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SUMMARY

In this article we review the species-specific genetic markers in the blood that are currently being used to authenticate the bison ancestry of animals entering the Ancestry Registry of the American Beefalo World Registry. Independently of that program we are also testing for these same genetic markers in a number of private herds in which attempts are being made to develop bison-cattle hybrids that are fully fertile and incorporate desirable traits of both ancestral species.

INTRODUCTION

Until the advent of the Basalo Hybrid Beefalo (BHB) in the early 1970s, there was little more than academic interest in genetic markers in the blood which might be used to distinguish between bison, cattle and their hybrids. BHB animals were said to be 3/8 American buffalo (Bison bison or Bos bison) and 5/8 domestic (Bos taurus). Despite the world-wide publicity which this new breed received, there was no published information on its derivation. This, coupled with the observation that these animals exhibited little, if any, superficial resemblance to bison, raised questions concerning their genealogy, especially in the scientific community.

Eventually we obtained blood samples from 7 fullblood and 141 halfblood BHBs. The blood samples were tested for the presence or absence of a number of bison-specific genetic markers detected by means of starch-gel electrophoresis, namely, bison-specific hemoglobin (Hb), transferrin (Tf) and two electrophoretic forms of the red blood cell (RBC) enzyme known as carbonic anhydrase (CA). We also tested the RBCs of these animals with antibody reagents which react with one or more species-specific antigenic markers on bison RBCs.

We estimated the percentage of animals of bison fractions ranging from 7/8 to 1/16 that would be expected to have at least one of these bison-specific genetic markers in their blood. These estimates, shown in Table 1, were on the conservative side for two reasons. We used a combined frequency of 0.84 for the two alleles that code for the bison-specific forms of CA, namely, CA1 and CA2, when we now know that the combined frequencies of those two alleles is closer to 0.92. For convenience in making those estimates, we assumed that our bison-specific antibody reagents were reacting with one species-specific antigen when it is likely that these reagents react with more than one bison-specific antigen.

As shown in Table 2, we found none of these bison-specific genetic markers in the blood of the 7 animals represented to us as fullblood BHBs and only one (the RBC antigenic marker) in 141 animals represented to us as halfblood BHBs. These results were reported in a brief note (Stormont, Morris and Suzuki)

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published in 1977.

Subsequent to these initial studies the World Beefalo Association was formed and not long thereafter a splinter organization, known as the International Beefalo Breeders Registry, came into existence. In the course of providing blood typing services for these two organizations, we put on record the blood types of virtually all the foundation BHBs plus additional purebred BHBs that entered these two registries, a total of 46 bulls and 54 cows. None of these animals possessed any of the aforementioned genetic markers. Many were also tested for electrophoretic forms of the RBC enzyme known as 6-phosphogluconate dehydrogenase (PGD) but none of them possessed the bison-specific form of that enzyme, thereby amplifying the conclusion that they probably had no bison ancestors.

Analysis of phenogroups in the B system of cattle blood groups provides the most definitive way of distinguishing between breeds of cattle (Stormont, 1984). Analysis of the B phenogroups in fullblood BHBs indicated that this breed was probably derived by breeding Charolais, Angus and Hereford cattle interse with a preponderance of Charolais breeding. We cannot, however, rule out the possibility some Holsteins and Shorthorns may have been used in the development of the BHB.

Let us now turn to a brief review of the species-specific genetic markers in the blood and the basis for their use in distinguishing between bison, cattle and their hybrids.

THE SPECIES-SPECIFIC GENETIC MARKERS

Those Defined by Serologic Tests.

One of the classical ways of distinguishing between two closely related species is to prepare antibody reagents which will react with (i.e., either agglutinate or hemolyse in the presence of complement) the RBCs of the one species but not the other, and the converse.

