Ichthyosis in the Belgian White and Red cattle breed

Ichthyosis bij het Witrood rundveeras van Oost-Vlaanderen

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ABSTRACT

Three neonatal Belgian white and red calves with a thickened and rigid skin, characterized by the presence of scaly plates separated by deep reddened fissures were presented for necropsy. The least affected calf still had a normal hair coat, whilst the other two cases were alopecic and additionally displayed eclabium, ectropion and microtia. Based on these findings, ichthyosis fetalis bovis was diagnosed. Histological examination of skin samples obtained after euthanasia revealed an exuberant orthokeratotic hyperkeratosis of the stratum corneum. In human harlequin ichthyosis, a disorder highly resembling bovine ichthyosis fetalis, a defective lipid transport in keratinocytes caused by a single locus autosomal recessive gene defect has recently been identified as the cause of the disorder. Pedigree information of the calves combined with additional information of three other calves of the same breed allowed the identification of 3 disease carrying bulls and suggests an autosomal recessive inheritance of bovine ichthyosis fetalis. As there is currently no cure for this lethal disease, a proper breeding program is the only option to minimize the incidence of ichthyosis in this specific cattle breed.

SAMENVATTING

Drie pasgeboren Oost-Vlaams Witrode kalveren met een gegeneraliseerde huidaandoening gekenmerkt door de aanwezigheid van dikke en stugge hoornplaten, van elkaar gescheiden door diepe rood gekleurde kloven, werden aangeboden voor lijkschouwing. Het minst erg aangetaste kalf had nog een normaal haarkleed, terwijl de beide andere kalveren bijna haarloos waren en verder eveneens eclabium, ectropion en microtia vertoonden. Voortgaande op deze bevindingen werd de diagnose van ichthyosis fetalis bovis gesteld. Het histologische aspect van de huidstalen verkregen na euthanasie, was dat van een buitensporige orthokeratotische hyperkeratose van het stratum corneum. Recentelijk werd aangetoond dat bij harlekijn ichthyosis, een menselijke variant van de ziekte, die sterke gelijkenissen vertoont met ichthyosis fetalis bij het rund, een monofactoriële autosomaal recessieve genmutatie de oorzaak is van deze aandoening door een verstoring van het vettransport ter hoogte van de keratinoocyten teweeg te brengen. De stamboekgegevens van deze kalveren werden aangevuld met stamboekgegevens van drie andere kalveren van hetzelfde ras met dezelfde aandoening. De gezamenlijke gegevens suggereren eveneens een autosomaal recessieve overerving van ichthyosis fetalis bovis en resulteerden bovendien in de identificatie van drie Oost-Vlaamse dekstieren die drager zijn van de afwijking. Aangezien voor deze letale aandoening momenteel geen enkele remedie voorhanden is, kan de incidentie van ichthyosis binnen dit ras enkel mits een doordacht fokbeleid tot een minimum herleid worden.

INTRODUCTION

Ichthyosis comprises a number of cutaneous keratinization disorders, most of which are genetic in origin, affecting both man and animals (Molteni et al., 2006). The term is derived from the ancient Greek word for fish ιχθυς (ichthys) and refers to the typical scale-like appearance of the skin due to hyperkeratinization of the epidermis. In animals including cattle, the pathomechanisms and underlying genetic defects of these rare disorders remain largely unresolved (Testoni et al., 2006). In contrast, significant
progress in the understanding of the molecular basis of this disease in the human being has recently been established (Akiyama, 2006). Apart from the situation in man, ichthyosis has most frequently been observed in cattle (Baker and Ward, 1985) and occasionally in rabbits, pigeons (Stünzi, 1985), chickens (Baker and Ward, 1985), llama (Belknap and Dunstan, 1990), mice (Sundberg et al., 1997), the Greater Kudu (Chittick et al. 2002), and certain dog breeds such as the West Highland White terrier, Irish terrier, Collie, Labrador Retriever, Jack Russell, American Pit Bull terrier, Boston terrier and Doberman pinscher (Hoskins, 2001). In the present paper, 3 cases of ichthyosis in Belgian white and red calves presented to our faculty in less than 2 years time will be discussed. Furthermore, genealogical data of three other calves with the same disease were implemented in the pedigree analysis. To the authors’ knowledge, this is the first report on this congenital anomaly in this specific cattle breed.

