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Pedigree analysis applied to an endangered buffalo population: possible management strategy

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Abstract

The Carabao breed (*Bubalus bubalis kerebao*) in Brazil may be endangered and at risk of losing specific qualities. This makes preservation and population studies extremely important. In this pedigree analysis on a Brazilian herd, low values for populational parameters and high mean endogamy were found. Mating optimization based on bulls of lesser kinship improves populational parameters and reduces inbreeding rates. Use of this tool, in addition to conservation programs, will help to mitigate genetic variability losses in the Brazilian Carabao herd, thus allowing its future enrollment in genetic improvement programs.

Keywords: genetic drift, inbreeding, marginal genetic contribution, population structure

Introduction

According to statistics on the total numbers of buffaloes in Brazil, the state of Pará stood out in 2011 with 38% of the Brazilian herd, corresponding to a total of 1.3 million animals (IBGE 2011). Officially, the Brazilian Buffalo Breeders Association (ABCB) recognizes four different breeds: Carabao, Jaffarabadi, Mediterranean and Murrah. The Carabao breed is a different subspecies, as confirmed by Marques et al (2011); and it is not included in the Brazilian buffalo production system, which is based on the Murrah, Mediterranean and Jaffarabadi breeds, as well as crosses between them (Borghese & Mazzi 2005; Seno et al

2006). The Brazilian Carabao can be considered to be a naturalized breed because it presents distinct traits relating to its origin in Southeast Asia (Marques et al. 2008).

The Brazilian Animal Genetic Resources Conservation Program under EMBRAPA (Brazilian Agricultural Research Corporation) began in 1981 with the ultimate objective of preserving endangered domestic animal breeds, such as the Carabao (Mariante et al 1999). According to Marques et al (2003), the conservation herd at the Amazon Animal Germplasm Bank (BAGAM) is possibly one of the only pure herds in the country (karyotype 2n = 48 chromosomes). However, due to the small number of animals in the founder herd and the matings that they underwent in the past, this genetic material is endangered (Albuquerque et al 2006). The few Carabao animals in the Amazon region are used as working animals and also for mating with other buffalo breeds for meat production.

There are no records of use of pedigree analysis among Carabao buffaloes, not even in its original region (Asia). Moreover, Flores et al (2007) mentioned that in the Philippines there was a tendency for matings involving the water buffalo (Carabao) and the river buffalo (especially the Murrah breed) to be undertaken in order to improve the production traits of the Carabao, essentially those of working animals. This means that, over time, the breed may become extinct in that country.

Knowledge of the genetic composition and contribution of each lineage, in each population, as well as the main sires and dams present in each of them, may be an important aid in optimizing mating, in order to complement traits, improve genetic gains and maintain variability. This would enable introduction of genetic improvement programs for this species. Unlike among cattle species, there are no intensive genetic improvement program involving buffalo species. The existing initiatives regarding this matter are due to the tenacity and perseverance of a few researchers and breeders (Marcondes et al 2007).

Practical recommendations for maintenance of genetic variability in captive populations have been made based on gene dropping analysis, effective number of founders, founder genome equivalents or other parameters (Caballero and Toro 2000). Alderson (1991) proposed calculating gene origin probabilities or assessing each potential candidate for breeding with reference to the founders, and then selecting animals with the highest effective number of founders as a way of equalizing founder contributions. Ballou and Lacy (1995) noted, however, that maximizing gene diversity is not simply a matter of equalizing founder contributions, because subsequent generations are a source of drift. The genetic tools of in situ conservation depend, in practice, on the existing situation and, for each single situation there are possible solutions and management tools for attempting to mitigate the effects of loss of genetic variability (Meuwissen 1997; Grundy et al 2000; Carvalheiro et al 2010).

The objectives of the present study were: to estimate parameters based on probability of gene origin (pedigree analysis) and those derived from inbreeding levels; and to simulate matings between active dams and available sires that formed part of the Amazon Animal Germplasm Bank (BAGAM), on Marajo Island, state of Pará, Brazil.

