	Bison
10	3	4	2	4	3	6	3	5	4	08-0923	Rhyan, Dr. Jack (Nat'l .	WY	3877	Bison quarantine study	Bison
10	3	4	2	4	3	6	3	5	4	08-1354	Corwin Springs	MT	92-08 / 8-161	field isolate	Bison
9	3	4	2	4	3	6	3	5	4	08-0923	Rhyan, Dr. Jack (Nat'l .	WY	3877	Bison quarantine study	Bison
9	3	4	2	4	3	5	3	5	4	08-1123	BQFS	MT	38-08 / 81ARG3848	Bison quarantine study	Bison
2	6	4	2	4	3	5	3	5	12	05-0444	(b)(6) [redacted]	TX	74EXP6760	2005 - TX herd # 1	cattle
8	3	7	2	4	3	4	2	6	8	05-0911	Yellowstone National Pa.	MT	81APF6425	surveillance	Bison
9	4	8	2	4	3	5	3	6	11	08-0151	Montana Fish Wildlife &.	MT	#17 / Madden / 08/03/1.	Bozeman	elk
9	4	8	2	4	3	6	3	6	11	08-0410	YNP	MT	8-407-08 / P08-071 / Bi.	Mammoth Hot Springs	bison
9	4	5	2	4	3	5	2	6	11	05-1282	APHIS, VS	MT	81 APH 5970 (1 yo M)	Corwin Springs	bison
10	4	7	2	4	3	7	3	6	11	09-0276	Anderson, Neil (MFWP)	MT	BR081145	field isolate	Elk
6	6	8	2	4	3	8	3	7	5	05-1132	USDA	MT	81APF6442	reactor	Bison
12	5	8	2	4	3	6	3	7	5	08-1221	Yellowstone National Pa.	WY	YNP-347	surveillance	Bison
12	6	7	2	4	3	6	3	6	5	09-0553	BQFS- Bison Quarantin.	MT	85-08	Bison quarantine study	Bison
2	3	9	2	3	4	7	1	4	9	02-0239	(b)(6) [redacted]	ID	AKN7636	2002 ID herd	cattle

National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388 Fax (515) 337-7315

Brucella Genotyping Final Report - Confidential

October 17, 2011

This is an updated genotyping report for n = 11 *Brucella abortus* biovar 1 isolates recovered from four female, 18 month-old Black Angus cattle owned by (b)(6) Livingston, Park County, MT. The isolates of *B. abortus* were generated from the following accessions associated with this herd:

Accession No. 11-045811, Case No. B11-0604, animal # **3309** (the same as animal ID #81TCD8221, below), isolate S1.

Accession No. 11-047974, Case No. B11-0611, animal ID #81TDC8283, isolate S1

Accession No. 11-047974, Case No. B11-0616, animal ID #81TDC8223, isolates S1, S2, and S3

Accession No. 11-047974, Case No. B11-0613, animal ID #**81TDC8221**, isolates S1, S2, and S3

Accession No. 11-047974, Case No. B11-0612, animal ID #81TDC8239, isolates S1, S2, and S3

The mammary secretions comprising Accession No. 11-045811 were received at the NVSL on September 14, 2011, while the tissue samples associated with Accession 11-047974 were received on September 23.

Genotyping of the resultant isolates, using VNTR (variable number tandem repeat; also referred to as MLVA) analysis of 10 individual loci within the *Brucella* genome, was performed the weeks of September 25 and October 9.

Results: All of the 11 *B. abortus* isolates recovered from the (b)(6) cattle possess the same VNTR profile (6-3-9-2-4-1-5-2-5-5); it is not unusual to observe that multiple isolates, from different animals within a herd, possess the exact same VNTR profile.

This VNTR profile is not shared with any other *B. abortus* isolates among the > 370 in the NVSL BioNumerics database. Identification of 'nearest neighbor' isolates was done using two statistical methods, an unweighted pair-group method with arithmetic mean (UPGMA) analysis, and a minimum spanning tree (MST) analysis.

The UPGMA analysis of the VNTR profiles is provided in Figure 1 (below); this indicates that several other isolates from the NVSL *B. abortus* genotyping database are 'nearest neighbors' to the (b)(6) herd isolates. These nearest neighbors include an isolate recovered in 2008 from a YNP bison, Case No. 08-0638; and another bison isolate, recovered in 2005, case No. 05-0999. Less closely related are a cattle isolate from the (b)(6) (MT, 2008) herd, with the profile 7-3-9-2-4-1-5-2-5-7; and isolates recovered from elk and bison in the GYA during the interval 2005 – 2010.

