Homozygous Pelger-Huet anomaly in three different crossbred rabbits: a case report

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ABSTRACT: In this case report, three different crossbreedings of pet rabbits were performed producing affected as well as healthy bunnies. All affected rabbits were smaller and had local alopecia, exophthalmus, and limb deviations compared to their healthy littermates; thus, a homozygous form of Pelger-Huet anomaly was suspected. This anomaly was confirmed by blood examination in which granulocytes with oval nuclei and a very coarse chromatin pattern, as well as lymphocytes with micronuclei were noticed. Karyotype analyses of the lymphocytes revealed many chromosomal aberrations in affected rabbits. Moreover, severe arterial abnormalities in the pelvic cavity and proximal part of the pelvic limbs were also found in these rabbits. Our findings suggest a multigenic origin of Pelger-Huet anomaly in rabbits, because only male and female offspring with the otter colour of fur were severely affected by this congenital disorder.

Keywords: laboratory animals; hereditary disease; chromosome; leukocytes; anatomical anomalies

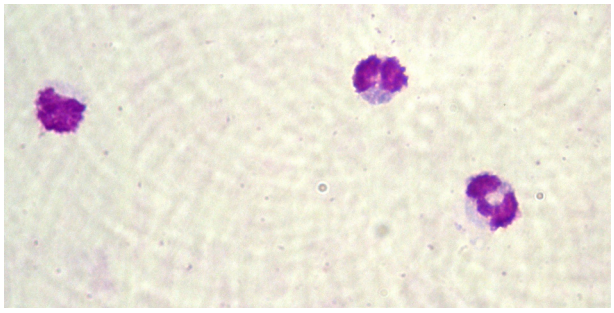
Crossbreeding in rabbits is often done with the intent to create a new breed or to improve the existing breed. Crossbreeding has been routinely performed by breeders to improve the phenotypes of rabbits, and thus increase the quality and quantity of their meat (Ozimba and Lukefahr 1991; Nofal et al. 1997; Oseni et al. 1997). On the other hand, pet rabbits are crossbred widely to reach a wanted phenotype (such as: fur colour and quality, decreased body size). However, in this way genetic aberrations can be introduced into the breed. Thus, crossbreeding may produce offspring with the desired phenotype, but these may actually carry undesired genes leading to hereditary defects that appear in subsequent generations.

One of these congenital disorders is the Pelger-Huet anomaly (PHA) which was described first in humans by Pelger (1931) and Huet (1932). Until now, this anomaly was observed in rabbits (Undritz 1939), dogs (Schalm 1965; Kiss and Komar 1967; Latimer et al. 1989; Al-Bassam et al. 2010), cats (Weber et al. 1981; Latimer et al. 1985), mice (Schultz et al. 2003), and horses (Gill et al. 2006).

PHA is an inherited heterozygous or homozygous autosomal dominant disorder of leukocyte development, which is characterised by persistent nuclear hyposegmentation of granulocytes and monocytes in the presence of a coarse, mature pattern of chromatin (Tomonaga 2005). Most blood granulocytes and monocytes have indented, band-shaped, or bilobed nuclei, but in the homozygous form of PHA, the nuclei are round to oval with very coarse chromatin patterns (Aznar and Vaya 1981). Similar morphological changes were found in the blood of the affected rabbits in this study (Figure 1, 2 and 4) as compared to their healthy littermates (Figure 3 and 5).

Recent studies have demonstrated that PHA is a blood laminopathy caused by mutations in genes encoding proteins of the lamin B receptor (Hoffmann et al. 2002; Best et al. 2003; Schultz et al. 2003). The lamin B receptor is required for morphological but not functional maturation of neutrophils

Supported by the Grant Agency for Science of the Slovak Republic, VEGA (Grants No. 018UVLF-4/2011 and No. 1/0406/12).



