Brazilian Journal of Genetics, 20, 1, 9-12 (1997)
(Revista Brasileira de Genética)

A new allele of peptidase-B in cattle

Maria Aparecida Cassiano Lara[1] *and Eucleia Primo B. Contel*[2]

ABSTRACT

Electrophoretic analyses of peptidase-B were carried out on red cell hemolysates from Holstein, Mantiqueira and Gyr cattle, using cornstarch, known in Brazil as Penetrose-30. We describe a new peptidase-B allele, denoted *Pep-B*^{3}, in Mantiqueira cattle, belonging to the *Bos taurus* group, which are the result of a cross of native cattle of Portuguese origin introduced in Brazil during colonial times (16th century) with Holstein and Caracu cattle. The genetic control of peptidase-B was determined by typing parents and progeny segregating for all three alleles, confirming that peptidase B is controlled by a single autosomal locus with three codominant alleles, denoted *Pep-B*^{1}, *Pep-B*^{2} and *Pep-B*^{3}. The use of the citrate-phosphate buffer system, at pH 5.9, on 14% gel, under the electrophoretic conditions standardized in this study permitted good visualization of all peptidase-B variants.

INTRODUCTION

Protein polymorphisms, which represent part of the genetic variability existing among living beings, have been extensively used in studies of racial characterization (Tagliaro *et al.*, 1995), of phylogenetic relations (Zafindrajaona and Lauvergne, 1993), and in studies of the linkage between these genetic markers and genes that affect traits of economic interest (Clamp *et al.*, 1992).

In cattle, some polymorphic gene systems may be considered racial markers since certain alleles exclusively occur in breeds of Indian origin (*Bos indicus*). The *Alb*^{C} allele of albumin (Ashton and Lampkin, 1965), the *Ca*^{Z} allele of carbonic anhydrase (Penedo *et al.*, 1982) and the *Pep-B*^{1} allele of peptidase-B (Del Lama *et al.*, 1992) are particularly important among them. Other systems in addition to these are considered to be excellent genetic markers because of the occurrence of certain alleles at distinct gene frequencies that permit the racial characterization of *Bos taurus* and *Bos indicus* groups. Among them are the *Am-1*^{C} allele of amylase (Ashton, 1965; Gebicke-Härter and Geldermann, 1977), the *Tf*^{E} and *Tf*^{D} alleles of transferrin (Baker and Manwell, 1980; Jain *et al.*, 1992), the *Gc*^{C} allele of the vitamin D-bound protein and *Ptf-2*^{S} allele of posttransferrin-2 (Van de Weghe *et al.*, 1982), and the *NP*^{H} allele of nucleoside phosphorylase (Panepucci *et al.*, 1990). Other studies have also demonstrated associations between these genetic markers and traits of economic interest, such as milk yield (Bovenhuis *et al.*, 1992), beef production (Rahman and Kalan, 1986) and adaptive traits (Carr *et al.*, 1974; Panepucci *et al.*, 1989). These data demonstrate the importance of looking for new genetic markers that might permit a better understanding of the origin of cattle breeds, as well as their application to animal breeding.

MATERIAL AND METHODS

A total of 472 Holstein, Mantiqueira and Gyr dairy cows were sampled at random from herds belonging to the Instituto de Zootecnia de Nova Odessa and to the Estação Experimental of Pindamonhangaba and Ribeirão Preto, all of them in the State of São Paulo.

[1] Seção de Reprodução e Inseminação Artificial, Instituto de Zootecnia, Caixa Postal 60, 13141-000 Nova Odessa, SP, Brasil.
[2] Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, USP, 14049-900 Ribeirão Preto, SP, Brasil. Send correspondence to E.P.B.C.

Blood samples were collected into 5-ml vacutainer tubes containing sodium EDTA as an anticoagulant. Red cells were separated by centrifugation at 2,000 *g* for 10 min, washed three times in 0.85% NaCl and stored at -20°C until the time for electrophoretic analysis.

Red cells were then incubated with an identical volume of 50 mM 2-mercaptoethanol for 20 min and the hemolysates were absorbed with 0.5 x 0.6 cm Whatman filter paper and applied to the gel.

The buffer system used in the cuvettes was phosphate/citrate, pH 5.9 (0.245 M NaH_2PO_4 and 0.15 M citric acid). The 14% gel (Val *et al.*, 1981) was prepared by 1:40 dilution. Electrophoresis was run for approximately 1.5 h at 8°C, with 5 V/cm applied to the gel. The gels were then cut horizontally and developed with l-leucylglycyl-glycine as substrate (Lewis and Harris, 1967).

