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Brucella Genotyping Test Report

Three *Brucella abortus* biovar 1 isolates were recovered and genotyped from a single adult cow (NVSL Acc # 496198), owner listed as Trans Ova Genetics-Barry Ellis from Baler, MT. The case was received at NVSL for culture and identification on May 11, 2007.

Genotyping was performed using the HOOF-print Variable Number Tandem Repeat (VNTR) locus analysis, and further analyzed using Simpson's Index of Diversity. Variation in the number of repeats is not the same among loci, where certain loci have higher levels of diversity than others. The Simpson's Index of Diversity represents likelihood that for a specific isolate, the number of repeat units for a chosen locus will be different from other isolates that may be encountered. A Simpson's Index that is closer to 1 correlates with a higher level of diversity, whereas an index closer to zero represents no diversity among isolates. The Simpson's Index of Diversity for each *B. abortus* VNTR locus used in the HOOF-print analysis is given in Table 1, and was calculated from **all** *Brucella abortus* isolates currently in the NVSL database.

Two major pieces of information can be interpreted from data in Table 1. First, three of the 10 loci (L-5, L-6 and L- 8) have no variation. This means that all genotyped *B. abortus* isolates in the NVSL database have the same number of repeat units at these loci, regardless of animal source or geographic location. Lack of variation suggests that loci L-5, L-6 and L- 8 lie within stable regions of the *Brucella* genome and do not frequently acquire mutations. Thus, these loci provide limited information for differentiating isolates within a disease outbreak.

Secondly, data from five of the 10 loci (L-2, L-3, L-7, L-9 and L-10) have higher Simpson's indices of diversity compared to the remaining five loci (L-1, L-4, L-5, L-6, and L-8; Table 1). Of the five loci with a higher Simpson's index of Diversity, loci L-7, L-9 and L-10 also have a high number of alleles for each locus, indicating that these loci are unstable in the *Brucella* genome and mutate very rapidly. This instability is also seen in Table 2, where isolates from the same animal and the same herd have observable variations at these loci. Therefore, genotyping comparisons to determine which isolates belong to the same outbreak cannot rely heavily on data from L-7, L-9 and L-10.

Table 1. Simpson's Index of Diversity for 10 VNTR loci (L-1 to L-10) within the *Brucella abortus* genome.

Locus number	L-1	L-2	L-3	L-4	L-5	L-6	L-7	L-8	L-9	L-10
Simpson's Index	0.301	0.700	0.716	0.358	**0.000**	**0.000**	0.802	**0.000**	0.789	0.760
No. of alleles per locus	8	5	6	5	1	1	10	1	10	8

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Comparison of the Montana Isolate to Other *Brucella* Isolates Recovered from Infected Animals

The three isolates from the single Montana cow (NVSL Accession 496198; Table 2) were compared to strains representing outbreaks in the U.S. from 1998 thru 2007. They include cases of *B. abortus* recovered from bison and elk in Idaho and the Greater Yellowstone Area of Montana and Wyoming, representative isolates from two infected cattle herds in Idaho from FY 2002 and 2005, three infected herds in Texas identified during FY 2004/2005, two herds from Louisiana, and a *B. abortus* vaccine strain recovered from an elk in Wyoming.

Table 2. Comparison of the Monatana *Brucella abortus* isolates (NVSL Acc # 496198) to other *B. abortus* strains recovered from infected livestock and wildlife in the United States.

					Brucella Genotyping Results									
NVSL Acc # or Lab ID	State	Epidemiology	Animal Species	Animal ID	L-1	L-2	L-3	L-4	L-5	L-6	L-7	L-8	L-9	L-10
496198	MT	New herd	cattle	81 TSK 4771 Supermammary LN	**5**	**4**	**3**	**7**	**2**	**2**	**5**	**2**	**10**	**6**
496198	MT	New herd	cattle	81 TSK 4771 Milk	**5**	**4**	**3**	**7**	**2**	**2**	**5**	**2**	**10**	**6**
496198	MT	New herd	cattle	81 TSK 4771 Prescapular LN	**5**	**4**	**3**	**7**	**2**	**2**	**5**	**2**	**13**	**6**
0355304	ID	2005 – ID herd	cattle	403	2	4	6	3	2	2	10	2	10	4
1361844	ID	2005 – ID herd	cattle	403	2	4	6	3	2	2	10	2	10	4
1361845	ID	2005 – ID herd	cattle	403	2	4	6	3	2	2	10	2	10	4
1361854	ID	2005 – ID herd	cattle	409	2	4	6	3	2	2	10	2	**9**	4
1355306	ID	2005 – ID herd	cattle	409	2	4	6	3	2	2	10	2	**9**	4
1355309	ID	2005 – ID herd	cattle	409	2	**5**	6	3	2	2	**9**	2	10	4
1361840	ID	2005 – ID herd	cattle	409	2	4	6	3	2	2	10	2	10	4
1361852	ID	2005 – ID herd	cattle	409	2	4	6	3	2	2	10	2	**9**	4
1385575	ID	2005 – ID herd	cattle	409	2	4	6	3	2	2	10	2	**9**	4
1455128	ID	2005 – ID herd	cattle	409	2	4	6	3	2	2	10	2	**9**	4
1355310	ID	2005 – ID herd	cattle	437	2	4	6	3	2	2	**8**	2	10	4
1361842	ID	2005 – ID herd	cattle	437	2	4	6	3	2	2	**9**	2	10	4
1455133	ID	2005 – ID herd	cattle	437	2	4	6	3	2	2	**8**	2	10	4
1262863	MT	Yellowstone	bison	81 APF 6442	7	4	6	8	2	2	5	2	9	5

