Monitoring of the genetic health of cattle in the Czech Republic

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ABSTRACT: A screening was carried out for *CVM*, *BLAD*, *DUMPS*, bovine citrullinaemia, glycogen storage disease V, and Robertsonian translocations in the cattle population of the Czech Republic. In 406 Holstein sires and 146 Czech Pied (Czech Simmental) sires entering the AI programme in the Czech Republic from 2003–2005, no heterozygous sire for *DUMPS*, bovine citrullinaemia and *BLAD* was found. The heterozygote was not found also in the beef sires of Charolais, Limousine, Beef Simmental, Blonde d'Aquitaine, Belgian Blue, Aberdeen-Angus, and Hereford breeds. In 111 elite Holstein females, 21 (18.9%) were heterozygotes for *CVM*, and were dominant homozygotes for *BLAD*, *DUMPS* and bovine citrullinaemia. In the myophosphorylase gene responsible for the glycogen storage disease V, in the Charolais (n = 30), Czech Pied (n = 53), and Belgian Blue, Limousine, Blonde d'Aquitaine, Aberdeen Angus, and Beef Simmental sires analysed, the heterozygote was not found. Robertsonian translocations were examined in 767 Holstein sires, 1 010 Czech Pied (Simmental) sires, 142 beef sires, and 48 dams. Of these, 10 sires of Czech Pied breed, 5 beef sires, and 13 females were found to be positive. The monitoring of *BLAD*, *CVM*, and Robertsonian translocations is recommended.

Keywords: cattle; *CVM*; *BLAD*; *DUMPS*; citrullinaemia; Robertsonian translocation; screening

Inherited disorders affect all species of domestic animals. They are hereditarily caused physical or functional anomalies, with a negative impact on health and productivity. It is an important task of breeders and veterinarians to eradicate these disorders, and control the genetic health of farm animals. Knowing the molecular basis of a defect, the direct detection of the heterozygous carriers is possible at the gene level after birth or even in embryos. Similarly, the detection of carriers of cytogenetic anomalies enables their exclusion from breeding and consequently, the maintenance of genetic health in the population.

In cattle, the most pressing problem in the genetics of health at present is the recessive and lethal Complex Vertebral Malformation (*CVM*) in the Holstein population. The defect can be traced back to the American elite sire Carlin-M Ivanhoe Bell. His father, Penstate Ivanhoe Star, born in 1963, was also found to be a carrier. Bell was formerly used extensively world wide, so the global impact on the mortality of Holstein calves is inevitable (Konersmann et al., 2003). It threatens, when a widely used elite sires producing large quantities of calves turn out, in retrospect, to have been carriers of a defective gene, the inbreeding in population intensifies the process (Citek and Blahova, 2004). This defect is caused by a mutation in an autosomal recessive gene (Revell, 2001; and more information at http://www.naab-css.org/educa-

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tion/CVMpressrelease-D.html). Typical signs of *CVM* are a shortened neck and forelimbs, contraction of the carpal joints, contraction and rotation of the fetlock joints. The hind limbs show contraction of the fetlocks, rotation of distal limbs, and elongation of the tarsus. Vertebral abnormalities are reported and cardiac abnormalities occur in approx. 50 per cent of cases (Agerholm et al., 2001; Revell, 2001). Several fertility traits associated with the foetal *CVM* status were reported. Many *CVM* foetuses are aborted at gestation day 159, while other *CVM* calves are prematurely born and usually stillborn (Agerholm et al., 2001). The rate of abortion was reported by Nielsen et al. (2003). In the Czech Republic, an improvement is hoped for because of the prudent arrangements made by the Holstein Cattle Breeders' Association.

Bovine Leukocyte Adhesion Deficiency (*BLAD*) is a lethal autosomal recessive disease in Holstein cattle characterized by a reduced level of expression of the β2 heterodimeric integrin. Integrins are adhesion molecules that mediate the passage of neutrophiles across membranes to destroy invading pathogens (Kehrli et al., 1992). As β2 integrin expression requires the intracellular association of the *CD11* and *CD18* subunits, defects in *CD18* prevent expression of all β2 integrins. The defective leukocyte adherence leads to inadequate mucosal immunity and *BLAD* affected cattle have recurrent mucosal infections, loss of teeth, impaired pus formation, delayed wound healing, and stunted growth (Nagahata, 2004). The molecular basis of *BLAD* is a single point mutation.

Deficiency of uridine – 5′-monophosphate synthase (*DUMPS*) is a recessive genetic disorder. *UMP* synthase is necessary for the de novo synthesis of pyrimidine nucleotides. Growth and development of the homozygous recessive calves are arrested, leading to embryonic mortality around 40 days post-conception (Shanks and Robinson, 1990; Robinson et al., 1993).