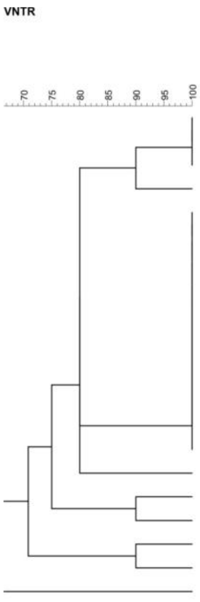
Different nearest neighbor isolates are identified using the MST analysis (Figure 2). Here, the 2008 (b)(6) herd isolate is the nearest neighbor to the (b)(6) isolates. Less closely related are clusters occupied by isolates from GYA elk, and at a greater remove, isolates from GYA bison obtained in the period from 2005 – 2010.

The different preferences for nearest neighbor isolates displayed by the two analyses reflect differences in the algorithms they use to cluster the categorical data constituting the VNTR profiles.

In the absence of detailed epidemiologic information about the isolates in the analysis, it is not possible to declare that one method's conclusions are necessarily superior to the other. However, data from the field indicate that the (b)(6) cattle operation is located on territory adjacent to that of the (b)(6) herd, and that elk are prevalent in this area and have contact with cattle. Accordingly, the arrangement of isolates in the MST analysis (Figure 2) may best reflect the transmission dynamics taking place among these elk and the animals in the (b)(6) herds.

James Higgins, PhD
Mycobacteria and Brucella Section
NVSL / APHIS / USDA
1920 Dayton Ave.
Ames, IA 50010
515-337-7034 (fax 7315)
James.A.Higgins@aphis.usda.gov

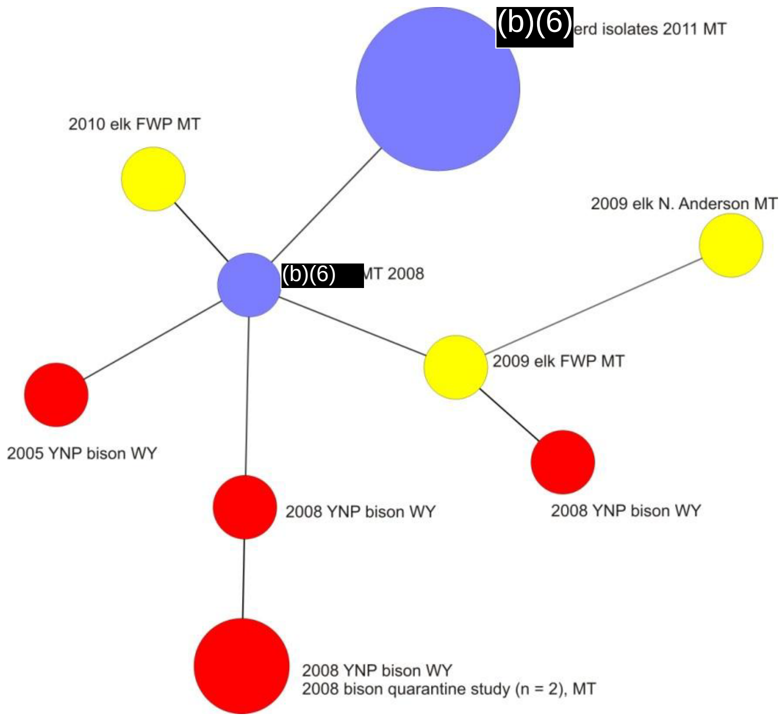
Figure 1. Dendrogram constructed from a UPGMA analysis of the VNTR profiles for the (b)(6) herd isolates (n = 11) and selected 'nearest neighbors' in the NVSL *Brucella abortus* genotyping database. The (b)(6) isolates are outlined in red box. Color blocks adjacent to VNTR matrix code for host species: red, bison; yellow, elk; blue, cattle.