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1272725	MT	Yellowstone	bison	81 APF 6470	2	5	3	3	2	2	6	2	16/17	12
1302822	MT	Yellowstone	bison	81 APH 5970	9	5	4	5	2	2	4/5	2	10	11
1407792	MT	Yellowstone	bison	2179	6	4	3	3	2	2	5	2	14	5
1431902	MT	Yellowstone	bison	14-06	6	4	3	3	2	2	5	2	14	5
1431904	MT	Yellowstone	bison	07-06	6	4	3	3	2	2	4	2	14	5
1466357	MT	Yellowstone	bison	sample 29-06	6	4	3	3	2	2	5	2	14	5
1531593	MT	Yellowstone	bison	77-06	6	4	3	3	2	2	5	2	14	5
1451690	WY	Yellowstone	bison	P06-003	8	4	3	9	2	2	6	2	14	12
	WY	Strain 19	elk	2004020178	5	4	4	2	2	2	8	2	12	6
718389	ID	strain 2308	elk	yellow 332	6	4	4	2	2	2	11	2	10	4
718390	ID	strain 2308	elk	yellow 332	6	4	4	2	2	2	10	2	10	4
718391	ID	strain 2308	elk	yellow 332	6	4	4	2	2	2	12	2	10	4
51837	ID	Rainey Creek – Feb 2000	elk	60237	2	6	7	3	2	2	5	2	12	8
180373	ID	Rainey Creek – 2002	elk	7217	2	6	7	3	2	2	5	2	12	8/9
163343	ID	Conant Creek - 2002	elk	60218/327	2	5	3	13	2	2	10	2	10	9
269054	ID	Rainey Creek – Mar 2002	elk	497/W 68	2	5	3	5	2	2	11	2	13	4
278724	ID	Rainey Creek – Jun 2002	elk	ERO3-177	2	6	7	3	2	2	5	2	13	8
300438	ID	Rainey Creek – Feb 2004	elk	4318	2	6	7	3	2	2	5	2	13	8
365387	ID	Rainey Creek – Feb 2005	elk	4985031105-2PA	2	7	6	3	2	2	7	2	16	7
543996	ID	2002 – ID herd	cattle	82VIU1922	2	5	3	10	2	2	10	2	10	10
543997	ID	2002 – ID herd	cattle	82ALK6226	2	5	3	10	2	2	10	2	9	10
543998	ID	2002 – ID herd	cattle	82AKN7633	2	5	3	10	2	2	10	2	9	9
544002	ID	2002 – ID herd	cattle	82VME6919	2	5	3	6	2	2	10	2	9	10
544002	ID	2002 – ID herd	cattle	82ALK223	2	5	3	10	2	2	10	2	9	10
354997	TX	2005 - TX herd # 1	cattle	74EXP6761	2	3	6	4	2	2	4	2	13	12
392810	TX	2005 - TX herd # 2	cattle	74FAA0148	4	3	5	4	2	2	8	2	13	6
327720	TX	2005 - TX herd # 3	cattle	74EME0146	2	3	6	4	2	2	4	2	13	12
98-27532	LA	1998 – LA herd # 1	cattle	AYR 3093	10	3	5	4	2	2	6	2	17	7
98-24255	LA	1998 – LA herd # 1	cattle	72 KS 8060	10	3	4	4	2	2	6	2	17	6
98-24441	LA	1998 – LA herd # 1	cattle	72 KS 2150	11	3	5	4	2	2	6	2	17	7
98-2436	LA	1998 – LA herd # 2	cattle	72 LP 0124	2	3	5	5	2	2	5	2	11	5
98-5100	LA	1998 – LA herd # 2	cattle	72 LP 2443	2	3	5	4	2	2	7	2	14	4

The three isolates from the Montana animal were identical at nine of the ten loci, but variation was seen at locus L-9. This locus shows 10 repeat units in two isolates recovered from the supermammary lymph node and milk, and 13 repeats in the isolate from the prescapular lymph node.

Minor variations in repeat units for certain loci can also be observed in other strains isolated from animals in the same herd. Table 2 shows three independent isolates from animal 403 that are identical. Conversely, isolates from animal 409 (same herd as 403) shows genotype variation at locus L-2, L-7 and L-9, and animal 437 (also from this same herd) shows genotype variation at locus L-7.

Conclusion

Analysis of this data indicates that the *B. abortus* strain recovered from the adult cow in Montana appears to be different from other *B. abortus* isolates in the NVSL database based on variation at the 10 VNTR loci. Specifically, loci L-1 and L-4 each have a unique allele (5 and 7 repeat units, respectively) not observed in any other *B. abortus* strain to date. Considering that these two loci exhibit a relatively low index of diversity, this variation from the other isolates in the database is considered to be significant. Although there appears to be some similarity between the genotype of the Montana isolates and those recovered from wildlife within the Greater Yellowstone area (GYA), conclusions cannot be drawn regarding possible relationships between Montana and GYA isolates. Several factors remain unknown which hinder the interpretation of this data, including; the possibility that multiple genotypes exist within infected populations of wildlife in the GYA, the stability/mutation rate of specific alleles and/or loci within the *Brucella* genome, and the likelihood that independent mutations may occur in two unrelated isolates resulting in identical genotypes. Therefore, this data must be interpreted with caution, and can only be used to augment concurrent traditional epidemiological investigations.