CASE REPORTS

Anamneses and gross anatomical features

A first Belgian white and red calf (calf 1) was presented to the Department of Morphology by a local veterinarian in May 2005. The full-term calf was born earlier that day by natural delivery with veterinary assistance. The farmer had called in the help of the veterinarian because he had the impression that his initial extraction attempts had resulted in injury of the calf. The male calf was born alive, exhibiting a generalized symmetric linear pattern of skin defects that was clearly not the result of a violent extraction. As the newborn showed signs of extreme pain and distress and was suffering from an apparent condition beyond human aid, it was euthanized soon after birth. On postmortem examination, a normal pelage in a distinguishable white and red pattern typical for the breed could be observed. The moderately thickened skin was remarkably rigid and unelastic, and characterized by several parallel reddened fissures in a dorsoventral orientation on the trunk and limbs, and in a star-like configuration on the forehead (Figure 1). No internal malformations were found after systematic anatomical dissection.

In May 2006, a second newborn male full-term calf (calf 2) from a different herd with similar but more severe lesions was presented to the Department of Morphology by the same veterinarian. She had assisted with the natural delivery of this calf upon request of the stockbreeder because a relatively large calf was felt on a first obstetrical examination. Shortly after its birth, the calf was euthanized for welfare reasons. Its entire skin was covered by thick horny plates separated by deep fissures (Figure 2). Except for the most distal parts of the limbs and lower lip, the entire body surface was completely hairless. However, at the level of the fissures, the tips of some hairs were protruding from the rims of the scaly plates (Figure 3). The typical white and red pattern of the breed could be discerned in the abnormally rigid and unelastic skin. There was a mild eversion of the lips (eclabium) and eyelids (ectropion) and the pinnae were short and stubby (microtia).

A third full-term Belgian white and red calf (calf 3) with similar defects was born by natural delivery in January 2007. Two veterinarians of the Department of Reproduction, Obstetrics and Herd Health had been called in for assistance with the delivery after the stockbreeder had felt a living fetus with an abnormal skin during vaginal examination of the dam in partus. In this female calf, the skin abnormalities including the fissures and the generalized alopecia were more severe than in the second calf described above, and the eclabium, ectropion and microtia were more pronounced (Figure 4). The pigmentation pattern of the skin could not be distinguished. The calf continuously tried to position itself in sternal recumbency, showed no sucking reflex and was excruciatingly vocalizing. It was euthanized for welfare reasons eight hours after birth.
Microscopical analysis

Skin samples of all three calves were taken from different locations, formalin fixed and processed for routine paraffin sectioning. The histological slices were stained with hematoxylin and eosin and evaluated with an Olympus BX61 light microscope. Control samples were taken from the skin of a 1 month old calf which was free of any skin abnormalities and had died from a torsion of the mesentery.

In all skin samples of the affected calves, the stratum corneum of the epidermis was remarkably thickened (Figure 5). This keratinized layer measured several hundreds to thousands of micrometers in thickness, depending on the severity of the case (e.g. flank samples: ± 800 µm in calf 1, ± 1600 µm in calf 2 and ± 1900 µm in calf 3) and on the location of the samples (calf 2: ranging from ± 500 µm thickness on the inside of the pinnae until ± 2700 µm at the level of the thigh). Measurements of the stratum corneum in the control calf ranged from 2 µm to 10 µm, with a thickness of ± 6 µm in the flank region.

In the affected calves the thickened stratum corneum was mainly orthokeratotic, but focal areas of moderate parakeratosis could occasionally be observed. On several slices, the keratinocytes exhibited vacuolisation of the cytoplasm (Figure 6). Depending on the orientation of the sections through the tissue, the lamellar to scaly organisation of the hyperkeratotic epidermis could be visualized. In the underlying dermis, the sweat glands and hair follicles were slightly dilated. Numerous apparently normal hairs were entrapped by their hyperkeratotic infundibula. An inflammatory reaction could not be discerned in any of the skin tissue layers.