VNTR

Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B		Case No.	Owner	State	Animal ID	Epidemiology
14	3	9	2	4	1	5	2	5	8	🟥	08-1108-S2	Yellowstone National Park	WY	YNP-355	(b)(6) - Submitter
14	3	9	2	4	1	5	2	5	8	🟥	08-1119	BQFS	MT	50-08 / 81AYE4071	Bison quarantine study
14	3	9	2	4	1	5	2	5	8	🟥	08-1121	BQFS	MT	6 / 81AYE4053	Bison quarantine study
9	3	9	2	4	1	5	2	5	8	🟥	08-0638	Yellowstone National Park	WY	YNP-82	field isolate
6	3	9	2	4	1	5	2	5	5	🟦	B11-0604	(b)(6)	MT	#3309 (= 81TDC8221)	DSA positive herd
6	3	9	2	4	1	5	2	5	5	🟦	B11-0612-S1	(b)(6)	MT	81TDC8239	
6	3	9	2	4	1	5	2	5	5	🟦	B11-0612-S2		MT	81TDC8239	
6	3	9	2	4	1	5	2	5	5	🟦	B11-0612-S3		MT	81TDC8239	
6	3	9	2	4	1	5	2	5	5	🟦	B11-0613-S1		MT	81TDC8221 (= #3309)	
6	3	9	2	4	1	5	2	5	5	🟦	B11-0613-S2		MT	81TDC8221	
6	3	9	2	4	1	5	2	5	5	🟦	B11-0613-S3		MT	81TDC8221	
6	3	9	2	4	1	5	2	5	5	🟦	B11-0616-S1		MT	81TDC8223	
6	3	9	2	4	1	5	2	5	5	🟦	B11-0616-S2		MT	81TDC8223	
6	3	9	2	4	1	5	2	5	5	🟦	B11-0616-S3		MT	81TDC8223	
6	3	9	2	4	1	5	2	5	5	🟦	B11-0611-S1		MT	81TDC8283	
11	3	9	2	4	1	5	2	5	6	🟥	05-0999	Yellowstone National Park	WY	unavail	surveillance
7	3	9	2	4	1	5	2	5	7	🟦	08-0416s1	(b)(6)	MT	1-1340 / #601 / 81TUG0218	new herd Montana - 20.
7	3	9	2	4	1	6	2	5	7	🟨	B10-0529S-1	MT FWP	MT	BR092820	field isolate
12	3	8	2	4	1	5	2	5	7	🟥	08-0647	Yellowstone National Park	WY	YNP-118	field isolate
9	3	8	2	4	1	5	2	5	7	🟨	B10-0273 S-1	MT FWP	MT	BR092770	field isolate
8	3	8	2	4	1	4	2	5	5	🟨	09-0286-S2	Anderson, Neil (MFWP)	MT	BR081161	field isolate

Figure 2. Minimum spanning tree (MST) generated from VNTR profiles for the (b)(6) herd isolates (n = 11) and 'nearest neighbors' in the NVSL *Brucella abortus* genotyping database. Larger circles / nodes contain multiple isolates, all sharing the same VNTR profile, while smaller nodes contain single isolates / VNTR profiles. Color code for host species: red, bison; yellow, elk; blue, cattle.



National Veterinary Services Laboratories
1920 Dayton Rd. , Ames, IA 50010
Phone (515) 337-7388 Fax (515) 337-7315

Brucella Genotyping Final Report - Confidential

November 26, 2010

This genotyping analysis was performed on a set of Montana cattle-derived *Brucella abortus* biovar 1 isolates from 2007 and 2008. The 2007 isolate was cultured from an animal owned by (b) (6), Baler, Montana (and later by Trans Ova), NVSL Case No. 07-0266, animal ID 81TSK 4771. The other set of isolates represented five colonies cultured from an animal (animal ID 1-1340/#601/81TUG0218) owned by (b)(6), Pray, Montana. These colonies are NVSL case Nos. 08-0416S1, 08-0416S2, 08-0416S3, 08-0416S4, and 08-0416S5. Genotyping was performed using VNTR (variable number tandem repeat; also referred to as MLVA) analysis of 10 individual loci within the *Brucella* genome.

Results:

The isolate from the cow owned by (b) (6) did not any match any other VNTR profiles in the NVSL *Brucella* genotyping database. While all five of the (b)(6) isolates had the same VNTR profile, none of the other isolates in the database matched the (b)(6) isolates.