When a new band was detected, especially in Mantiqueira cattle, the progeny of all individuals that carried that phenotype was typed in order to exclude the possibility that the event observed was a secondary band. The progeny of mating between parents carrying the three alleles was also analyzed.

Gene frequencies and their respective standard deviations and Hardy-Weinberg equilibrium were determined using the Fregen computer program of the Genioc system (Cabello and Krieger, 1991).

RESULTS

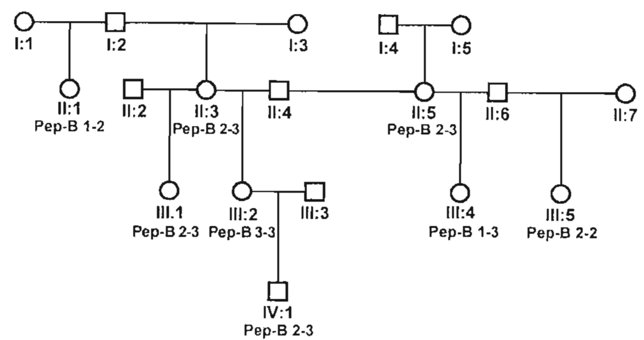
The new method permitted the detection of a new peptidase-B allele, denoted *Pep-B*^{3}, in Mantiqueira cows. The electrophoretic pattern of this enzyme, visualized with the substrate l-leucylglycyl-glycine, is illustrated in Figure 1.

Analysis of parents and progeny confirmed that peptidase-B polymorphism is determined by a single locus with three alleles: *Pep-B*^{1}, *Pep-B*^{2} and *Pep-B*^{3}. Their products present differences in electrophoretic mobility, with Pep-B3 being the most anodal enzyme and Pep-B2 the intermediate one.

Estimated gene frequency, standard deviations and χ^2 values, which permitted us to determine Hardy-Weinberg equilibrium, are presented in Table I. Only the *Pep-B*^{2} allele was detected in Holstein cows, as expected, and *Pep-B*^{1} and *Pep-B*^{2} were detected in the others, in addition to *Pep-B*^{3} which was exclusively found in Mantiqueira cows. The χ^2 test revealed that the Gyr and Mantiqueira herds were in Hardy-Weinberg equilibrium at this locus.

DISCUSSION

We identified a new allele of red cell peptidase-B, denoted *Pep-B*^{3}, in Mantiqueira cattle, as well as alleles *Pep-B*^{1} and *Pep-B*^{2}. The method described here permitted good visualization of all variants of this enzyme. The choice of the type of support used was due to the fact that cornstarch, known in Brazil as Penetrose-30, is locally manufactured and can be easily acquired



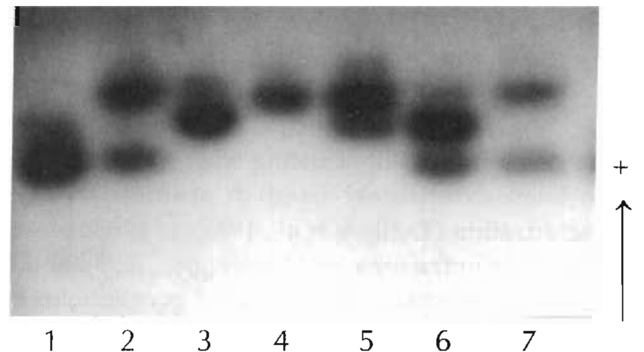


Figure 1 - Electrophoretic pattern of cattle red cell peptidase-B obtained on 14% cornstarch gel, citrate/phosphate buffer (0.245 M NaH_2PO_4 and 0.15 M citric acid), pH 5.9, and developed with l-leucylglycyl-glycine as substrate. The data are presented from left to right. Pep-B 1-1 (lane 1 - provenance Gyr); Pep-B 2-2 (lane 3); Pep-B 3-3 (lane 4); Pep-B 1-2 (lane 6); Pep-B 1-3 (lanes 2 and 7) and Pep-B 2-3 (lane 5). The segregation of the three alleles in a family of the Mantiqueira cattle can be observed in the pedigree shown above.

Table I - Gene frequency estimates and respective standard deviations of the genetic system of red cell peptidase-B from cattle belonging to two taurine breeds (Holstein and Mantiqueira) and one Zebu breed (Gyr) selected for dairy purposes.