At the level of the fissures the stratum corneum was torn apart, exposing the remaining epidermal cell layers directly to the exterior environment. In this region the epidermis had a desiccated aspect and displayed several signs of cell death such as pyknosis and karyorrhexis.
Genealogical data

Genealogical data from the Belgian red and white Breed Stud Book were consulted in order to analyse the pedigrees of the three affected calves. Additional pedigree information of three other calves of one farm showing the same condition was used for the identification of more carriers and is also shown in Figure 7.

Pedigrees of the six calves and the suggestion of a simple autosomal recessive inheritance by Molteni et al. (2006) allowed the identification of three disease carrying bulls. A first bull (Usine) sired the first two affected calves and a second bull (Kantovani) sired the third calf. Further investigation of the three additional pedigrees revealed a third carrier (Tamboer). Information of the studbook taught us that these bulls were used in 27 percent of the first inseminations in Belgian red and white breed in 2004 and 2005 (P. Vercauteren, personal communication, 2007). Furthermore, a closer look to the pedigree showed a clear link between calf three on one side and calf one and two on the other side, since Kantovani is a full brother of Korneel (one of the male ancestors of the third calf). Korneel and also Ibel are other suspected carriers, although this could not be confirmed based on the current pedigree information. Kantovani is certainly the common ancestor of 4 calves (calf 3, 4, 5 and 6). The inbreeding coefficients are: 12.90% (calf 2), 0.80% (calf 3), 6.29% (calf 4), 6.25% (calves 5 and 6). In case of calf 1, we couldn’t calculate the coefficient, because the pedigree of the mother is not known.

DISCUSSION

In cattle two different types of congenital ichthyosis have been described so far: a severe form called ichthyosis fetalis bovis and a milder form ichthyosis congenita bovis (Stöber, 2002). Notwithstanding the differences in external appearance of the three calves discussed in the present paper, all three cases can be classified as forms of ichthyosis fetalis. This most extreme and fatal form of ichthyosis has already been reported in the Norwegian Red Poll, Friesian, Brown Swiss and Chianina breeds (Stöber, 2002; Molteni et al., 2006) and is thought to be caused by a single locus autosomal recessive gene defect (Molteni et al., 2006). The affected calves are either aborted or are born at full term in a moribund state and die within a few days after birth (Stünzi, 1985; Molteni et al., 2006). The entirely affected skin is characterized by typical thick horny plates separated by deep fissures (Testoni et al., 2006). Generalized alopecia is most commonly found, but sometimes hairs may be over-abundant. The tight inelastic skin may cause eversion at the
Ichthyosis is a skin disorder characterized by thick, scaling skin. Hyperkeratosis is a common feature, and ichthyosis congenita is the milder form of the disease. The skin defects do not involve direct lethality. In a recent report by Testoni et al. (2006) a Chianina calf affected by ichthyosis congenita survived for at least eight months, but was finally euthanized because of severe inflammation and bacterial contamination of the skin and underlying muscles. Apart from the skin defects, other abnormalities such as microtia, cataract and thyroid deficiencies have also been associated with this disorder (Chittick et al., 2002; Testoni et al., 2006).

Ichthyosis congenita has been reported in Jersey, Pinzgauer, Holstein-Friesian and Chianina calves (Testoni et al., 2006). It mainly affects male calves, a recessive sex-linked gene defect is suspected as cause of this disorder (Baker and Ward, 1985), although others believe an autosomal recessive gene defect is the origin of this condition (Molteni et al., 2006; Testoni et al., 2006). Despite the investigations in an Italian research project on a candidate gene for bovine ichthyosis (Molteni et al., 2004), little is currently known on the exact cause and pathogenesis of this disorder in cattle.