The 'nearest neighbor' to the (b) (6) *B. abortus* strain was an isolate recovered from a Montana elk, submitted by Neil Anderson in 2009, animal ID BR081161, Case No. 09-0286. This elk isolate displayed differences in repeat number for 2 of the 10 loci. Based on observations made from conducting VNTR analyses of field isolates of *Brucella*, this degree of heterogeneity does not necessarily rule out a possible epidemiological link between the two animals. However, further information on the natural history of the infection in the elk is required before any definitive conclusions can be made regarding the transmission of this particular strain of *B. abortus*.

The nearest neighbor to the *B. abortus* strains from the animal owned by (b)(6) was an isolate recovered from a Montana elk submitted by the department of Fish, Wildlife and Parks, in January 2010, animal ID No. BR092820, Case No. B10-0529. This isolate differed from the (b)(6) isolates at only one VNTR locus ('VNTR 2'), by one repeat (6 repeats v 5 for the (b)(6) isolates). Given the wide disparity in VNTR profiles that can be observed among field isolates from a specific cohort of animals, the high degree of similarity between the profiles for these strains is suggestive of a link between these two isolates, but again, detailed epidemiologic evidence is required to fully support such a hypothesis.

Figure 1 (below) shows the clustering of isolates generated by a UPGMA (unweighted pair-group method with arithmetric mean) analysis of the VNTR profiles. [A total of 33 cattle, 35 elk, and 43 bison isolates from the NVSL database were incorporated into this analysis; the Montana cattle isolates are indicated in the Figure by the light blue highlighting]. Horizontal red lines are used to demarcate the clusters containing the Montana cattle isolates.

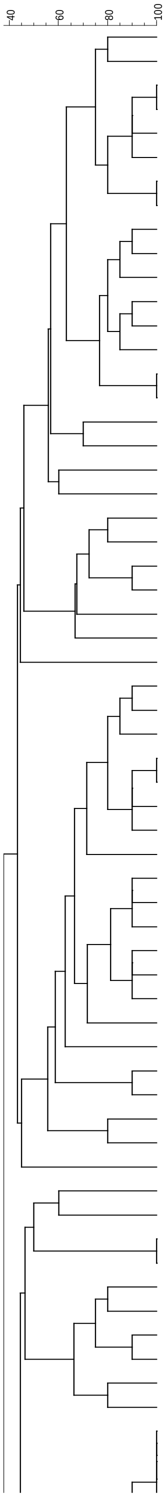
To confirm that the groupings generated by this statistical method are valid, a second method, termed MST (minimum spanning tree) analysis, also was utilized. The tree diagram generated by the MST analysis is presented in Figure 2 (below); this diagram incorporates the same isolates as were used in the 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 boxes in the top portion of the diagram indicate the location of the spheres containing the Montana cattle *B. abortus* isolates.2

Although the MST analysis uses a different algorithm than the UPGMA analysis, it corroborates the clustering results for the Montana cattle isolates obtained using the UPGMA technique. As a general conclusion, the MST tree indicates that the Montana cattle isolates are part of a larger cluster that comprises Yellowstone-derived bison isolates of *B. abortus* (green spheres) and field isolates from Texas cattle from the late 90s, in the top-center portion of the tree. Another major cluster, comprised of cattle isolates from Texas from 2005 – 2006, as well as isolates from cattle owned by (b)(6), is located in the bottom portion of the tree.

James Higgins, MS PhD
Mycobacteria and Brucella Section
NVSL / APHIS / USDA
1920 Dayton Ave.
Ames, IA 50010
515-337-7034 (fax 7315)
James.A.Higgins@aphis.usda.gov