Materials and methods

Material studied and area descriptions

Birth data from Carabao animals, collected during the period ranging from May 1976 to September 2008, were used. From 1976 to 1996, the data used originated from the Lower Amazon Experimental Field (CEBA), owned by EMBRAPA Eastern Amazon, which was located on the left bank of the Amazon River, in the municipality of Monte Alegre, state of Pará, Brazil, at the following coordinates: 2° 00' 30'' S latitude; 54° 04' 13'' W longitude; and at an altitude of 30 m. The animals were raised under an extensive regime, in an *in situ* conservation system, complying with a physical production system model in which the main sanitary and animal management practices were observed. The climate type was characterized as *Ami* (tropical rainy with a short dry period). The annual mean temperature was 26.6°C, the mean relative air humidity was 84%, and the annual rainfall was around 2,100 mm. Feeding mainly took place on plain pastures composed of native grasses, with prevalence of the genera *Panicum, Echynochloa, Luziola, Oriza, Paspalum, Leersia* and *Hymenachne*.

In June 1996, the animals were transferred to the Emerson Salimos Experimental Field (CEMES), in Salvaterra, Pará, Brazil, where BAGAM was established. The municipality of Salvaterra is located at the following coordinates: 48° 30' 54" W longitude and 00° 45'21" S latitude, in the geographical mesoregion of Marajó, on the right margin of the Paracauari River, around 17 km from the town of Salvaterra. The local climatic type is characterized as *Ami*, with annual mean temperature of 27°C and annual mean rainfall of 2,943 mm. The vegetation of the pastures was mainly composed of native grasses. There were small areas of cultivated pasture composed of Amazonian quicuio (*Brachiaria humidicola*) and smooth canarana erecta (*Echinochloa pyramidalis*). There were also forage fields of elephant grass cv. Cameron. The same extensive regime and conservation system as used by CEBA was continued.

The pedigree file used in this study comprised data on 445 kinships relating to 87 animals born at CEBA and 358 animals born at BAGAM over the last four decades, which were the offspring of 131 dams and 5 different sires, with a mean offspring/mother ratio of 2.7, ranging from 1 to 9 progenies. Control over the herd, over the course of these decades had given rise to improvements that had had a direct effect on the number of animals followed (Table 1), thereby allowing the pedigree analysis. Approximately 66% of the records studied originated between 1999 and 2008.

Parameters based on probability of gene origin, inbreeding coefficient, kinship and mean generation interval were calculated by means of the PEDIG® software (Boichard 2007), which presents programs in Fortran for different parameters to be calculated. To describe the genetic variability in both strains, we used parameters based on the probability of gene origin. These parameters were the effective number of founders (Nf), the effective number of ancestors (Na) and the number of remaining genomes (Ng). In this context, "founder" referred to an animal whose genealogy was unknown.

The effective number of founders (N_{fun}) represented the number of equally contributing founder animals that would produce the same genetic variability found in the population investigated. N_{fun} was calculated as $N_{fun} = 1/\Sigma$ (p²i), where p²i was the ratio of alleles in the reference population contributed by founder i.

The effective number of ancestors (Na) determined the minimum number of ancestors (both founders and non-founders) required to explain the complete genetic diversity in the reference population. It was obtained by calculating the marginal contribution of each ancestor

(Boichard et al 1997) as $1/\Sigma$ (p²k), where p²k was the marginal contribution of ancestor k in the population.

The number of remaining genomes (Ng) represented the number of founders with the same contribution that would lead to the same genetic diversity found in the population and would not cause loss of alleles due to genetic drift. We calculated Ng as $1/\Sigma$ (p²i/ri), where ri was the expected ratio of alleles from founder i that would remain in the reference population and p²i was the expected ratio of alleles from founder i that contributed to the reference population.

The calculation to determine the increase or decrease in the number of sires and dams between periods was given by the N_{fun}/Na ratio. The indicative genetic derivation process, which quantified the loss of founder allele between generations, was given by the Ng/N_{fun} ratio.

To determine the quality of the pedigree file, the mean number of founders and the equivalent number of generations were calculated. The inbreeding coefficient was obtained by using the VanRaden (1992) algorithm.

To simulate mating optimization for the BAGAM herd, the active dams (N = 78), active sires (N = 2), reserve sires (N = 3) and young sires (N = 15) were taken into consideration. Breeding pairs were assembled with kinships of 12.5%, 25%, 37.5% or 50% and mating was simulated between these sires and the herd's active dams. Following this, the inbreeding levels were compared and the new populational parameters (N_{fun} , Na and Ng) were compared with parameters based on using the two active sires (id numbers: 180 and 199).

