The occurrence of dermatosparaxis in a commercial Drakensberger cattle herd in South Africa

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ABSTRACT
Dermatosparaxis is a heritable collagen dysplasia causing skin extensibility and fragility. In Belgian Blue cattle this mutation has been described as a 3 base pair (bp) change followed by a 17 bp deletion in the gene coding for procollagen 1 N-Proteinase (pNPI). An outbreak in a commercial Drakensberger herd in South Africa followed the introduction in late 2000 of a 3-year-old bull that developed skin lesions in 2001 and was culled in 2002. Some of his offspring were similarly affected, 1 of which was kept as a breeding bull after his sire's death. Two affected calves were referred to the Onderstepoort Veterinary Academic Hospital in October 2005. Detailed examination revealed only skin abnormalities limited to the lateral extremities of the thorax, abdomen and pelvis, viz. either acute lacerations of varying sizes, slow healing defects or thin scars in chronic cases. During a subsequent farm visit, 13 animals with similar wounds were seen in the herd of 146 animals. Electron microscopic examination of skin biopsies revealed haphazard arrangement and loose packing of dermal collagen fibrils within collagen fibres. The fibrils showed size variation and slightly irregular outlines on cross-section, consistent with mild dermatosparaxis. DNA samples of affected calves were analysed using primers designed to amplify the region of the pNPI gene that contained the mutation described in Belgian Blue cattle, but this mutation could not be demonstrated in any of the animals tested. It is concluded that a form of dermatosparaxis with a different gene mutation from that described in Belgian Blue cattle exists in Drakensberger cattle in South Africa. This possibly also explains the milder and more delayed clinical signs and the milder dermal collagen ultrastructural abnormalities.

Key words: cattle, collagen, dermatosparaxis, Drakensberger, heritable, South Africa. ultrastructure.


INTRODUCTION
Dermatosparaxis (tearing of skin), also known as cutaneous asthenia or Ehlers–Danlos syndrome, is a heritable collagen dysplasia causing hypextensibility and fragility of the skin. In some species, joint laxity and blood vessel abnormalities have also been described. The condition has been known in humans since the 17th century, and was first described as a collagen abnormality by Ehlers (Denmark, 1901) and Danlos (France, 1908)17. This autosomal recessive genetic defect has been reported in humans, dogs, mink, cattle, sheep, cats and horses17, but in South Africa only in humans and white Dorper sheep16. Cattle breeds known to be affected include Belgian Blue, Holstein, Charolais, Hereford, Simmental and crossbred cattle13,15. Clinical signs and severity of the condition vary between species15, and several different clinical forms (with different ages of onset) of the disease have been described in humans5. In Dorper sheep the onset is from 3 weeks to 2 months of age, and severe skin lesions develop that necessitate euthanasia16, although different clinical forms have been described in other breeds of sheep17. In horses, a milder and more delayed clinical form exists, occurring by 6 months of age, and skin lesions (2–30 cm diameter) develop mostly over the dorsolateral thoracic, lumbar and sacral areas13,14.

In Belgian Blue (and Holstein) cattle onset is soon after birth, and it is reported that in some instances the skin of a foetus can be removed completely by manipulation during dystocia. In these breeds the condition is reported to be lethal, and typical clinical signs include initial subcutaneous oedema of the limbs, eyelids and dewlap and large skin defects in the same areas that are prone to infection5. These lesions heal slowly with thin, papyraceous scars13. It is further reported that early diagnosis can be made by veterinarians by the ease of penetration of the skin by needles during drug administration5. In the Hereford breed, onset is at approximately 2 months of age, and severe skin fragility leading to large open lesions exposing the dermis on the head and body, as well as joint laxity and thickened skin have been described15. Apart from the above clinical abnormalities, abnormal lowing and poor vision has been reported in calves15.