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

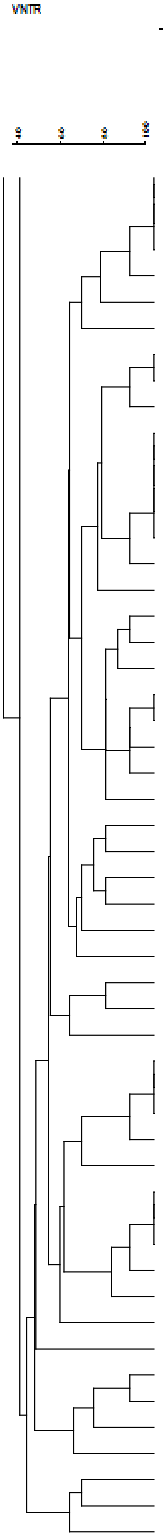
VNTR



VNTR

Hoof 1	Hoof 3	Hoof 4	Hoof 8	VNTR 16	VNTR 17	VNTR 2	VNTR 21	VNTR 5A	VNTR 5B	Case No.	Owner	State	Animal ID	Epidemiology	Species
2	4	3	2	3	3	3	3	4	5	B10-0654	(b)(b)(6) [redacted]	ID	82APC7447 (W-133)	field isolate	Cattle
2	3	3	2	3	3	3	3	4	6	B10-0173	[redacted])	ID	82VTT9873	3718 milk	Cattle
2	4	3	2	3	3	3	3	5	4	B10-0203	MT FWP	MT	BR092077	field isolate	Elk
2	4	3	2	3	3	3	3	5	4	B10-0325	MT FWP	MT	BR092992	field isolate	Elk
2	8	3	2	3	3	3	3	5	4	05-0014	Edwards, Hank	WY	00W2173	undetermined	elk
2	9	3	2	3	3	3	3	5	4	09-0298	Anderson, Neil (MFWP)	MT	BR081181	field isolate	elk
2	6	3	2	3	3	3	3	4	4	06-0034	(b)(6) [redacted]	ID	409	2005 - ID herd	cattle
2	6	3	2	3	3	3	3	4	4	06-0423	Aborted fetus from Bruce.	ID	409 fetus	2005 - ID herd	cattle
2	7	3	2	4	3	3	1	5	9	00-0669		ID	60237-89	Rainey Creek - Feb 2000	elk
2	7	3	2	4	3	3	3	5	9	09-0005	Wyoming Game and Fi.	WY	GF042208	elk fetus from Franz feedground	elk
2	7	3	2	4	3	3	1	5	7	05-0338	(b)(6) [redacted] TD	WY	83XX 4453	reactors	Cattle
2	7	3	2	4	3	3	3	4	8	02-0246	ID. Dept. of Fish & Gam.	ID	7217 fetus # 2 215	field isolate	elk
2	7	3	2	4	3	3	3	5	8	04-0003	IDFG Wildlife Health Lab.	ID	ER03-177 / 2233-160	Rainey Creek - Jun 2002	elk
2	7	3	2	4	3	3	3	5	10	08-0666	(b)(6) (b)(6) [redacted]	WY	83VTH1249/ Y-113/ sa.	seropositive herd 2008	cattle
2	6	3	2	4	3	3	3	5	7	05-0757	ID Dept of Fish & Game .	ID	4985031105	Rainey Creek - Feb 2005	elk
2	6	3	2	4	3	3	3	5	7	09-0006	Wyoming Game and Fi.	WY	GF042908	elk fetus from Franz feedground	elk
2	5	3	2	5	3	4	3	6	4	09-0007	Wyoming Game and Fi.	WY	GF031908	from vaginal implant transmitter recovere.	elk
2	4	3	2	5	3	5	3	5	4	09-0008	Wyoming Game and Fi.	WY	GF031408	from vaginal implant transmitter recovere.	elk
2	4	5	1	4	3	3	3	5	4	98-0614	(b)(6) [redacted]	TX	74DRA8471	1998 - TX herd # 2	cattle
2	5	6	2	4	3	3	1	5	4	98-0617	[redacted]	TX	74DNY4218	1998 - TX herd # 2	cattle
12	3	5	2	3	3	3	3	5	7	98-0564	[redacted]	TX	74EAF0453	1998 - TX herd # 1	cattle
8	3	5	2	3	3	3	3	5	10	99-0978	None Listed	TX	None Listed	1999 - TX herd # 1	cattle
9	3	5	2	3	3	3	1	5	9	98-0559	(b)(6) [redacted]	TX	74EAF0421	1998 - TX herd # 1	cattle
8	3	5	2	3	3	3	1	5	9	99-0983	None Listed	TX	None Listed	1999 - TX herd # 1	cattle
11	3	6	2	3	3	3	1	5	7	98-0563	(b)(6) [redacted]	TX	74EAF0470	1998 - TX herd # 1	cattle
5	3	7	2	3	3	3		5	6	05-1065	[redacted]	TX	fetus C	MX origin ?	cattle
12	5	8	2	3	6	3	3	5	4	08-0924	Rhyan, Dr. Jack (Nat'l .	WY	3892	Bison quarantine study	Bison
2	4	4	2	3	3	5	3	4	9	05-0908	Fish, Wildlife & Parks	MT	unavail	South Fork Indian Creek	elk