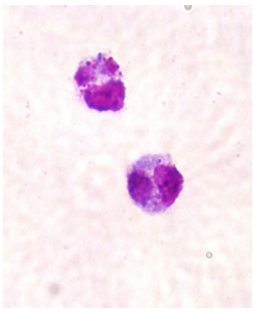


Figure 1 and 2. Blood smears, two different adult rabbits with the homozygous Pelger-Huet anomaly. The nuclear hyposegmentation of neutrophil granulocytes. May-Grunwald and Giemsa stain; magnification power 1000×

(Johanson et al. 1980; Cohen et al. 2007; Grondin et al. 2007; Hoffmann et al. 2007); thus, the physiological function of morphologically malformed neutrophils could be intact (Latimer et al. 1989). Here, we report a case of congenital Pelger-Huet anomaly in three different crossbreed pet rabbits which presumably carried the homozygous allele of the Tan (a^t) gene. A limitation of this study is that the genotypes of all six healthy parents were predicted by the phenotypes of their offspring only.

It is well known that homozygous PHA cases exhibit low birth weight, delayed growth, and increased intrauterine and early post-natal death (Nachtsheim 1943, 1950; Undritz 1943, Haverkamp Begemann and van Lookeren Campagne 1952). Although it is believed that the homozygous form of PHA is lethal in the uterus, those few animals that are somewhat less severely affected may live for a relatively long time. However, this phenomenon was described previously only in humans with PHA where bones were not deformed (Alexeieff 1967; Aznar and Vaya 1981; Gastearena et al. 1982). All affected rabbits were smaller and exhibited local alopecia, exophthalmus, and limb deviations compared to their healthy littermates (Figure 6 and 7).

MATERIAL AND METHODS

In this study, the hereditary transmission of homozygous PHA was proven directly as follows:

Blue of Vienna (predicted genotype: C/C E^D/E^D B/B d/d a/a H/H R/R) sire mated Slovak grey-blue Rex (predicted genotype: C/C E^D/E^D B/B d/d A/a^t r/r) dam. All six offspring were healthy, but when Slovak grey-blue Rex (predicted genotype: C/C E^D/E^D B/B d/d A/a^t r/r) sire mated the same dam, they produced a litter of seven bunnies (two males and two females with normal phenotypes, and one male and two females with an affected phenotype). Two affected bunnies, one male and one female lived until the 4^{th} and 6^{th} month of life, respectively, but the last affected female was euthanized at the age of 205 days with a body weight of 3.4 kg.

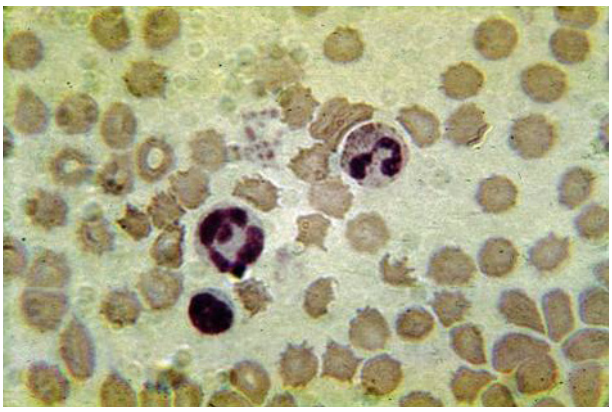


Figure 3. Blood smear, normal nuclear segmentation of neutrophil granulocytes in an adult rabbit with a normal phenotype. May-Grunwald and Giemsa stain; magnification power 1000×

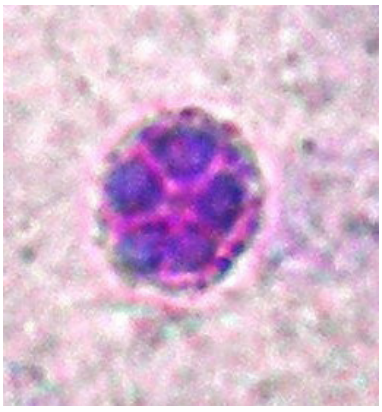


Figure 4. Blood smear, adult rabbit with the homozygous Pelger-Huet anomaly. Note the oval nucleus of a neutrophil granulocyte with a coarse chromatin pattern (detail). May-Grunwald and Giemsa stain; magnification power 1500×