Breed	N	*Pep-B*^{1}	*Pep-B*^{2}	*Pep-B*^{3}	χ^2
Holstein	100	0	1.000	0	-
Gyr	255	0.7667 ± 0.0191	0.2333 ± 0.0191	0	0.435
Mantiqueira	117	0.1154 ± 0.0224	0.8205 ± 0.0258	0.0641 ± 0.0154	0.669

at low cost. The buffer system and electrophoretic conditions used permitted good separation, as well as the identification of all phenotypes of red cell peptidase-B.

On the basis of the review published by Harris and Hopkinson (1976), who pointed out that the different peptidases (EC 3.4.11 or 13* and 3.4.13.9) can be identified by their different electrophoretic properties and affinities for specific substrates, and considering that the peptidase activity studied here was developed with the same substrate (l-leucylglycyl-glycine) as previously used by Lewis and Harris (1967) for human hemolysates, and by Saison (1973) and Del Lama *et al.* (1992) for cattle, we conclude that the enzyme studied here corresponds to that described by these investigators, who called it Pep-B.

In addition, care was taken to incubate the hemolysates with 2-mercaptoethanol prior to use, in order to avoid oxidation of sulfhydryl radicals present in the molecule of the enzyme by oxidized glutathione, a substance found in large amounts in red cells. This minimized formation of secondary bands.

We detected peptidase-B monomorphism in Holstein cattle, in agreement with data reported by Saison (1973), who described the absence of genetic variation in Charolais, Holstein, Simmenthal and Limosine cattle, as later confirmed by Del Lama *et al.* (1992), who suggested the fixation of $Pep\text{-}B^2$ in these taurine breeds.

The genetic variability detected in Gyr cows agrees with the data reported by Del Lama *et al.* (1992), who first described genetic polymorphism of red cell peptidase-B. These investigators reported the occurrence of the $Pep\text{-}B^1$ allele in Zebu cattle and suggested its use as a genetic marker for this racial group. However, our results demonstrate $Pep\text{-}B^1$ in Mantiqueira cows, which belong to the *Bos taurus* group.

Red cell peptidase-B from Mantiqueira cows was studied for the first time in the present investigation. The origin of this cattle is somewhat obscure. According to Guaragna *et al.* (1984), the Mantiqueira breed was formed from crosses between Holstein sires and Caracu cows, a breed introduced on the occasion of the discovery of Brazil. Thus, we raise the hypothesis that the $Pep\text{-}B^3$ allele is a marker of the Portuguese breed.

A possible explanation for the occurrence of the $Pep\text{-}B^3$ allele in Mantiqueira cattle is that these cows are being selected for adaptive traits which are often contrary to the objectives of selection for yield traits in specialized breeds, a fact that would lead to the elimination of genetic variability at the peptidase locus, as detected in the present study for Holsteins and in other studies in the literature (Saison, 1973).

ACKNOWLEDGMENTS

The authors wish to thank the directors of the Estação Experimental de Zootecnia, Dr. José Ramos Nogueira and Dr. Luciano Ricardo Marcondes da Silva, and their staff for the assistance provided during blood collection, and Drs. Roberto Hauck Reichter and Lewis Joel Greene for valuable suggestions. We are particularly grateful to the scientific investigator Guilherme Paes Guaragna, in charge of the project "Breeding of Mantiqueira Type Dairy Cattle" for encouraging us to study these animals.

We are also indebted to Refinaria de Milho Brasil for their kind gift of the starch used in the present study.

This research was supported by CAPES, CNPq and by Instituto de Zootecnia.

Publication supported by FAPESP.

RESUMO

A peptidase-B eritrocitária foi estudada eletroforeticamente em bovinos das raças Holandesa, Mantiqueira e Gir, utilizando-se o amido de milho conhecido no Brasil como Penetrose-30. Detectamos um novo alelo desta enzima, denominado $Pep\text{-}B^3$, no bovino Mantiqueira. Este gado é descendente de raças que foram introduzidas no Brasil no período da colonização (século XVI) e do gado Caracu e Holandês.

Determinamos o controle genético da peptidase-B através da análise da progênie de pais que possuíam os três alelos, confirmando que esta enzima é controlada por um único loco autossômico com três alelos,denominados $Pep\text{-}B^1$, $Pep\text{-}B^2$ e $Pep\text{-}B^3$. A utilização de um tampão citrato-fosfato, pH 5,9 com o gel na concentração de 14% e nas condições eletroforéticas padronizadas neste trabalho, permitiu uma boa visualização de todas as formas da peptidase-B.

REFERENCES

Ashton, G.C. (1965). Serum amylase (Thread protein) polymorphism in cattle. *Genetics 51*: 431-437.