In humans, different types of acquired ichthysisiform dermatosis are described along with hereditary forms of ichthyosis. The latter have been classified into 10 groups, based on their clinical appearance, etiology and pathomechanisms (DiGiovanna and Robinson-Bostom, 2003). Two specific subtypes belonging to the group of congenital autosomal recessive ichthyoses (CAR1), i.e. harlequin ichthyosis and lamellar ichthyosis, most closely resemble bovine ichthyosis fetalis and ichthyosis congenita respectively (Chittick et al., 2002). The genetic cause of harlequin ichthyosis has recently been identified by Akiyama et al. (2005). They demonstrated that substantial mutations in the ABCA12 gene (ATP Binding Cassette transporter subfamily A (locus 2q34)) are a constant finding in infants with harlequin ichthyosis and that corrective gene transfer into ABCA12 defective keratinocytes leads to complete functional recovery in vitro. Previously, Russell et al. (1995) already linked mutations in transglutaminase 1 (TGM1 (locus 14q11.2)) with the classic lamellar ichthyosis phenotype, whilst Akiyama (2006) reported that minor missense mutations in ABCA12 can also lead to a similar phenotype indicated as lamellar ichthyosis type 2.

Both the ABCA12 and TGM1 proteins play an essential role in creating an intercellular lipid barrier in the stratum corneum (Akiyama, 2006). Rather than a simple inert barrier layer, the stratum corneum forms a complex interface with the environment. It is arranged in a typical bricks and mortar array, with the keratin-laden stratum corneum cells being the bricks surrounded by the intercellular mortar of lipid lamellae (DiGiovanna and Robinson-Bostom, 2003). The lipid lamellae are derived from lamellar bodies originating from the Golgi network in the epidermal granular cells (Hovnanian, 2005). These secretory organelles do not only contain the necessary components to build the extracellular lipid membranes, but they are also enriched with hydrolytic enzymes essential for the desquamation process of the stratum corneum (Elias, 2004, Candi et al., 2005). Members of the ABCA subfamily play an essential role in the active transport of lipids across cellular membranes against a concentration gradient (Peelman et al., 2003; Hovnanian, 2005). In the epidermis, ABCA12 is supposed to be involved in the extrusion of the content of the lamellar bodies into the intercellular space (Akiyama, 2006). After secretion, these lipids are riveted in parallel structures (Elias, 2004), which are in turn firmly linked to the cornifying cell envelope of the keratinocytes by means of the TGM1 enzyme (Nemes et al., 1999; Candi et al., 2005). Missense mutations in the TGM1 or ABCA12 genes hamper a proper construction of the intercellular lipid layers of the stratum corneum. This leads to an inadequate barrier function and impaired desquamation process, which results in lamellar ichthyosis (Akiyama, 2006). More serious mutations in the ABCA12 gene provoke a complete standstill of the lipid transport towards the extracellular matrix. Ultrastructurally, the cytoplasm of the keratinizing cells becomes congested with abnormal lipid-containing droplets and vacuoles (Akiyama et al., 2005). The barrier function of the stratum corneum is severely affected and the desquamation process is substantially disturbed, leading to the harlequin ichthyosis phenotype (Akiyama, 2006).

The fact that the phenotype of bovine ichthyosis fetalis most closely resembles human harlequin ichthyosis (Chittick et al., 2002) and the finding of lipid vacuoles in the stratum corneum both in the present study and in the case description by Testoni et al. (2006) suggest that processes similar to severe ABCA12 deficiency may be involved in bovine ichthyosis fetalis.

Pedigrees show manifestation of the disease in males as well as in females and absence of the disease in parents of affected animals. These observations are characteristic features of a recessive autosomal inheritance of the disorder, which has already been suggested by Molteni et al. (2006).