Ultrastructural changes in the Hereford have been described as randomly distributed, loosely packed collagen fibrils within collagen fibres, leading to fibres with a diffusely, bizarre, randomly-linked and linear (‘hieroglyphic’) appearance on cross-section; in contrast with the normal round appearance17. Owing to the use of artificial insemination and inbreeding in Belgian Blue cattle, the defective gene, after its 1st appearance, spread quickly and widely within this breed5. This spread, however, also led to early recognition of its autosomal recessive mode of inheritance5. The molecular cause of the condition was shown to be a defect in the procollagen protease enzyme, leading to abnormal orientation of collagen fibrils within collagen fibres, with resultant weak fibres5. The genetic mutation has been studied in detail in humans and also in Belgian Blue cattle, and described in the latter as a 3 base pair (bp) change followed by a 17 bp deletion in the beginning of the coding sequence of the gene coding for the enzyme procollagen 1 N-Proteinase (pNPI)5. This mutation has an effect similar to the type VII C form of Ehlers–Danlos syndrome in humans, changing the reading frame of the message and resulting in the synthesis of a truncated protein. It has been recognised that heterogeneity may occur with Ehlers–Danlos syndrome in humans, due to the fact that other mutations from those previously described, are causally involved in the disorder5.

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The Drakensberger breed is classed as a composite indigenous breed or as a locally developed breed of South Africa. In a study using red cell antigen loci it was found that the Drakensberger clustered amongst others with the dairy types like the Ayershire, Holstein and Shorthorn, but the history and origin of the breed is difficult to determine accurately.

OUTBREAK HISTORY

The herd of commercial Drakensberger cattle in which this condition occurred resides on a small farm adjacent to the town Virginia, in the northern Free State province of South Africa. The farm is situated between the town and a large inactive gold mine. Management on the farm is limited with no records on calving dates or masses being kept and there is year-round breeding with a multi-sire system. Cattle are identified by branding, but only the year of birth is used. During the last half of 2000, the farmer bought a 3-year-old Drakensberger bull from a stud herd for breeding purposes. In July 2001 this bull started to develop skin lesions, after being in a fight with another bull. The lesions seen on this bull were described by the veterinarian as initially large oedematous areas on the skin of the neck, thorax and preputium that drained some serous fluid on lancing. The epidermis was initially intact but eventually became necrotic and sloughed, leaving the underlying dermis exposed and then healing slowly. The lesions responded poorly to treatment, and new lesions developed from time to time. After several attempts at treating this bull, he was culled during 2002.

Calves born to this bull started to develop lesions at about 4 months of age according to the owner. These lesions were similar to those seen in the bull, and were also non-responsive to treatment, although they appeared mostly on the lateral extremities of the thorax and abdomen. Skin biopsies were taken during October 2003 of an affected calf. A diagnosis of eosinophilic dermatitis (suspected insect bite hypersensitivity) was made. One of the male calves of the original bull, born in 2001, was kept on the farm as a breeding bull. This animal also developed some skin lesions, although milder than those seen in his sire. This latter bull’s offspring also seemed to develop skin lesions when they reached about 4 months of age according to the owner.

Several attempts at treating these lesions were unsuccessful, but no mortalities occurred amongst the affected cattle. The owner further reported that after the slow healing process of the primary lesions, the animals seemed to develop only occasional and much milder lesions once they had reached about 2 years of age. Both the farmer and the veterinarian were under the impression that the incidence of the disease was slowly increasing during 2005, and a heritable skin condition was suspected at this stage. Because of this, the 2nd bull was culled, and the help of the Production Animal Clinic of the Onderste- poort Veterinary Academic Hospital (OVAH) was solicited during October 2005. Since the middle of 2006 no new cases have been reported in the herd.

MATERIALS AND METHODS

Two Drakensberger calves, a 6-month-old bullock and a 7-month-old heifer, were admitted to the Production Animal Clinic of OVAH in October 2005, showing large open skin lesions. Following their admission, full clinical examinations as well as haematology and serum chemistry were performed, the latter by the clinical pathology laboratory of OVAH using standard techniques.

Dermal samples were taken using a 6 mm biopsy punch from the centre and periphery of the affected areas, as well as from unaffected areas of skin, after local anaesthesia. Lesions were treated conservatively by applying antiseptics, fly repellants and aqueous cream daily.

The 2 calves were discharged 3 weeks after admission and a thorough investigation of the herd on the farm followed during which a full history was taken. All animals in the herd were examined for skin abnormalities, weighed (using a weigh tape), body condition scored and whole blood samples taken for genotyping. Known relationships between animals in the herd were recorded.

Proportions and means were compared using the Fisher exact and Student’s t-test, respectively, and data were analysed using NCSS 2004 (Kaysville, Utah, USA).