This can often be done by preparing an antiserum in domestic rabbits against the one species (the homologous species) and absorbing all the antibodies in this antiserum that will react with the RBCs of the other species (the heterologous species). When this is done, it is generally the rule that such antisera will still contain some antibodies specific for the RBCs of the homologous species. But sometimes we are not always successful in producing these species-specific antibody reagents. Such was the experience of Owen, Stormont and Irwin (1958) in the initial study of blood groups in American buffalo.

We have been successful in producing bison-specific antibody reagents not only using antisera produced in rabbits but also antisera produced in cattle by immunizing with bison RBCs. On the other hand, all of our cattle-specific reagents have been prepared by using bison RBCs to absorb antisera produced in rabbits.

Positive reactions with the bison-specific antibody reagents are indicated by the letter B whereas positive reactions with the cattle-specific reagents are indicated by the letter C. In testing bison-cattle hybrids with these reagents three types are possible, namely, B, BC and C. Any animal of type BC is an authentic hybrid, whereas any animal of type B qualifies along with those of type BC as having at least one bison-specific genetic marker.

Those Defined by Electrophoretic Tests.

The Hemoglobins. Braend and Stormont (1963) reported that all of 113 bison blood samples, obtained from two non-interbreeding populations, were of exactly

the same Hb type, a two-zoned pattern in which the leading zone migrated to a position just behind the fast zone (B) of cattle Hb whereas the trailing or slow zone migrated to the same position as the slow zone of cattle Hb, namely, zone A. Thus, this monomorphic, two-zoned pattern of bison Hb is clearly distinct from the three common Hb types, A, AB and B, found in the taurine breeds of cattle.

We use the symbol bi to indicate the presence of bison Hb. The possible types of Hb encountered in the hybrids are bi, Abi, biB, A, B and AB. Any animal of type Abi or biB is an authentic hybrid, whereas any animal of type bi or Abi or biB has at least one bison-specific genetic marker.

The Transferrins. In the aforementioned report, Braend and Stormont pointed out that all 113 bison were of the same Tf type, a three-zone pattern which, under the conditions of their runs, appeared to be indistinguishable from the three-zone pattern of Tf type A of domestic cattle.

Using a system of buffers which were more effective in revealing differences in Tf types, Stormont (1964) was able to distinguish the bison Tf type from all of the Tf types coded for by the common Tf alleles (TF^A, Tf^D1, Tf^D2 and Tf^E) of taurine breeds, thereby adding another bison-specific genetic marker which could be tested for in the hybrids.

Any animal of Tf phenotype Abi or biD_1 or biD_2 (or biD where the distinction between D_1 and D_2 may not be clear) or biE are authentic hybrids, whereas any animal possessing any one of those types, or bi Tf alone, qualifies as having at least one bison-specific genetic marker.

The Carbonic Anhydrases. Sartore, Stormont, Morris and Grunder (1969) described two electrophoretic forms of CA in domestic cattle and three in bison.

The alleles for the two forms of cattle CA were designated CA^F and CA^S and these produce phenotypes F, FS and S. The alleles for the three forms of bison CA were designated CA^1, CA^2 and CA^3 and these produce phenotypes 1, 1-2, 1-3, 2, 2-3 and 3. When the bison CA types were compared with those of cattle it was evident that the zones coded for by the bison alleles CA^1 and CA^2 occupied positions which were clearly ahead of the fast zone (F) of cattle. In contrast, zone 3 of bison occupied an electrophoretic position which appeared to be the same as that occupied by the slow zone (S) of cattle. Thus, only the zones CA1 and CA2 of bison could be utilized as bison-specific genetic markers.

In the various bison-cattle hybrids any of the six bison CA phenotypes and any of the three cattle CA phenotypes are possible. However, since zone CA-3 of bison is indistinguishable from CA-S of cattle, we use only the symbol S in designating those phenotypes in which either 3 of bison or S of cattle could be present.

The distinguishable phenotypes in the hybrids are 1, 1-2, 1-F, 1-S, 2, 2-F, 2-S, F, FS and S. Any animal of phenotype 1-F or 2-F is an authentic hybrid whereas an animal of any of the following phenotypes has at least one bison-specific genetic marker: 1, 1-2, 1-F, 1-S, 2, 2-F and 2-S.