It has been shown that an inbreeding coefficient below 3.125% has no negative effect on health status.
whilst a coefficient above 6.25% could cause deleterious health problems (I. Moyaert, personal communication, 2007). It can be concluded that inbreeding coefficients of calf 2, 4, 5 and 6 clearly indicate that the farmer has increased the risk for health problems by inbreeding. Only one calf (calf 3) had a low inbreeding coefficient of 0.8%, which can easily be explained by the farmer’s decision to look for a new female blood line. Nevertheless, the pedigree of the dam and Kantovani has still a common ancestor, namely Ibel. This important finding suggests that preventing inbreeding is not the only method to decrease the incidence of ichthyosis. It also shows that even a mere 0.8% chance of homozygosity for a deleterious recessive gene is not completely safe. Moreover, the line between ‘healthy’ inbreeding coefficients and ‘non healthy’ ones is rather arbitrary and depending on the total genetic load of the considered population.

Currently, the hereditary forms of ichthyosis in man and animals are incurable diseases. Treatment in human medicine is mainly focused on a symptomatic alleviating therapy based on hydration, lubrication, keratolysis and long-term antibiotic therapy (DiGiovanna and Robinson-Bostom, 2003). Corrective gene transfer as performed by Akiyama et al. (2005) in vitro is a promising technique that can open new possibilities for therapeutic applications. However, major technical problems remain to be solved including the tendency for cells to lose the inserted corrective gene (DiGiovanna and Robinson-Bostom, 2003).

In animal forms of ichthyosis, euthanasia remains the only acceptable option because of economical and ethical aspects. However, a well thought-out breeding program can restrict and avoid the spread and prevalence of the genetic disorder by excluding proven carriers, both males and females, from breeding. These case reports have clearly shown that prevention of inbreeding by decreasing inbreeding coefficients below the threshold of 3.125% is not enough, since one of the affected calves had a “safe” inbreeding coefficient of 0.8%. The presence of multiple disease carriers in the Belgian red and white studbook requests transparent registration of potential carrier bulls and sincere communication between breeders and official breeding organisations, this in close cooperation with local veterinarians and scientists diagnosing and mapping emerging disorders.

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**Uit het verleden**

De oude geneesheren werden ook wel eens piskijkers genoemd en mochten ze het horen, ze zouden er trots op geweest zijn. Via het onderzoek (vooral van de geur en het kleur) van de urine van hun beklagenswaardige patiënten wisten zij immers tot allerhande diagnosen te komen. Op oude schilderijen en prenten werden doctors in de medicijnen steevast voorgesteld met een pisfles tegen het licht houdend en aandachtig bestuderend. Maar hoe zou dat gezeten hebben bij hun tijdgenoten, de ongediplomeerde en Latijn onkundige paardenmeesters?

Een tipje van de sluier werd opgelicht in *Den Lust-Hof van het Cureren der Peerden, beschreven door Mr. Jacobus de Smet, Peirde-Meester in Borgerhout, buyten de Stadt Antwerpen* (eerste druk, Antwerpen 1686). Daarin vinden we het volgende kenmerk van de goede vakman: *een goet Peirde-meester moet altydts riecken aen den strondt, ende een Doctoor der menschen die magh wel snuffelen aen de pisse, soo dat ieder een syn honingh daer uyt haele.* Want wie dat niet doet is geen *goet Meester.* Door dat onderzoek moet men tot een oordeel komen van hoe het *van binnen gestelt* is. En de medicatie moet op dit oordeel gebaseerd zijn, zo voegde Jacobus de Smet er nog ten overvloede aan toe.

Overigens was meester de Smet goed op de hoogte van de paardenanatomie en hij had een rudimentair begrip van fysiologie. Zo wou hij in zijn boek *mede bewys doen van den ommelloop des Bloedts.* Hij stond mede aan het begin van een diergeneeskunde gesteund op anatomische kennis en schreef dit expliciet: *My dunkt dat het wel noodigh waer al eer iemandt hem begint te bemoeyen met het meesteren ofte Cureren van eenige Peerden, dat sy al voren goede kennis hadden van alle Lidtmaeten (onderdelen) van deselve, om dat sy daer uyt souden considereren uyt wat oorspronck dat de quael gesproten is om hem voorts daer naer te reguleren met de Medecynen.*

De citaten zijn afkomstig uit de vierde, van *vele fauten* gezuiverde en vermeerderde druk te Antwerpen, zonder jaartal.

Luc Devriese