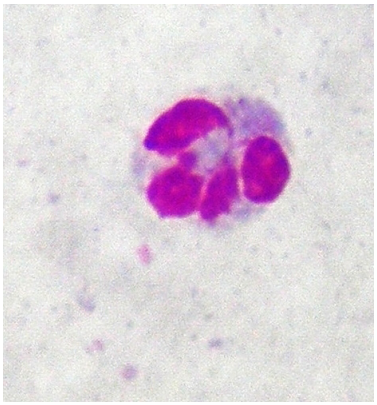


Figure 5. Blood smear, normal nuclear segmentation of a neutrophil granulocyte in an adult rabbit with a normal phenotype (detail). May-Grunwald and Giemsa stain; magnification power 1500×

Dwarf pearl rabbit (predicted genotype: C/C E^D/E^D B/B d/d A/a^t R/r) sire mated Dwarf Slovak grey-blue Rex (predicted genotype: C/C E^D/E^D B/B d/d A/a^t r/r) dam. This crossbreeding produced a litter of four bunnies (one male and one female with normal phenotypes, and two males with an affected phenotype). Both affected males were euthanized at the age of 185 days weighing 1.2 and 1.4 kg, respectively.

In the last crossbreeding, Netherland rabbit (predicted genotype: C/C E^D/E^D B/B D/D a/a s/s Y^1/Y^1 Y^2/Y^2) sire mated Tan rabbit (predicted genotype: C/C E^D/E^D B/B D/D a^t/a^t S/S y^1/y^1 y^2/y^2) dam and produced a litter of seven healthy bunnies (four males and three females). Then, two females and one male rabbit (predicted genotypes: C/C E^D/E^D B/B D/D a/a^t S/s Y^1/y^1 Y^2/y^2) were randomly chosen and a brother-sister crossing was performed. Altogether,

Figure 7. An adult rabbit with the homozygous Pelger-Huet anomaly

Figure 6. An adult rabbit with the homozygous Pelger-Huet anomaly, and littermate with a normal phenotype (on the right side of the photo)

they produced 12 bunnies (five males and three females with normal phenotypes, and three males and one female with an affected phenotype). One male rabbit died at the age of four months, but other affected littermates were euthanized at the age of 175 days with body weights ranging from 1.5 to 1.7 kg.

Before euthanasia, blood was collected using *v. auricularis media* puncture from all affected rabbits and two rabbits with a normal phenotype (one male and one female) from the 3rd crossbreeding, and subsequently, survey radiographs were obtained. Thin blood films were prepared and stained with Giemsa stain for evaluation of white blood cell morphology. Cultivation of lymphocytes from the bone narrow and peripheral blood for chromosomal preparations was performed according to Parkanyi et al. (2004). The corrosion casts were prepared using Duracryl Dental (Spofa-Dental, Czech Republic) as described by Mazensky and Danko (2010).

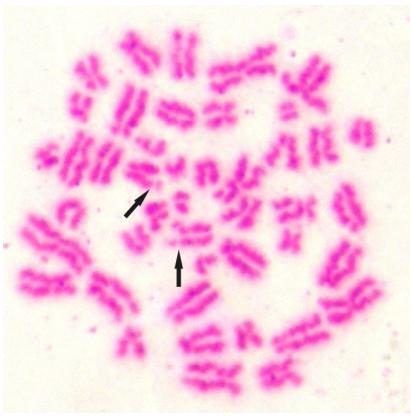


Figure 8. C-metaphase, adult rabbit with the homozygous Pelger-Huet anomaly. Karyotype analyses of lymphocytes from the bone narrow with chromosomal aberrations (arrows). Giemsa-Romanowski stain; magnification power 1000×