2	4	4	2	3	3	7	3	4	9	B10-0252	MT FWP	MT	BR092578	field isolate	Elk
2	4	4	2	3	3	7	3	4	14	B10-0566	MT FWP	MT	BR090476	field isolate	Elk
2	4	4	2	3	3	4	3	4	12	B10-0091	MT FWP	MT	BR090731	field isolate	Elk
2	4	4	2	3	3	4	3	4	12	B11-0011	TEI- Flying D Ranch	MT	9-273/FC 0124	field isolate	Bison
2	4	4	2	3	3	4	3	4	5	09-0443	Neil Anderson	MT	BR080548	field isolate	elk
2	4	4	2	3	3	4	3	4	10	B10-0514	MT FWP	MT	BR090381	field isolate	Elk
2	4	5	2	3	3	5	3	4	11	09-0328	Anderson, Neil (MFWP)	MT	BR081200	field isolate	Elk
2	3	4	2	3	3	5	3	4	10	02-0235	(b)(6) [redacted]	ID	82VIU1921	2002 ID herd	cattle
2	3	10	2	3	3	5	3	4	10	02-0236	[redacted]	ID	82ALK6225	2002 ID herd	cattle
2	3	6	2	3	3	5	3	4	10	02-0241	[redacted] s	ID	82VME6918	2002 ID herd	cattle
2	3	13	2	3	3	5	3	4	9	02-0123	Idaho Fish & Game Wild.	ID	60217	Conant Creek	elk
2	3	7	2	3	3	5	3	4	9	02-0124	Animal Health Labs	ID	60218	Conant Creek	elk
2	3	10	2	3	3	5	3	4	9	02-0237	(b)(6) [redacted]	ID	82AKN7632	2002 ID herd	cattle
2	5	4	2	3	3	5	3	4	13	B10-0517	MT FWP	MT	BR090384	field isolate	Elk
2	4	6	2	3	3	5	3	5	5	04-0418	Cimpl Packing Co.	SD	83VNY6729	(b)(6)(b)(6) [redacted] isolate - SDSU possible x-conta.	cattle
1	2	4	2	3	3	6	3	4	9	05-0018	Edwards, Hank	WY	01W307	field isolate	elk
1	2	4	2	3	3	7	3	4	9	05-0019	Edwards, Hank	WY	01W311	field isolate	elk
3	5	4	2	3	3	4	3	4	7	09-0295	Anderson, Neil (MFWP)	MT	BR081174	field isolate	elk
3	5	4	2	3	3	5	3	5	7	09-0354	Anderson, Neil	MT	BR081308	field isolate	elk
2	3	9	2	3	4	7	1	4	9	02-0239	(b)(6) [redacted]	ID	AKN7636	2002 ID herd	cattle
7	5	8	2	4	3	8	3	8	6	08-0187	Montana Fish Wildlife &.	MT	#53 / (b) (6) [redacted] / 12/24/1.	Bozeman	elk
4	5	4	2	4	3	7	3	5	6	05-1285	(b)(6) [redacted]	TX	74FAA0148 (milk)	2005 - TX herd # 2	cattle
8	2	3	2	4	3	4	3	4	6	05-1002	Yellowstone National Pa.	WY	3203	surveillance	Bison
8	2	3	2	4	3	4	3	4	6	05-1005	Yellowstone National Pa.	WY	3221	surveillance	Bison
7	4	2	2	4	3	2	3	5	10	06-0861 A	B. abortus strain 19	TX	74EDJ9879	(b)(6) [redacted]	Cattle
5	4	2	2	4	3	2	3	5	7	06-0513	(b)(6) [redacted] .	TX	74FDL6104	field isolate, Calliham, TX	Cattle
6	4	2	2	4	3	2	3	5	6	05-1317	King Ranch Inc.	TX	NL	field isolate	Cattle
6	7	2	2	4	3	2	3	5	6	06-0025	(b)(6) [redacted]	TX	NL	field isolate	Cattle
6	4	2	2	4	3	2	3	7	4	03-0072	B. abortus strain 2308	ID	yellow 332 (N03-060)	Canyon, ID	elk
7	4	4	2	4	3	2	3	7	4	09-0269	B. abortus RB51	TX	Sample 1 / 74SQY3675	(b)(6) [redacted]	Cattle
7	3	4	2	4	3	6	3	6	12	08-0404	YNP	MT	8-340 / P08-010	Yellowstone National Park	bison
7	3	4	2	4	3	6	3	6	12	08-0933	Rhyan, Dr. Jack (Nat'l .	WY	81-08	Bison quarantine study	Bison
7	3	4	2	4	3	6	3	6	12	08-1080	USDA/APHIS/VS/BQFS	MT	91-08 / 81APM1605	Bison quarantine study	Bison