Ashton, G.C. and **Lampkin, G.H.** (1965). Serum albumin and transferrin polymorphism in East African cattle. *Nature 205*: 209-210.

Baker, A.C.M. and **Manwell, C.** (1980). Chemical classification of cattle. 2. Phylogenetic tree and specific status of the zebu. *Anim. Blood Grps. Biochem. Genet. 11*: 127-150.

Bovenhuis, H., Arendonk, J.A.M.V. and **Korver, S.** (1992). Associations between milk protein polymorphisms and milk production traits. *J. Dairy Sci. 75*: 2549-2559.

Cabello, P.H. and **Krieger, H.** (1991). *Genioc-Programoteca*. Fundação Instituto Oswaldo Cruz, Rio de Janeiro.

Carr, W.R., MacLeod, J., Woolf, B. and **Spooner, R.L.** (1974). A survey of relationship of genetic markers, tick-

infestation level and parasitic diseases in zebu cattle in Zambia. *Trop. Anim. Hlth Prod. 6:* 203-214.

Clamp, P.A., Beever, J.E., Fernando, R.L., McLaren, D.G. and **Schook, L.B.** (1992). Detection of linkage between genetic markers and genes that affect growth and carcass traits in pigs. *J. Anim. Sci. 70*: 2695-2706.

Del Lama, S.N., Del Lama, M.A., Mestriner, M.A. and **Mortari, N.** (1992). Peptidase-B polymorphism in cattle erythrocytes. *Biochem. Genet. 30*: 247-255.

Gebicke-Härter, P.J. and **Geldermann, H.** (1977). Inheritance of amylases in blood serum of cattle. *Biochem. Genet. 15*: 59-73.

Guaragna, G.P., Gambini, L.B. and **Figueiredo, A.L.** (1984). *Mantiqueira - 30 Anos Depois*. Ed. Instituto de Zootecnia, Nova Odessa, SP, Brasil, pp. 27.

Harris, H. and **Hopkinson, D.A.** (1976). *Handbook of Enzyme Electrophoresis in Human Genetics*. North-Holland, Amsterdam.

Jain, A., Singh, A. and **Khan, F.H.** (1992). Transferrin polymorphism in Jersey cattles. *Indian J. Dairy Sci. 45*: 300-302.

Lewis, W.H.P. and **Harris, H.** (1967). Human red cell peptidases. *Nature 215*: 351-355.

Panepucci, L., Alencar, M.M. and **Oliveira, G.P.** (1989). Estudo preliminar da relação entre marcadores genético-bioquímicos e resistência ao carrapato em gado de corte. *Rev. Soc. Bras. Zoot. 18*: 172-178.

Panepucci, L., Alencar, M.M., Vicente, V. and **Mortari, N.** (1990). Comparative gene frequencies of nucleoside phosphorylase from cattle of *Bos taurus* and *Bos indicus* derivation in Brazil. *Comp. Biochem. Physiol. 95B*: 501-503.

Penedo, M.C.T., Mortari, N. and **Magalhães, L.E.** (1982). Carbonic anhydrase polymorphism in Indian Zebu cattle. *Anim. Blood Grps. Biochem. Genet. 13*: 141-143.

Rahman, M.F. and **Kalan, M.A.** (1986). Association of transferrin types with weight gain in cattle. *Indian Vet. J. 63*: 1001-1003.

Saison, R. (1973). Red cell peptidase polymorphism in pigs, cattle, dogs and mink. *Vox Sang. 25:* 173-181.

Tagliaro, C.H., Franco, M.H.L. and **Brito, B.G. de** (1995). Protein polymorphism and genetic relationships in Mouro in relation to other breeds of pigs reared in Southern Brazil. *Rev. Bras. Genet. 18*: 69-73.

Val, A.L., Schwantes, A.R., Schwantes, M.L.B. and **Luca, P.H. de** (1981). Amido hidrolisado em milho como suporte eletroforético. *Ciênc. Cult. 33*: 992-996.

Van de Weghe, A., Van Zeveren A., Bouquet, Y. and **Varewyck, H.** (1982). Phenotype frequencies of vitamin D binding protein (Gc) and of posttransferrin-2 (Ptf-2) in Belgian cattle breeds. *Anim. Blood Grps. Biochem. Genet. 13*: 25-31.

Zafindrajaona, P.S. and **Lauvergne, J.J.** (1993). Comparaison de populations de zébu malgache à l'aide des distances génétiques. *Genet. Sel. Evol. 25*: 373-395.

(Received April 25, 1996)