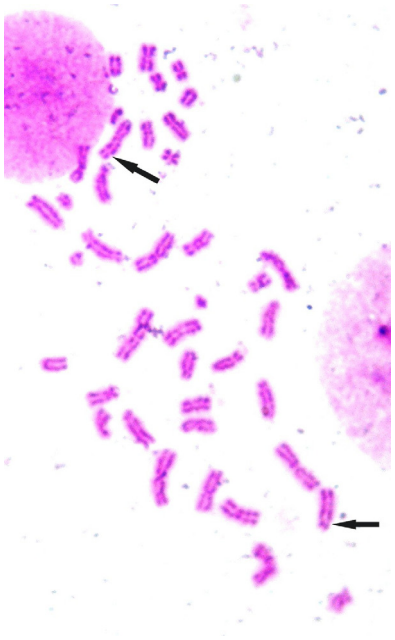


Figure 9. C-metaphase, adult rabbit with the homozygous Pelger-Huet anomaly. Karyotype analyses of lymphocytes from the peripheral blood with chromosomal aberrations (arrows). Giemsa-Romanowski stain; magnification power 1000×

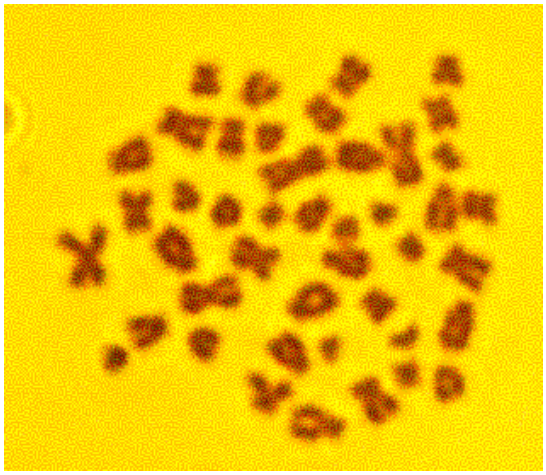


Figure 10. C-metaphase, adult rabbit with a normal phenotype. Karyotype analyses of peripheral blood lymphocytes (2*n* = 44, XX). Giemsa-Romanowski stain; magnification power 1000×

RESULTS AND DISCUSSION

Clinical signs of depression were seen in all affected rabbits similarly as described in the case of a five year-old male Basenji dog with PHA (Al-Bassam et al. 2010). From the aforementioned findings, we can presume that only rabbits with the otter colour of fur (genotype: a^t/a^t) were affected by the homozygous form of PHA. PHA occurs in congenital and acquired forms. The acquired form is caused by drug administration or infectious diseases (Dusse et al. 2010), but this was ruled out due to the absence of clinically ill rabbits and because no treatments were applied in any of cases. On the other hand, as mentioned previously, the congenital form of PHA is caused by mutations in the gene encoding the lamin B receptor which affect the structure of this protein (Hoffmann et al. 2002; Best et al. 2003). This receptor is an inner nuclear membrane protein that interacts with heterochromatin, and thus is responsible for the maturation of neutrophil granulocytes (Cohen et al. 2007; Hoffmann et al. 2007). It is presumed that its disruption causes abnormalities in nuclear heterochromatin morphology (Vale et al. 2011), result-

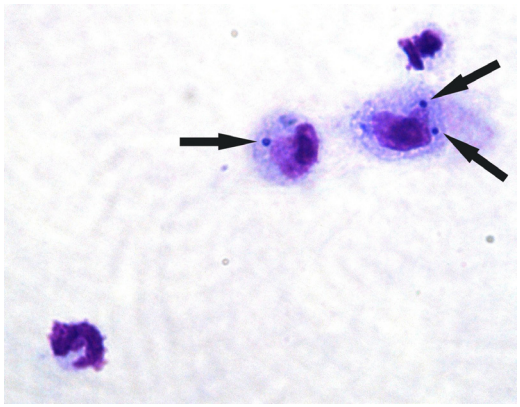


Figure 11. Blood smear, adult rabbit with the homozygous Pelger-Huet anomaly. Lymphocytes and monocytes have micronuclei (arrow) in their cytoplasm. May-Grunwald and Giemsa stain; magnification power 1000×