Figure 1. (continued)



VNTR Hoof 1	VNTR Hoof 3	VNTR Hoof 4	VNTR Hoof 8	VNTR VNTR 16	VNTR VNTR 17	VNTR VNTR 2	VNTR VNTR 21	VNTR VNTR 5A	VNTR VNTR 5B	Case No.	Owner	State	Animal ID	Epidemiology	Species
7	3	4	2	4	3	6	3	6	12	08-0933	Rhyan, Dr. Jack (Nat'l .	WY	81-08	Bison quarantine study	Bison
7	3	4	2	4	3	6	3	6	12	08-1080	USDA/APHIS/VS/BQFS	MT	91-08 / 81APM1605	Bison quarantine study	Bison
7	3	4	2	4	3	6	3	6	12	08-1120	BQFS	MT	54-08 / 81AYE4054	Bison quarantine study	Bison
8	3	4	2	4	3	6	3	6	12	08-1122	BQFS	MT	91-08 / 81APM1605	Bison quarantine study	Bison
7	3	6	2	4	3	6	3	6	11	08-0386	University of Montana	MT	#33 / Bison	Yellowstone National Park	bison
11	3	12	2	4	3	6	3	6	8	08-0934	Rhyan, Dr. Jack (Nat'l .	WY	97-08	Bison quarantine study	Bison
14	3	9	2	4	3	5	3	6	9	08-1119	BQFS	MT	50-08 / (b)(6) [redacted]	Bison quarantine study	Bison
14	3	9	2	4	3	5	3	6	9	08-1121	BQFS	MT	6 / 81AYE4053	Bison quarantine study	Bison
9	3	9	2	4	3	5	3	6	9	08-0638	Yellowstone National Pa.	WY	YNP-82	field isolate	Bison
7	3	9	2	4	3	5	3	6	8	08-0416S1	(b)(6) [redacted]	MT	1-1340 / #601 / 81TUG.	new herd Montana - 2008	cattle
7	3	9	2	4	3	5	3	6	8	08-0416S2	[redacted] (b)(6)	MT	1-1340 / #601 / 81TUG.	new herd Montana - 2008	cattle
7	3	9	2	4	3	5	3	6	8	08-0416S3	[redacted]	MT	1-1340 / #601 / 81TUG.	new herd Montana - 2008	cattle
7	3	9	2	4	3	5	3	6	8	08-0416S4	[redacted] (b)(6)	MT	1-1340 / #601 / 81TUG.	new herd Montana - 2008	cattle
7	3	9	2	4	3	5	3	6	8	08-0416S5	[redacted]	MT	1-1340 / #601 / 81TUG.	new herd Montana - 2008	cattle
7	3	9	2	4	3	6	3	6	8	B10-0529	MT FWP	MT	BR092820	field isolate	Elk
9	3	8	2	4	3	5	3	6	8	B10-0273	MT FWP	MT	BR092770	field isolate	Elk
8	3	6	2	4	3	5	3	6	6	08-0392	University of Montana	MT	#39 / Bison	Yellowstone National Park	bison
11	3	6	2	4	3	5	3	6	6	08-0869	Yellowstone National Pa.	WY	YNP-292	field isolate	Bison
8	3	6	2	4	3	5	3	6	13	08-0921	Rhyan, Dr. Jack (Nat'l .	WY	32-08	Bison quarantine study	Bison
5	3	6	2	4	3	5	3	6	10	08-0382	University of Montana	MT	#27 / Bison	Yellowstone National Park	bison
5	3	6	2	4	3	5	3	6	10	08-1147	Yellowstone National Pa.	WY	YNP-206	field isolate	Bison
5	3	6	2	4	3	5	3	6	12	08-0876	Yellowstone National Pa.	WY	YNP-289	field isolate	Bison
5	3	6	2	4	3	5	3	6	9	08-1019	USDA/APHIS/VS/BQFS	MT	20-08 / 81ARG3869	Bison quarantine study	Bison
7	3	6	2	4	3	5	3	6	5	B10-0973	Clarke, P. Ryan-bull bis.	MT	YNP95021	field study	Bison
9	3	7	2	4	3	4	3	6	6	09-0286	Anderson, Neil (MFWP)	MT	BR081161	field isolate	elk