Skin specimens were fixed in 10% buffered formalin and then routinely processed for light microscopy. Sections were cut at 4 microns and stained with haematoxylin and eosin. Specimens were subsequently processed for transmission and scanning electron microscopy. For transmission electron microscopy (TEM) 1 mm tissue cubes were rinsed in Millonig’s phosphate buffer, post-fixed in 1 % osmium tetroxide in Millonig’s buffer and again rinsed in Millonig’s buffer before dehydrating through a series of graded alcohols. The samples were then infiltrated with a mixture of propylene oxide and an epoxy resin, embedded in absolute resin and polymerised overnight at 60 °C. Ultra-thin sections were prepared and stained with lead citrate and uranyl acetate and viewed in a Philips CM10 transmission electron microscope operated at 80 kV. Scanning electron microscopy (SEM) samples were dehydrated in graded alcohols, critically point dried, sputter coated with palladium and examined in a Philips XL20 scanning electron microscope. Normal skin specimens from age-matched controls of the same breed were not compared.

The primer pair described in Colige et al. to detect the presence of a 17 bp deletion in the gene coding for pNPI (procollagen I N-proteinase) in the Belgian Blue cattle was used to determine if a similar deletion occurred in affected Drakensberger cattle. A total of 18 animals was tested, 7 of these were affected with lesions ranging from acute to chronic and 11 had no lesions. DNA extracted from a bovine tissue culture (MDBK/NBL-1; American Type Culture Collection) was used as a control sample. DNA for genotyping was extracted from whole blood samples collected in EDTA using the method of Budowle et al.. A total of 01 μl of a 25 mM stock solution of forward primer (5’-CACCAGCGTTGAGCCCCCTGCT-3’) labelled with PET® (Applied Biosystems) and unlabelled reverse primer (5’-CAGCCCCATCGCGATTGC TGGAG-3’) was used in a PCR programme consisting of 95 °C for 45 seconds, 56 °C for 45 seconds and 72 °C for 60 seconds. The PCR product was analysed on an ABI 310 Genetic Analyzer (Applied Biosystems) and the results interpreted using STR (Board of Regents, California) on a personal computer. Using this technique, only the 101 bp fragment would occur in normal animals, both the 101 bp and the 84 bp fragments would occur in non-affected, carrier animals and only the 84 bp fragment (17 bp deletion) would occur in affected animals if the mutation was similar to the mutation responsible for dermatosparaxis in the Belgian Blue cattle.

RESULTS

Epidemiology

At the time of the visit to the herd in October 2005 there were 146 cattle on the farm. Thirteen animals (8.9%) presented with open wounds of different sizes and clinical stages (including the 2 calves referred to OVAH). Some animals developed fresh wounds during our investigation due to trauma induced by the handling facilities.

The prevalence of skin wounds amongst females was 8.1 % (9/111) and amongst males was 11.4 % (4/35) (P = 0.77). The Odds Ratio (OR) for males compared to females was 1.45.

Animals with wounds at the time of the
farm visit were all born in 2001, 2002, 2004 or 2005 (Table 1). There were no wounds amongst animals born before 2001 and in 2003, and wounds were most prevalent in animals born in 2001.

The animal with the lowest body weight and number of wounds weighed 140 kg, and there were 8 other calves weighing less than 140 kg without wounds. There was a male calf with wounds born during 2005 that weighed 210 kg, and 1 born during 2004 that weighed 290 kg, subjectively representing good growth compared to their peers within this herd. The mean mass of animals with wounds was 298 kg (95% C.I. = 218–378 kg) and of those without wounds was 357 kg (95% C.I. = 332–381 kg), $P = 0.17$. Mean body condition score was 2.78 and 2.86 for animals with and without wounds, respectively ($P = 0.51$).

Owing to the lack of records neither familial relationships between animals in the herd, nor temporal distribution of the occurrence of lesions could be established.

**Clinical signs**

Detailed clinical examination of the calves admitted to the hospital revealed only skin abnormalities. All other organ systems examined were normal, and body condition scores were 2 and 2.5 (5-point scale) for the heifer and bull calf, respectively.