Results and Discussion

Until the 1990s, the number of females in the data file (101 animals, between 1976 and 1998) was greater than the number of males (48 animals, between 1976 and 1998). Subsequently, there was an inversion, mainly because, over the last decade, 167 males were registered (56.4%) and only 129 females were registered (43.6%). This happened possibly because only information regarding active parents, and not those regarding all animals born in the herd, were recorded in the field spreadsheets, as observed throughout the last two decades. In June 1996, 105 animals were transferred to BAGAM (58 females, 3 males and the rest of them from other categories). The improvement in the data quality was possibly due to the effective establishment of the Conservation Center in Salvaterra (Marajo Island, Pará), with institutional and financial support provided by the EMBRAPA Animal Genetics Resources Platform, which can also be confirmed from the data shown in Table 1.

Table 1. Number of parents, known or unknown, of Carabao animals belonging to EMBRAPA

 Eastern Amazon.

Period	Municipality	N	Known sires	Known dams	Unknown
May 1976 to May 1996	Monte Alegre	87	0	0	174
June 1996 to October 1998	Salvaterra	62	2	50	0
April 1999 to September 2008	Salvaterra	296	4	107	2

N = number of animals in the pedigree file.

These animals were used to establish the present-day breeding herd that is examined in this study. It can be seen that the extensive production regime used contributed towards absence of description or even loss of much of the fatherhood information data from past generations. Therefore, the parameters studied are a direct consequence of this structure. However, it is important to be aware that the more complete the genealogical data file is, the more accurate and informative the population variability estimates will be. Throughout the past decades, in addition to the increase in animal numbers, there was also an improvement in zootechnical control over the herd. These simultaneous changes had a direct influence on the number of animals cared for and made this study possible. Valera et al (2005), analyzing information from horses, and Sorensen et al (2005) and McParland et al (2007), analyzing information has in relation to genetic diversity and pedigree quality studies.

The generation intervals according to gamete pathways are presented in Table 2. The generation interval was around 7.7 years. However, until 2000, the generation interval was greater than 7.7, and thus the mean generation interval was considered to be 8 years for determination of generations and for studying endogamy coefficients according to generation. Female buffaloes are known to be long-lived. It is common, especially in meat production herds in northern Brazil, to keep these females until they have had 15 or 16 births over the course of their productive lives. Furthermore, the generation intervals have a tendency to be higher in conservation centers, since the animals are kept in the herd for as long as possible, especially those considered to be the founders of the herd, as a way of ensuring higher participation by these animals in shaping the population genetics. In this manner, the gene loss is not so intensive, as in the Brazilian Carabao.

In this regard, considerable reductions in the father-son and mother-son generation intervals can be observed between the two periods defined in this study. The Carabao herd is part of a conservation program. The results from the population analysis were closer to those from horses than from cattle breeds, which can be explained by the way in which the species are exploited and bred. Another important point relates to the prolonged continuation of sires and dams in the herd. It takes longer to replace animals and this, according to Malhado et al (2007), is worse among buffaloes because of their longevity as reproducers. However, the mean generation interval observed (7.7 years) was not very different from the 7 years calculated by Malhado et al (2008), in a study on Mediterranean buffaloes; or the 7.2 and 7.1 years, respectively, calculated by Faria et al (2002) and Vercesi Filho et al (2002a) among registered animals from breeds such as Polled Nellore and Tabapuã.

Daniad	0	Gamete	pathways	
Period	Sire-son	Sire-daughter	Dam-son	Dam-daughter
Total	7.9	7.9	7.8	7.3
1996 to 2000	10.3	8.4	10.4	7.1
2001 to 2008	6.3	7.6	6.2	7.4

Table 2. Mean	generation	interval,	in years,	for the fo	our gamete	pathways.

The parameters for genetic and populational variability are shown in Table 3. Thirty-two animals were identified as founders, but the effective number of founders was smaller (5.3), which represented about 16% of the total number of founders.

 Table 3. Main populational parameters calculated for Carabao buffaloes, EMBRAPA Eastern Amazon, Brazil.