5	3	7	2	4	3	5	3	6	6	07-0266	(b) (6) [redacted] (later TransO.	MT	81 TSK 4771	new herd - Montana 2007	cattle
10	3	7	2	4	3	4	3	6	12	08-0389	University of Montana	MT	#36 / Bison	Yellowstone National Park	bison
12	3	7	2	4	3	5	3	6	12	08-0966	Yellowstone National Pa.	WY	YNP- 398	field isolate	Bison
6	3	7	2	4	3	6	3	6	10	B10-0118	MT FWP	MT	BR091887	field isolate	Elk
12	3	3	2	4	3	4	3	6	10	08-0928	Rhyan, Dr. Jack (Nat'l .	WY	4058	Bison quarantine study	Bison
6	3	8	2	4	3	5	3	7	13	08-0390	University of Montana	MT	#37 / Bison	Yellowstone National Park	bison
4	3	8	2	4	3	5	3	7	11	08-0929	Rhyan, Dr. Jack (Nat'l .	WY	45-08	Bison quarantine study	Bison
8	3	9	2	4	3	6	3	7	13	08-1256	Corwin Springs	MT	24-08	surveillance	Bison
6	3	3	2	4	3	5	3	5	5	06-0281	Bison Quarantine Study	MT	07-06/bison	seropositive YNP bison calf euthanized at.	bison
6	3	3	2	4	3	5	3	5	5	06-0429	Bison Quarantine Feasib.	MT	sample 29-06	YNP B. abortus-exposed bison calf as pa.	bison
6	3	3	2	4	3	5	3	5	5	06-0887	USDA/APHIS/VS	MT	77-06 (1.5 yo F)	Corwin Springs, Belgrade	bison
7	3	3	2	4	3	5	3	5	5	06-0279	Bison Quarantine Study	MT	14-06/bison	seropositive YNP bison calf euthanized at.	bison
9	3	5	2	4	3	5	3	5	9	B10-0584	MT FWP	MT	BR090822	field isolate	Elk
10	3	4	2	4	3	6	3	5	4	08-0863	Yellowstone National Pa.	WY	YNP-180	field isolate	Bison
10	3	4	2	4	3	6	3	5	4	08-0923	Rhyan, Dr. Jack (Nat'l .	WY	3877	Bison quarantine study	Bison
10	3	4	2	4	3	6	3	5	4	08-1354	Corwin Springs	MT	92-08 / 8-161	field isolate	Bison
9	3	4	2	4	3	6	3	5	4	08-0923	Rhyan, Dr. Jack (Nat'l .	WY	3877	Bison quarantine study	Bison
9	3	4	2	4	3	5	3	5	4	08-1123	BQFS	MT	38-08 / 81ARG3848	Bison quarantine study	Bison
2	6	4	2	4	3	5	3	5	12	05-0444	(b)(6) [redacted]	TX	74EXP6760	2005 - TX herd # 1	cattle
8	3	7	2	4	3	4	2	6	8	05-0911	Yellowstone National Pa.	MT	81APF6425	surveillance	Bison
9	4	8	2	4	3	5	3	6	11	08-0151	Montana Fish Wildlife &.	MT	#17 / Madden / 08/03/1.	Bozeman	elk
9	4	8	2	4	3	6	3	6	11	08-0410	YNP	MT	8-407-08 / P08-071 / Bi.	Mammoth Hot Springs	bison
9	4	5	2	4	3	5	2	6	11	05-1282	APHIS, VS	MT	81 APH 5970 (1 yo M)	Corwin Springs	bison
10	4	7	2	4	3	7	3	6	11	09-0276	Anderson, Neil (MFWP)	MT	BR081145	field isolate	Elk
6	6	8	2	4	3	8	3	7	5	05-1132	USDA	MT	81APF6442	reactor	Bison
12	5	8	2	4	3	6	3	7	5	08-1221	Yellowstone National Pa.	WY	YNP-347	surveillance	Bison
12	6	7	2	4	3	6	3	6	5	09-0553	BQFS- Bison Quarantin.	MT	85-08	Bison quarantine study	Bison

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