Skin abnormalities in the 2 calves were asymmetrical large tears in the epidermis exposing the dermis on the lateral trunk (thorax, abdomen and bony protrusions of the hips) that seemed to be at different clinical stages. The bull calf presented with a very large (50 × 30 cm) open wound, with fresh skin edges and covered with thin scabs (Fig. 1). According to the owner, this lesion was approximately 2 weeks old at the time of admission. The heifer calf presented with bilateral but asymmetrical epidermal defects that seemed to be older, with contracted edges that were poorly defined and varying in size from 2 to 50 cm in length (Fig. 2). According to the owner, the biggest lesion on the heifer was approximately 4 weeks old at the time of admission. Apart from the lesions present, the skins of these animals also seemed soft and thin. Stretching of the skin revealed a sensation that the skin consisted of 2 parallel layers that could be separated without difficulty, particularly the skin of the caudo-lateral abdomen.

Haematology revealed a mild mature neutrophilia in the heifer ($4.82 \times 10^9$ neutrophils/μl; normal range: 0.6–4.0 × 10$^9$ neutrophils/μl). Serum chemistry of both calves showed high creatinine kinase (CK) levels (186 and 221 units/μl, respectively; normal range: 12–146 units/μl), low urea levels (1.1 and 2.7 mmol/μl, respectively; normal range: 3.6–10.7 mmol/μl) and a low Albu-
min/Globulin ratio (0.79 and 0.76, respectively; normal range: 0.9–1.4).

**Microscopy**

Lesions visible on light microscopical examination were limited to the superficial dermis to the depth of the hair bulb with the deeper dermis appearing unaffected. The supporting framework of the

Table 1: Age distribution of affected animals.

<table>
<thead>
<tr>
<th>Year of birth</th>
<th>Number of animals with wounds</th>
<th>Total number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 2001</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>2001</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>2002</td>
<td>2</td>
<td>18</td>
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<td>2003</td>
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<td>2004</td>
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<td>34</td>
</tr>
<tr>
<td>2005</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>146</td>
</tr>
</tbody>
</table>
superficial dermis showed a decrease in the number of fibres as well as an abnormality in the collagen fibres themselves. The collagen fibres of the sub epithelial collagen showed a marked variation in thickness and were arranged in a haphazard pattern. Many of the fibres were short and there were small fragments of collagen scattered between the remaining fibres. Areas in which there were very few fibres were also present throughout the superficial dermis.

Transmission electron microscopy (TEM) of the reticular dermis of skin biopsies from the affected areas of the 2 calves admitted to OVAH revealed the presence of collagen fibres containing abnormal fibrils distributed among fibres with a normal architecture. The abnormality was demonstrated in the haphazard arrangement of collagen fibrils within the collagen fibres (Fig. 3). Some fibrils displayed a curved shape in longitudinal profile and were not as tightly packed within the fibre. The fibrils also showed a variation in size (Figs 3 and 4) and slightly irregular outlines of individual fibrils were evident in cross-sections (Fig. 4). Active fibroblasts with dilated endoplasmic reticulum, containing finely granular material, were present. The striated periodicity of the fibrils was normal. Scanning electron microscopy reflected the TEM findings in demonstrating the loose, convoluted appearance of the fibrils within the fibres and the size difference between individual collagen fibrils (Fig. 5). The affected skin exhibited the abnormal features as described while the collagen fibres of the unaffected skin appeared normal with closely packed parallel collagen fibrils (Figs 6 and 7).

Genotyping
All animals tested in this study, including the bovine tissue culture control, amplified only the 101 bp fragment (Fig. 8), indicating that the mutation responsible for the condition seen in this Drakensberger herd is probably not the same as the mutation responsible for dermatosparaxis in the Belgian Blue cattle.

DISCUSSION
Dermatosparaxis was the most likely condition that fitted the history, epidemiology and clinical signs in this outbreak. Differential diagnoses for localised anasarca in cattle include increased hydrostatic pressure (heart failure, vascular obstruction), hypoproteinaemia (nutritional-, intestinal-, hepatic- or renal disease), vascular damage (infectious diseases such as besnoitiosis and lumpy skin disease, hypovitaminosis A) and uroporitoneum (mostly male animals)13.

These were ruled out with reasonable confidence by the epidemiology and clinical chemistry results of animals involved in this outbreak. Other hereditary skin diseases of cattle with roughly similar clinical signs include only familial acantholysis that has only been described in Angus cattle in New Zealand13, and that was ruled out by electron microscopic examination. It is difficult to speculate on the mode of inheritance of this condition but given the
similarities to dermatosparaxis in other breeds and species and given that the age groups affected by the condition coincided with the introduction of similarly affected bulls in the herd, as well as fairly low prevalence of skin lesions in the herd (<10 %) at the time of examination, an autosomal recessive mode of inheritance could be suggested. According to the farmer these were not the only bulls present in the herd at the time and one would expect a reasonably high prevalence of carrier females present in the herd.