Genetic variability parameters evaluated

Number of main founders/ancestors	100
Sex of the reference-population	Female
Total number of individuals	445
Number of individuals without progeny	309
Size of the reference-population (known parents)	62
Total number of founders	32
Effective number of founders (N_{fun})	5.3
Effective number of ancestors (Na)	4.73
Effective number of remaining genomes (Ng)	3.49
Marginal contribution (1 ancestor) %	41.91
Marginal contribution (5 ancestors) %	72.78
Marginal contribution (10 ancestors) %	82.26
Number of ancestors explaining 50% genetic variability of population	2

The mean progeny number among the females with important contributions was only five. Eleven ancestors were responsible for more than 80% of the herd's genetic variability (Table 4), and just two animals accounted for 50% of the herd's genetic variability. These results indicate that only a small number of male siblings or related individuals were and still are being used for breeding, which is very similar to what happens in the few Marajo farms. These farms use animals from different areas, but all of them located on the same island. In EMBRAPA Eastern Amazon's herd of buffaloes of commercial breeds, Marcondes et al (2010) pointed out that only 20 ancestors would account for about 70% of the existing genetic variability.

Table 4. Accumulated contribution relating to the main individuals analyzed.

Individual	Sex	Year	Accumulated contribution	Sire	Dam	Ν
А	М	2000	0.4194	В	L	120
В	Μ	1988	0.5685	0	0	85
С	Μ	1995	0.6371	0	0	90
D	F	1990	0.7016	0	0	7
E	F	1991	0.7278	0	0	9
F	Μ	1987	0.7520	0	0	35
G	F	1996	0.7722	0	0	4
Н	F	1995	0.7903	0	0	4
Ι	F	1990	0.8065	0	0	9
J	F	1995	0.8226	0	0	5
Κ	F	1995	0.8387	0	0	4

Individual, Sire and $Dam = individual \ coding$; $N = number \ of \ progenies$; M = males; F = females; $Year = birth \ year$.

The values observed confirm that the Carabao breed in Brazil finds itself in a dramatic situation that justifies continuation of the conservation procedures and the efforts made in the BAGAM herd, through sustaining the breed on the EMBRAPA Animal Genetic Resources Platform. These values were lower than those observed for the Venezuelan cattle breed called Criollo Limonero, which also belongs to a conservation program and is endangered. Villasmil-Ontiveros et al (2008) studied 2552 records, among which the total number of founder animals was 386; N_{fun} 63.5 animals; Na 38 animals; Ng 27.7 animals; and 18 animals accounted for 50% of the genetic variability. Although these numbers are higher, they are still considered to be just as critical as those found in the Carabao breed. Similar results were found by Goyache et al (2003) in a study on the European Xalda sheep breed in a conservation program. This breed is at risk of losing its genetic diversity because of excessive use of only a few individuals as parents. The herd's N_{fun} was 9.9 animals. The mean inbreeding value in the whole population was 1.5%. However, according to these authors, the inbreeding values and effective size of the population would be unable to provide realistic

information about the risk of diversity loss. In Switzerland, the Franches-Montagnes horse breed is the only native breed, which makes its survival of crucial importance. The Na was found to range from 18.9 to 20.1 animals, and 50% of the genetic variability of this population was attributed to only seven individuals (Poncet et al 2006), which highlights the critical situation of the Swiss breed, just as it does for the Brazilian Carabao.

In the case of buffaloes of the Murrah and Mediterranean breeds and crosses from these breeds, also known as river buffaloes (*Bubalus bubalis bubalis*), belonging to EMBRAPA Eastern Amazon, Marcondes et al (2010) observed N_{fun} and Ng values that were also considered critical (respectively, 24 and 21 animals). In other species of domestic animals that are not considered endangered, the following N_{fun} values can be observed, for example: 39.5 animals for Spanish Arab horses (Cervantes et al 2008); 144 animals for the Polled Nellore cattle (Faria et al 2002); 39.6 animals for Andalusian horses (Valera et al 2005); and 87.2 animals for Nellore cattle (Vozzi et al 2006).

Barros et al (2011) found *Na* similar to N_{fun} at the conservation center for the Marota breed in the state of Piauí (Marota is a northeastern Brazilian native goat breed). When this happens, it means that the animals that contributed towards establishment of the breed are still playing an effective role in the current herd. In other words, no animals other than those considered to be the herd's founders were making any effective contributions towards the genetic composition of the herd. In the case of the Carabao, the situation observed is similar (values of 4.73 and 5.3, respectively), mainly because CEBA's active females are still in the BAGAM herd today. It is important to note that the ideal is that these effective numbers should be as close as possible to the numbers observed for the founder population.