Bovine citrullinaemia is an autosomal recessive error of urea metabolism as a result of a deficiency of the activity of argininosuccinate synthase (*ASS*). Affected (homozygous) calves are unable to excrete ammonia and display neurological symptoms that become progressively worse, leading to death within one week of birth (Grupe et al., 1996).

Glycogen storage disease V, also known as deficiency of muscle glycogen phosphorylase, or myophosphorylase, is a muscle disease induced by point mutation in the respective gene (Tsujino et al., 1996; Soethout et al., 2002). It causes exercise intolerance, myalgia and recurrent myoglobinuria. The disorder was reported first by Angelos et al. (1995) with the monogenic autosomal recessive pattern of inheritance. In humans, the defect was originally known as McArdle's disease, causing similar symptoms. Recently, Johnstone et al. (2004) have studied the occurrence of the glycogen storage disease in New Zealand.

The Robertsonian translocation is the most common cytogenetic anomaly in cattle. Among them, the 1;29 translocation is the most frequent. That is why it has been monitored for years to prevent its spread in the population, which results in an increase in embryonic mortality, and reduction in fertility (Molteni et al., 2005). The 1;29 translocation has been described by Gustavsson and Rockborn (1964), in the Czech Republic at the first by Lojda (1974). More than 40 Robertsonian translocations have been found in cattle. Rubes et al. (1996) found new translocations 16;20 in the progeny of a German Red Pied sire and a Czech Red Pied cow. They found a potential relationship between the 16;20 and 14;20 translocation and lower *in vitro* embryo development (Rubes et al., 1999).

The aim of this paper is to report on the monitoring of the genetic health of the cattle population in the Czech Republic. Firstly, the screening of young bulls entering artificial insemination (AI) programme was carried out, the loci of *BLAD*, *DUMPS*, bovine citrullinaemia and glycogen storage disease V were genotyped, and a definite number of elite cows were examined for *CVM*. Secondly, the young bulls and females were investigated for Robertsonian translocations.

MATERIAL AND METHODS

The screening for lethal recessive disorders

The genotyping of *BLAD*, *DUMPS* and bovine citrullinaemia was carried out in 582 sires of the Holstein and Czech Pied (Simmental) breeds, and also a small number of beef sires were involved. Glycogen storage disease V was tested in 98 sires of Charolais, Czech Pied (Simmental) and other beef breeds. Young sires entering the progeny testing in the Czech Republic in 2003 and 2004, and young bulls in the rearing houses were involved.

Also, *BLAD*, *DUMPS*, bovine citrullinaemia and *CVM* were analysed in 111 elite females too, and

the dams or potential dams of sires were included into the analysis.

DNA was isolated from whole blood or sperm. *BLAD*, *DUMPS*, bovine citrullinaemia and glycogen storage disease V were genotyped by polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP). The sequences of primers for PCR and restrictases were taken from literature. The sequences were as follows:

BLAD (Shuster et al., 1992, Tammen, 1994)
5′-GTC AGG CAG TTG CGT TCA A-3′
5′-GAG GTC ATC CAC CAT CGA GT-3′

DUMPS (Schwenger et al., 1993)
5′-GCA AAT GGC TGA AGA ACA TTC TG-3′
5′-GCT TCT AAC TGA ACT CCT CGA GT-3′

bovine citrullinaemia (Dennis et al., 1989)
5′-GTG TTC ATT GAG GAC ATC-3′
5′-CCG TGA GAC ACA TAC TTG-3′

glycogen storage disease V (Soethout et al., 2002)
5′-CCA GGA AGA CCC TCA TTC CA-3′
5′-AGG GAA ACA CAC ACA CAG-3′

For restriction analysis, the following enzymes were used: *BLAD Taq I* or *Hae III* (Tammen, 1994), *DUMPS Ava I* (Schwenger et al., 1993), bovine citrullinaemia *Ava II* (Dennis et al., 1989), glycogen storage disease V *StyI* (Soethout et al., 2002). Fragments were visualised on agarose gels stained with ethidium bromide. *CVM* was genotyped in a service done by the Laboratory of Immunogenetics of the Czech and Moravian Breeder's Society. The mutation in *SLC35A3* gene diagnostic for *CVM* was analysed out using the allelic specific PCR.

The examination of Robertsonian translocations

The sires reared in the Czech Republic are examined for the occurrence of the Robertsonian translocations before their admission to breeding. Also females suspected for the translocation are examined, if they were intended to produce sires. The results of the examinations from 1996 to 2005 are reported here.