The 6-Phosphogluconate Dehydrogenases. Contrary to results published by Naik and Anderson (1970), Suzuki, Auditore, Morris and Stormont (1979) observed that the enzyme 6-phosphogluconate dehydrogenase is monomorphic in bison, just as it is in cattle, with the bison zone (labelled B) migrating ahead of the cattle zone (labelled C), thereby providing another genetic marker which can be used to distinguish between the two species and their hybrids.

In the hybrids three phenotypes (B, BC and C) are possible. Any animal of phenotype BC is an authentic hybrid whereas any animal of phenotype B or BC possesses at least one bison-specific genetic marker.

6PGD is a homo-dimer. That is to say, each molecule of 6PGD is composed of two identical polypeptide chains. In hybrids of phenotype BC we see not only the homo-dimeric zones, B and C, but also see intermediate between those zones a

hybrid zone. The hybrid zone is a hetero-dimer in which each molecule is composed of one B polypeptide chain and one C polypeptide chain.

Other Markers. In addition to tests for the aforementioned markers, we also electrophorese the blood plasma of all hybrids in polyacrylamide gels after the method described by Gahne, Juneja and Gromulus (1977) in attempts to distinguish between bison, cattle and their hybrids by analysis of the Gc (vitamin D binding protein) and post-transferrin-2 (PTf-2) zones. The reason we do this is that J. Kraay, in a poster demonstration presented at the 19th International Conference on Animal Blood Groups and Biochemical Genetics, Gottingen, 23-27 July, 1984, presented line drawings showing distinct differences between bison and cattle with respect to these two systems of proteins. At times, especially when the blood samples are very fresh, we can see real differences between the PTf-2 zones of cattle and that of bison but we have not been able to make a clear distinction between the Gc zones of the two species. In routine runs in polyacrylamide gels we have found that the PTf-2 zones of the hybrid cannot be reliably distinguished from those of the parental species.

In a study of 19 systems of isozymes in the tissues (kidney, liver and muscle) of a variety of mammalian species, including Bos bison and Bos taurus, Baccus, Ryman, Smith, Reuterwall and Cameron (1983) reported that bison and cattle were distinctly different with respect to only one enzyme, namely, GOT-1, which is consistent with the earlier observations of Suzuki et al (1979). They also recorded two forms of glutamate oxalate transaminase (GOT-1) in bison, one of which was the same as that observed in cattle. But the numbers of animals studied (7 bison and 6 cattle) were hardly sufficient to decide whether the two species differ at the GOT-1 locus.

DISCUSSION

Whether anything can be gained by searching for additional systems of genetic markers in the blood that might be used to distinguish between bison, cattle and their hybrids is debatable. We take the position that the tests provided by our laboratory are more than adequate for the purpose of screening animals being considered for registration in the Ancestry Registry of the American Beefalo World Registry. ABWR has no requirement for bison-specific genetic markers in animals less than 3/8 bison. For animals 3/8 bison up to but not including 1/2 bison, one or more bison-specific markers is required. For 1/2 bison up to 3/4 bison, two or more bison-specific markers are required. For animals over 3/4 bison, three or more bison-specific markers are required, as published in Vol. 2, No. 4 (July 26, 1985) of the Beefalo Nickel. As indicated by the data in Table 1, there is virtually no chance that an animal which is truly 3/8 bison, or more, would fail to exhibit at least one of the four kinds of bison-specific markers upon which the data in that table are based.

After our experiences in testing BHBs for bison-specific genetic markers it is, of course, gratifying to find that there are now many hybrid Beefaloes which do possess bison-specific genetic markers and that there is a registry (ABWR) to represent those animals. The Officers, Board of Directors and members of this organization realize that if the Beefalo is to gain credibility in the eyes of animal scientists and the general public it must exhibit some genes that trace to bison ancestors.