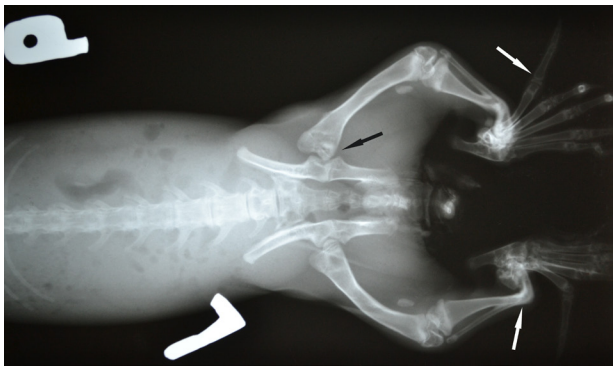


Figure 12. Radiographic images of an adult rabbit with the apparent homozygous Pelger-Huet anomaly. Note the chondrodystrophy and angular limb deformities and both hind paws with a fifth digit (arrows)

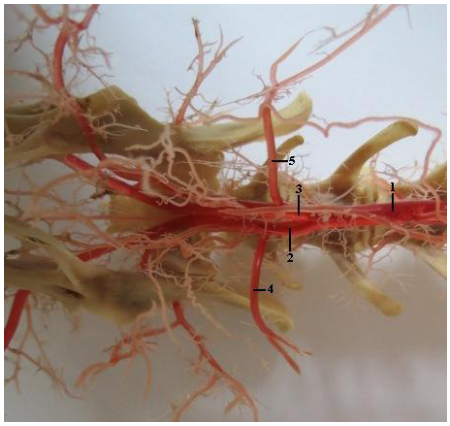


Figure 13 and 14. Irregular origins of bilateral arteria circumflexa ilium profunda in two different adult rabbits with the homozygous Pelger-Huet anomaly. 1 = *aorta abdominalis*; 2 = *arteria iliaca sinistra*; 3 = *arteria iliaca dextra*; 4 = *arteria circumflexa ilium profunda sinistra*; 5 = *arteria circumflexa ilium profunda dextra*. Macroscopic image, ventral view; horizontal field width 9.98 × 14.18 cm

ing in altered nuclear activity, impaired structural dynamics, and aberrant cell signalling (Schreiber and Kennedy 2013). The expression of the lamin B receptor in lymphoblastoid cells is reduced in heterozygous cases and is very low in homozygous cases of PHA as compared to normal lymphoblastoid cells (Hoffmann et al. 2002). This could explain the chromosomal aberrations found in lymphocytes from the bone narrow and peripheral blood in affected rabbits (Figure 8 and 9) and their healthy littermates (Figure 10). Micronuclei (Figure 11) are formed when chromosomes or their fragments are not incorporated into one of the daughter nuclei during mitosis. Moreover, their association to the lamin B receptor mutation has been established (Utani et al. 2007, 2010).

In addition to the morphological malformations of neutrophil nuclei, homozygous PHA in humans is characterised by skeletal abnormalities, developmental delay and seizures (Haverkamp-Begemann and van Lookeren Campagne 1952; Aznar and Vaya 1981; Siegert et al. 1983; Hoffmann et al. 2002). Severe chondrodystrophy, developmental anomalies and increased pre- and post-natal mortality

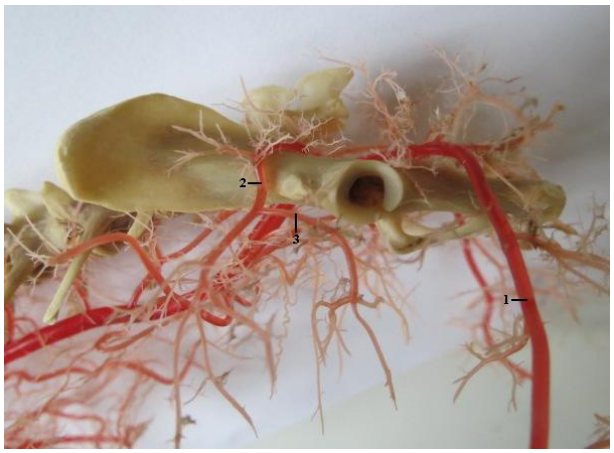


Figure 15. An irregular origin of *arteria circumflexa femoris lateralis dextra from arteria iliaca dextra* in an adult rabbit with the homozygous Pelger-Huet anomaly. 1 = *arteria femoralis dextra*; 2 = *arteria glutea cranialis dextra*; 3 = *arteria circumflexa femoris lateralis dextra*. Macroscopic image, lateral view; horizontal field width 10.37 × 14.02 cm