The presence of skin lacerations did not affect growth or production, despite the difference in mean mass of animals with and without wounds. This difference was not statistically significant (using Student’s t-test) and was confounded by the fact that the healthy part of the herd was older than the animals with wounds. This age difference was most likely due to the fact that the herd produces weaner calves for the feedlot industry and most of the affected animals would have been sold at around 7 months of age. There was no evidence to suggest that body condition was affected by the presence of wounds. If the normal pre-weaning growth rate of beef calves is considered, it seems most likely that the 8 animals that weighed less than 140 kg at the time of examination were younger than 4–5 months of age, although exact birth dates were not known. The fact that no lesions were found in these 8 calves supported the observation made by the farmer that calves were only affected once they reached about 4 months of age. This is different from previous reports of dermatosparaxis in cattle, but similar to the manifestation in horses.

Although the odds of a male animal being affected were 1.45 times higher than that of a female animal, it has to be kept in mind that most males in the herd are sold as weaners at the age of ±7 months (while most of the females are kept as replacement animals), making the male component of the herd much younger than the females, and this gender predilection was probably confounded by the age distribution within the herd. Previous reports of dermatosparaxis in cattle have also found no correlation with gender.

The only significant clinical signs were the skin abnormalities. The mild and chronic systemic inflammatory response (neutrophilia and decreased albumin to globulin ratio) was most likely due to secondary infection of the large wounds. The serum chemistry abnormalities were most likely caused by transport (increased CK as a result of muscle injury
during transport to the hospital over a distance of almost 300 km) and starvation (decreased urea as a result of ongoing rumen metabolism in the absence of non-protein nitrogen intake). Clinical signs recorded in this outbreak were less severe than those previously described in cattle, and the absence of mortality due to the disease also differed from previous reports. In these Drakensberger cattle, lesions were limited to the lateral extremities of the thorax, abdomen and bony protrusions of the pelvis. This, as well as the later onset of clinical signs represents evidence that the condition in Drakensberger cattle is not exactly the same as that seen in Belgian Blue, Holstein, Hereford and other breeds of cattle, and that it more closely resembles the disease described in horses. The abnormalities found in the electron microscopic examination of the dermis of the 2 hospitalised calves was consistent with dermatosparaxis. Longitudinally sectioned collagen fibrils are normally closely associated (Figs 6 and 7), with their packing into smooth-surfaced bundles being near parallel. Normal fibrils in transverse sections usually appear circular in outline. The abnormal fibrils found in the skin lesions of the 2 calves exhibited a wide diameter range and irregular contours in cross-sectional profile (Fig. 4). These findings indicate a mild form of dermatosparaxis, as not all the collagen fibres in the 2 calves exhibited abnormal fibrils. In addition, when compared with the 2 calves, the ultrastructural findings in ovine and feline dermatosparaxis have shown a greater percentage of distorted collagen fibrils and most of the transverse fibril profiles have displayed flanged contours. Similarly, the abnormalities seen in Drakensberger cattle seem to be less marked than those in Hereford, where so-called ‘hieroglyphic’ appearance of collagen fibres was described in TEM examination. This is further evidence that the form of dermatosparaxis seen in Drakensberger cattle is milder than that described in other breeds. The mutation of the pNPI gene described in Belgian Blue cattle could not be demonstrated in this case in any of the affected cattle that were tested. This suggests that the mutation in the Drakensberger breed is probably not the same as that in the Belgian Blue, and once again supports evidence that the mutation in Drakensberger cattle is probably unique and needs further investigation. On the other hand, it has been suggested that heterogeneity may occur in Ehlers–Danlos syndrome in humans. If the same applies to Dermatosparaxis in cattle, the possibility of an historical relation between the Drakensberger and the Holstein, a breed known to be affected by Dermatosparaxis, also needs further investigation. This investigation provides strong evidence that a milder, delayed form of dermatosparaxis with a different genetic mutation to that described in other cattle breeds exists in Drakensberger cattle in South Africa.

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REFERENCES

12. Ramsay K, Harris L, Kozé A 2000 Landrace breeds: South Africa’s indigenous and locally developed farm animals. Farm Animal Conservation Trust, Pretoria