Sire A alone was responsible for 42% of the herd's genetic contribution (Table 4). Moreover, the animals born between October 2003 and September 2008 (n = 148) were related to progeny traced to only two half-sibling sires (less than 25% of the genes in common) and 82 dams. The N_{fun}/Na ratio was 1.12. Vercesi Filho et al (2002a) found a value of 0.36 when observing cattle of the Indubrasil breed, while a higher value of 1.67 was reported for Criollo Limonero (Villasmil-Ontiveros et al 2008); 1.44 for Tabapuã (Vercesi Filho et al 2002b); 1.48 for Guzerá (Faria et al 2004a); and 1.77 for Sindi (Faria et al 2004b). These findings once again indicate the huge vulnerability of the Carabao breed, since the bottleneck effect would indicate that the number of reproducers would decrease due to selection. The Ng/N_{fun} ratio was 0.66, which was higher than the values observed for Guzerá (Faria et al 2004b), respectively 0.39 and 0.43, but lower than the values of 1.31 and 1.59 that were obtained for the Nellore and Gir breeds by Faria et al (2009). Therefore, in the Carabao breed, a reasonable part of the loss of genetic variability may have been due to the bottleneck effect and genetic drift, which brings the need for deeper studies.

The mean endogamy coefficient of the herd was 1.85%, and the mean endogamy coefficient of the endogamic animals was 11.95%. The percentage of endogamic animals was 25.4% (69 individuals out of a total of 272), and all of them were born in the fourth generation. The maximum level of inbreeding was 31.25%, and the majority of the inbred individuals were found in the range of 10 to 15%. It is worth highlighting that these individuals were born between June 2004 and September 2008, in the fourth generation. Despite the increasing amount of information on the Carabao's pedigree, much of it was lost over the years, which may compromise the conclusions regarding the inbreeding coefficients. In the first generation, no inbreeding was registered (F) because this generation was composed of the very first

founders' offspring. However, in the second and third generations, there was a greater likelihood of inbreeding because of the lack of information regarding fatherhood in the pedigree data file. According to Faria et al (2009), because this was a conservation herd with a small number of animals, development of generations would allow increments in F.

Malhado et al. (2008) reported that the mean inbreeding coefficient for the Mediterranean breed was 1.9%, which was closer to the 1.85% obtained in the present study. On the other hand, in a study on a herd of Murrah, Mediterranean and crossbred animals, Marcondes et al (2010) observed a mean inbreeding rate of 0.5%. Therefore, in comparison with breeds in conservation programs, the mean inbreeding coefficient of breeds in commercial herds has a tendency to be reduced. Cecchi et al (2006) stated that the results relating to the number of inbred animals and to the mean inbreeding coefficient may be underestimated when the pedigree data file is of low quality. In relation to pedigree quality graphs (Figures. 1 and 2), especially before 2003, and the absence of inbreeding until the third generation in Carabao buffaloes, it can be concluded that a higher number of related individuals and/or individuals with a coefficient higher than 31.25% may exist than has been presented in this study.



Figure 1. Mean number of ancestors in Carabao pedigree, according to birth year and sex.

With regard to individual inbreeding coefficient, the highest proportion of inbred animals was grouped in the range of 10 to 15%, differently to what was observed by Costa et al (2005) and Villasmil-Ontiveros et al (2008), who found that almost 50% of the inbred individuals were in the range of 5 to 10%. In the results from the present study, the births occurred between October 2003 and September 2008 (n = 148) and corresponded to the progeny of only two half-brother bulls and 82 females. Even more critical is the situation presented by Nomura et al (2001), in which 43% of the Japanese cattle raised for meat (Japanese Black) born in one year descended from only five bulls, in a population of more than 55,000 females. The effective size of the Japanese livestock population, despite the significant herd size, was only 17.2 animals. Even though the data from the present study showed a high relationship among the Carabao animals studied, with low values for the probability of gene origin (which leads to loss of genetic variability, possibly due to the bottleneck effect and genetic drift), the

herd still does not show deleterious effects relating to endogamy. Therefore, the existing low diversity needs to be preserved.

The following parameters were also calculated: mean number of ancestors (Na) and numbers of equivalent generations for males and females over the years (Figures. 1 and 2), which would indicate the quality of the pedigree file studied. Thus, improvement in the pedigree file was observed, since the numbers of ancestors doubled, with regard to both females and males after 2003.