Figure 16. The typical arrangement of aorta abdominalis in an adult rabbit with a normal phenotype. 1 = *aorta abdominalis*; 2 = *arteria iliaca externa sinistra*; 3 = *arteria iliaca externa dextra*; 4 = *arteria circumflexa ilium profunda sinistra*; 5 = *arteria circumflexa ilium profunda dextra*; 6 = *arteria iliaca interna sinistra*; 7 = *arteria iliaca interna dextra*; 8 = *arteria sacralis mediana*. Macroscopic image, ventral view; horizontal field width 11.72 × 15.46 cm

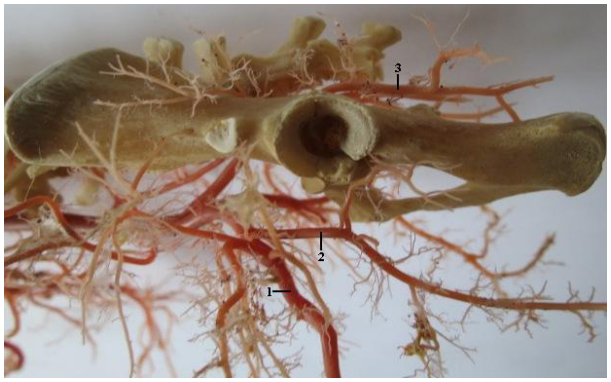


Figure 17. The typical arrangement of arteria femoralis in an adult rabbit with a normal phenotype. 1 = *arteria femoralis sinistra*; 2 = *arteria profunda femoris sinistra*; 3 = *arteria iliaca interna sinistra*. Macroscopic image, lateral view; horizontal field width 9.91 × 16 cm

were observed in rabbits with the homozygous form of PHA (Undritz 1943; Nachtsheim 1950). Stillborn kittens affected by PHA were small, had a hydrocephalus-like skull, shortened and thickened limbs with a severe medial deviation and cup-shaped methaphyses (Latimer et al. 1985). In our study, similar radiographic skeletal abnormalities were seen in all affected rabbits (Figure 12). Moreover, radiographic findings in all affected rabbits were consistent with chondrodysplasia as in the case of the aforementioned study. Sparseness of hair, presence of scales, decreased body size and bony and soft tissue fusion between digits were observed in mice by Schultz et al. (2003) too. These authors stated that these skin lesions are manifestations of epidermal hyperplasia with orthokeratotic hyperkeratosis and abnormal piliary canals.

In carnivores, Latimer et al. (1989) found necrotic lesions in central areas of the physis which were associated with a failure of vascular invasion. Severe vascular abnormalities were found in our affected rabbits (Figure 13, 14 and 15) as compared to their healthy littermates (Figure 16 and 17), and the physiological arterial arrangement in the pelvic cavity and proximal part of the pelvic limbs (Ahasan et al. 2013). To our knowledge, this is the first reported case of fast-flow vascular malformations in rabbits affected with PHA. Congenital vascular abnormalities are always present at birth, tend to grow proportionately with the body, persist throughout life and may not be visible clinically. However, they can result in severe health problems and even pre-mature death.

In mice and humans, Hoffmann et al. (2002) performed a genome-wide linkage screen and found that PHA is linked with chromosome 1. Our findings suggest a multigenic origin of PHA in rabbits, because only male and female offspring with the otter colour of fur (genotype: a^t/a^t) were severally affected by this congenital disorder. It is well known that the "a^t" gene is recessive to the "A" (agouti) gene, but dominant to the "a" gene. In conclusion, further research is required to elucidate how the homozygous allele a^t/a^t is associated with the homozygous form of PHA in rabbits.

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Received: 2013–05–21
Accepted after corrections: 2014–02–25

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