REFERENCES

BACCUS, R., RYMAN, N., SMITH, M. H., REUTERWALL, C. and CAMERON, D. 1983. Genetic variability and differentiation of large grazing mammals. J. Mamm. 64, 109-120.

BRAEND, M. and STORMONT, C. 1963. Haemoglobin and transferrin types in American buffalo. Nature 197, 910-911.

GAHNE, B., JUNEJA, K. and GROMULUS, J. 1977. Horizontal polyacrylamide gradient gel electrophoresis for the simultaneous phenotyping of tranferrin, post-transferrin, albumin and post-albumin in the blood plasma of cattle. Anim. Blood Grps biochem. Genet. 8, 127-137.

MALIK, S. N. and ANDERSON, D. C. 1970. Study of glucose 6-phosphogluconate dehydrogenase and 6-phosphogluconate dehydrogenase in American buffalo (Bison bison). Biochem. Genet. 4, 651.

OWEN, R. D., STORMONT, C. and IRWIN, M. R. 1958. Studies on blood groups in the American buffalo (bison). Evolution 12, 102-110.

SARTORE, G., STORMONT, C., MORRIS, B. G. and GRUNDER, A. A. 1969. Multiple electrophoretic forms of carbonic anhydrase in red cells of domestic cattle (Bos taurus) and American buffalo (Bison bison). Genetics 61, 823-831.

STORMONT, C. 1964. A further comparison of bison and cattle transferrins. Federation Proceedings 23, 557.

STORMONT, C. 1980. The B and C systems of cattle revisited. Pp 31-43 in Frontiers in Immunogenetics. Ed. by W. H. Hildemann. Elsevier/North Holland.

STORMONT, C. 1984. Genetic markers in the blood and their application in animal breeding. Pp 319-327 in Proceedings of the 2nd World Congress on Sheep and Beef Cattle Breeding. South African Stud Book and Livestock Improvement Association. Bloemfontein, R.S.A.

STORMONT, C. J., MORRIS, B. and SUZUKI, Y. 1977. Analysis of blood groups in Beefalo and other bison-cattle hybrids. Anim. Blood Grps biochem. Genet. 8, Suppl. 1, 33.

SUZUKI, Y., AUDITORE, K. J., MORRIS, B. and STORMONT, C. 1979. Transient electrophoretic forms of red cell 6PGD in American buffalo (Bison bison) and domestic cattle (Bos taurus). Biochem. Genet. 17, 445-448.

Table 1. Percentage of animals expected to have at least 1 of the 4 bison-specific markers, namely, the RBC antigenic marker, Hb, Tf and CA.

Bison fraction	Percentage with one or more markers
7/8	..... 100.00
3/4	..... 100.00
1/2	..... 100.00
31/64	$1 - (0.03125^3 \times 0.18625)$ 99.99
7/16	$1 - (0.125^3 \times 0.265)$ 99.95
3/8	$1 - (0.25^3 \times 0.37)$ 99.42
5/16	$1 - (0.375^3 \times 0.475)$ 97.50
1/4	$1 - (0.5^3 \times 0.58)$ 92.75
3/16	$1 - (0.625^3 \times 0.685)$ 83.28
1/8	$1 - (0.75^3 \times 0.79)$ 66.67
1/16	$1 - (0.875^3 \times 0.895)$ 40.04

Table 2. Comparison of observed and expected numbers of animals of various bison fractions for the presence of at least one of the four bison-specific markers.

Fraction	No. tested	No. with at least one marker	No. expected
7/8	2	2	2
3/4	6	6	6
1/2	7	7	7
31/64	1	1	1
7/16	1	1	1
7/16*	1	0	1
3/8	4	2	4
3/8*	7	0	7
5/16	3	0	3
1/4	12	9	11
3/16*	141	1	117
1/8	9	3	6
1/16	2	1	1

* Basolo Hybrid Beefalo (BHB) Breeding.