The number of equivalent generations (Figure 2) was correlated with the number of generations that could effectively be accounted for in the calculations, and this also improved after 2003. Although the data file contained animals born four decades ago, three generations could not be included in the calculations.



Figure 2. Number of equivalent generations in Carabao pedigree, according to birth year and sex.

New populational parameters were calculated for each kinship degree after the mating simulation (Table 5). As the kinship level among the sires increased, there was a decrease in the genetic variability of the future progeny. This decrease ranged from 25% in the number of effective founders (N_{fun}) to 30% in the number of remaining genomes (Ng). However, the mean inbreeding coefficient of the future progeny (F) would be increased by 34% in an extreme situation (use of two sires with kinship of 50%, or, for example, full-blood siblings). All of the populational parameters improved in relation to the situation before simulation, with only one mating season, with the exception of the genetic contributions from ten principal ancestors (b), for which bulls with mean kinship of 12.5% were used.

Villasmil-Ontiveros et al (2008) reported on a rotational mating system, with around 60 bulls, which produced approximately 2500 offspring belonging to five different families. After five generations of use, the inbreeding coefficient remained below 5%. Adoption of optimized matings with kinship control would be an outlet for inbreeding control and, consequently, for the loss of genetic variability in Carabao buffaloes, with the aim of having a higher number of dams and, therefore, increasing the effective size of the population.

Over recent years, a growing number of methods have been proposed for minimizing the loss of genetic diversity in small populations (Fernández and Caballero 2001). Most of these are based on the fundamental rule that the group of breeding individuals should be chosen so as to minimize the average coancestry among them (e.g. Ballou and Lacy 1995; Fernández and Toro 1999; Toro et al 1999). Minimization of the average coancestry has been theoretically proven to be the most efficient method for preserving genetic diversity (Caballero and Toro 2000, 2002). In one such method proposed by Meuwissen (1997), the genetic level of selected animals was maximized while their average coancestry was constrained to a predefined value. This rule is easy to implement in any breeding scheme, but reproductive limitations (e.g. in the Carabao breed) may require some modifications of the optimal solution.

The mean inbreeding that resulted from the matings of active females with two active males (with 25% kinship between them) was 11%. When sires that are less closely related were used in reproduction, the mean F was reduced to 7.4%. In other words, there was a 33% decrease in the mean level of inbreeding in the future progeny. The genetic variability resulting from these simulations (represented by new N_{fun} , Na and Ng values), in addition to the genetic contributions from the main ancestors and the mean kinship between males and females) in the progeny, was found to become higher as the kinship between the two bulls became smaller (Table 5).

Table 5. Values for effective number of founders (Nfun), effective number of ancestors (Na), effective number of remaining genomes (Ng), genetic contributions from the main ancestors (a), genetic contributions from ten principal ancestors (b), mean kinship between males and females (R) and mean coefficient of inbreeding among the progeny (F) in the Carabao herd, found through simulation.

Kinship level (males)	N _{fun}	Na	Ng	a	b	R	F(%)
Before*	5.30	4.73	3.49	41.91	82.26	n.a.	11.95
12.5 %	6.70	6.15	4.01	31.09	84.78	14.80	7.40
25.0 %	6.70	5.31	3.34	37.18	83.01	17.60	8.80
37.5 %	5.60	5.28	3.00	36.64	84.79	22.20	11.10
50.0 %	5.00	4.90	2.79	37.37	85.90	22.40	11.20

* Before = parameters before simulation; n.a. = not applicable

Use of a new mating strategy will enable reduction of the loss of genetic variability, improvement of the populational indexes and reduction of the endogamy within the Carabao population. There are several marker-based and pedigree-based parameters and modern molecular genetic tools for describing genetic variation (Toro et al 2011) that can be applied, to the Carabao breed in the future.

Conclusions

- The low values observed in the populational parameters indicate that this herd developed over a narrow genetic base, thus showing that only a small number of sires were and are being used in reproduction. There is loss of alleles between generations, in addition to the possible bottleneck effect, and these together contribute towards the critical situation observed in the Carabao breed in Brazil;
- Mating optimization based on bulls of lesser kinship improves the populational parameters and reduces the inbreeding in an endangered population.

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Go to top