The modified method of investigation of live dividing lymphocytes from peripheral blood by Moorhead et al. (1960) was used. Heparinized blood was treated by a cultivating medium, glutamine, a solution of non essential amino acids, mitogenic activator phytohemaglutinin and bovine serum, and cultivated for 72 hours at 37°C. The mitose was stopped by adding 0.02% solution of colchicine, and hypotonic shock was induced by KCl to dissipate chromosomes. After fixation by a mixture of methanol and acetic acid and staining by Giemsa, the mounts were examined. At least 20 mitosis were evaluated.

RESULTS AND DISCUSSION

The screening of the lethal recessive disorders

The genotyping of *DUMPS* and bovine citrullinaemia was carried out in 406 Holstein sires, and *BLAD* was also analysed when the status for it was still not known (Table 1). The panel consisted of 224 sires having begun in 2003 and 2004 the progeny testing for their breeding value in the Czech Republic, and of 182 young sires in rearing houses before their entry into an insemination programme. Also, 146 Czech Pied sires (Czech Simmental) were genotyped for the loci mentioned. They were young animals in rearing houses entering the AI programme, and the samples were collected from March 2003 to May 2005. The proportion of Holstein animals in the monitored panel is designedly relatively high, because the Holstein population is more often affected by genetic disorders. As there is a share of Holstein genes in the Czech Pied cattle, it is also of interest to check it for the presence of recessive alleles. Also, the sires of Charolais (n = 8), Limousine (n = 6), Beef Simmental (n = 2), Blonde d'Aquitaine (n = 3), Belgian Blue (n = 9), Aberdeen-Angus (n = 1), and Hereford (n = 1) cattle were genotyped.

No one heterozygous sire for *DUMPS*, bovine citrullinaemia and *BLAD* was found in the genotyped panel. Thus, the situation regarding these genetic disorders in the Czech cattle population seems to be good at present. It is a positive result, considering that *BLAD* used to be a serious problem in the Holstein population in the Czech Republic a few years ago (Hradil, 1994), when 65 positive sires from 377 and 4 positive cows from 61 were found. In Red Holstein, used to improve the Czech Simmental population, 34 bulls from 64 by one imported sire tested positive. Some observations

Table 1. Number of animals tested for recessive disorders

Breed	Recessive disorder				
	BLAD	*DUMPS*	bovine citrullinaemia	glycogen storage disease V	*CVM*
Sires					
Holstein	406	406	406	–	–
Czech Pied	146	146	146	53	–
Charolais	8	8	8	30	–
Limousine	6	6	6	4	–
Beef Simmental	2	2	2	1	–
Blonde d'Aquitaine	3	3	3	3	–
Belgian Blue	9	9	9	6	–
Aberdeen-Angus	1	1	1	1	–
Hereford	1	1	1	–	–
Females					
Holstein	111	111	111	–	111

The genotyping did not detect heterozygous carrier, with the exception of 21 Holstein females which were positive for CVM

showed that heterozygous carriers of *BLAD* mutation yielded more milk in the first lactation and more milk protein than their non-carrier half-sisters (Lubieniecki et al., 1999). This could be a factor in the world-wide spread of *BLAD*.

Evidently, measures for eradication of *BLAD* in the cattle population in the Czech Republic were efficient, considering that after ten years not one positive young sire was found.

As *CVM* is the serious problem in the health genetics at present, since 2002 the *CVM* status of sires used in the AI programme in the Czech Republic must be declared, and the use of positive sires is restricted. Therefore, according to the breeders data, in the analysed group of young sires only 4 were heterozygous carriers of *CV* allele (*CV*). That is why we focused on the females. The elite Holstein cows or heifers, mothers of sires or potential mothers coming from the best stock herds in the Czech Republic were genotyped for *CVM*. In the panel of 111 females, 21 were found to be heterozygotes (*CV*), and 90 were homozygous non-carriers (*TV*). Consequently, the frequency of heterozygotes was 18.9%, and the frequency of recessive allele in the survival animals was 9.5%. Genotyping the elite females enables to prepare the optimal mating design, and helps to improve rapidly the genetic health of the Holstein population in the Czech Republic. All the females were also genotyped for *BLAD*, *DUMPS* and bovine citrullinaemia, not one heterozygous carrier was found.

Further, 30 Charolais sires (Table 1) were genotyped for the glycogen storage disease V, as previous studies revealed the recessive allele only in this breed (Soethout et al., 2002; Jolly et al., 2004). In our group, the heterozygous animal was not found. Also the Czech Pied sires (n = 53), Belgian Blue (n = 6), Limousine (n = 4), Blonde d'Aquitaine (n = 3), Aberdeen Angus (n = 1), and Beef Simmental (n = 1) were genotyped for *GSD V* with negative results. Similarly, Bilstrom et al. (1998) analysed glycogen storage disease V in 60 Piedmontese and 34 Saler cattle as a negative control, and did not find heterozygotes. Thus, our results confirm the previous findings.

The examination of Robertsonian translocations

During the reported period 1996 – April 2005, a total of 1 967 animals have been examined, namely 1 919 sires, and 48 females (Table 2). The examined male population consisted of 767 Holstein sires,

Table 2. Robertsonian translocations

Year	Sires					Females	
	examined				with translocation	examined	with translocation
	Holstein	Czech Pied	beef	total			
1996	23	95	8	126	1 Highland; 1 Czech Pied	20	1 Czech Pied
1997	68	149	21	238	3 Blonde d'Aquitaine	3	1 Czech Pied
1998	75	139	10	224	1 Czech Pied	1	–
1999	87	115	8	210	–	0	–
2000	87	131	26	244	1 Charolais	5	1 Charolais
2001	91	96	26	213	–	4	1 Charolais
2002	61	93	8	162	–	1	1 Czech Pied
2003	92	94	12	198	7 Czech Pied	5	3 Czech Pied
2004	136	72	14	222	1 Czech Pied	9	5 Czech Pied
2005	47	26	9	82	–	0	–
Total	767	1 010	142	1 919	15	48	13

1 010 Czech Pied (Simmental) sires, and 142 sires of beef breeds.

In the analysed sires, 15 were found to be a carrier of the translocation, namely 10 sires of Czech Pied breed, and 5 sires of beef breeds, 1 Highland, 3 Blonde d'Aquitaine, and 1 Charolais. The relative frequency was 0.99%, and 3.52%, respectively.

This rather high frequency in beef sires is surprising, and emphasizes the importance of cytogenetic control, since the use of the non-detected carriers (both in natural service and in artificial insemination) in relatively small herds of beef cows could affect the reproduction. Similarly, the control in the Czech Pied and Holstein populations is relevant, as a carrier widely used in the AI programme could damage the herd's fertility. Moreover, the sires with translocation transfer it to their progeny. Havrankova et al. (1987) found the 1;29 translocation in 50% of sons and 53% of daughters of heterozygous carriers. In the examined population, they did not find lower fertility, even though many authors (Dyrendahl and Gustavsson, 1979, e.g.) report its reduction by 5–10%.

Similarly, McWhir et al. (1987) stated that the translocation was inherited by 50% of offspring of the heterozygous carrier, and heterozygous male 1;29 carriers left fewer calves than karyotypically normal bulls when used in natural service. Therefore, the carriers of the Robertsonian translocation should not be used in breeding, as a half of progeny will be also carriers, and fertility could be more or less damaged. Because the Robertsonian translocations occur in many breeds, examination should be carried out on all sires in the breeding programme including those imported.

In this paper, the translocation was found in 13 females out of 48 (Table 2). The pathological karyotype had 11 Czech Pied and 2 Charolais females. The frequency of 27.08% was remarkably high, but only females suspected for the translocation due to its occurrence in the fater's karyotype were involved in the analysis. In contrast, the examination of translocation is performed routinely in all males in rearing houses before their being licenced for insemination, and that is why the frequency in males was substantially lower. The analysis of suspected elite females, and exclusion of carriers prevents the spread of translocation in the population and consequent reproduction problems as discussed.

CONCLUSIONS

Recessive inherited disorders and abnormal karyotypes in cattle have very low frequency, nevertheless, in some cases they can influence the economics of cattle breeding significantly. The massive

spread of genetic defects like *CVM* or *BLAD* in recent years was caused by the extensive use of elite sires who were latent heterozygous carriers. Artificial insemination facilitated the world-wide spread of genetic defects. Molecular genetic methods of testing enable the control of genetic health in populations.

Based on the results, the situation regarding the analysed recessive disorders of *BLAD*, *DUMPS*, bovine citrullinaemia and glycogen storage disease V, respectively, seems to be good. Nevertheless, the monitoring of *BLAD* in young Holstein sires is recommended, because the disorder was widespread in the Czech population in the 1990s, and long-term monitoring is necessary to ensure the eradication of the recessive allele from the population. The rigorous control of *CVM* status in Holstein sires entering breeding is required, as the disorder is at present a very serious problem. In special circumstances, when the breeder wants to get the sons of an exceptional sire, it is possible to service the heterozygote, but the progeny of the mating must be genotyped, and only negative young sires should be used in breeding. In such a case, it would be better to genotype embryos fathered by the heterozygous sire, and only those found negative should be used for transfer. The use of *CVM* carriers in the conventional AI programme in the whole population is not recommended.

Arising out of these results, the cytogenetic analysis of young sires is recommended to prevent the spread of Robertsonian translocations and future fertility damage in the cattle population. As demonstrated in this paper, the translocations occur in many breeds, therefore, all sires should be tested. In both recessive disorders and translocations, the analysis of selected elite dams could help to improve the genetic health of the population.

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