

Subclinical laminitis in captive female Esfahan mouflon (*Ovis orientalis isphahanica*): gross and light microscopic pathology

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Abstract:

The herd consisted of 25 captive Esfahan mouflons and their offspring. The mouflons were wild and originated from their natural habitat in Esfahan province. They had been kept in small enclosures with rough concrete floors. The diet consisted of alfalfa hay, corn silage, and a commercial concentrate. The mouflons showed severe claw overgrowth and detectable subclinical form of laminitis such as sole hemorrhage and yellow wax discoloration. Both prevalences were unexpectedly high (73.9%). The majority of foot lesion samples included in this study were taken from mouflons examined in the course of veterinary practice over a period of 4 months in a farm for clinical and histopathological purposes. Pseudocarcinomatous epidermal hyperplasia which are characterized by extreme proliferation of epithelial cells with large amounts of whorl-like structures that show no specific pattern, like dyskeratosis or central keratinization. Our findings were supportive of researchers who have suggested that subclinical laminitis is a multifactorial disease that involves a complex interaction between nutrition and management. Feeding management, proper design of facilities and routine trimming seems to prevent subclinical laminitis is predisposed for more serious lesions in Esfahan mouflons.

Case History

Wild sheep constitute a part of wildlife population of small ruminants in Iran. The common races are Transcaspian ural (*Ovis orientalis arkal*), Afghan ural (*Ovis orientalis cycloceros*) Armenian mouflon (*Ovis orientalis gmelini*), Larestan mouflon (*Ovis orientalis laristanica*), and Esfahan mouflon (*Ovis orientalis isphahanica*) (Firouz, 2005; Ziaie, 2009). The Esfahan mouflon is one of the races of Iranian wild sheep that inhabits the central and southwestern mountains of Iran (Firouz, 2005; Ziaie, 2009). Some ruminants, which were domesticated long ago and have been used intensively, are still able to survive in ever-decreasing natural habitats (Keller et al., 2009). Wild ruminants such as deer and mouflons are farmed, but less than cattle, or sheep in Iran.

Sub-acute ruminal acidosis (SARA) is the most important nutritional disease of ruminant animals (Nocek, 1997; Nordlund et al., 2004; Marie Krause et al., 2006; Enemark, 2009; Plaizier et al., 2009). Compromises in ruminant animals health due to ruminal acidosis is a concern for animal welfare reasons. Lameness is probably the most important animal welfare issue in captive exotic animals, and ruminal acidosis has been recognized as a major risk factor for laminitis (Nocek, 1997; Nordlund et al., 2004).

The subclinical form of laminitis (SL) (pododermatitis aseptica diffusea) is not well described, and certainly not well understood (Donovan et al., 2004; Plaizier et al., 2009). Domestic cattle show no visible signs of disease; however, they experience a low-grade recurrence of laminitic

events that impair claw horn growth and quality (Donovan et al., 2004). Moderate ecchymotic to severe hemorrhages on the cleaned sole of domestic cattle is considered to be an indicator of SL (Greenough and Vermunt, 1991; Bergsten and Frank, 1996; Smilie et al., 1996). This condition is considered to predispose to more serious lesions and lameness (Bradley et al., 1989; Greenough and Vermunt, 1991; Frankena et al., 1992). Both acute and subclinical forms of laminitis are thought to have environmental and nutritional components (Bergsten and Frank, 1996; Donovan et al., 2004). It has been proposed that examining claws for sole lesions may be used as a method to determine the prevalence of SL in a herd. However, to the best of our knowledge, reports of field studies conducted in Iran to determine the occurrence and prevalence of sole lesions in a herd have not been published. This cross-sectional study was carried out to investigate the prevalence and potential risk factors of sole hemorrhages (SH) in female Esfahan mouflon (*Ovis orientalis isphahanica*). The result of this study will help veterinarians and farmers to appreciate the welfare need of wild ruminants.

The herd was consisted of 23 captive female Esfahan mouflons, 2 captive male Esfahan mouflons and their offspring. The mouflons were wild and originated from their natural habitat in Esfahan province. The female mouflons and their offspring had been kept in small enclosures with rough concrete floors in Karaj, Iran. The mouflons had lambed 2 months before the study and were otherwise healthy. The animals fed a total-mixed ration. The diet was consisted of alfalfa hay, corn silage, and a commercial concentrate. They had free access to feed bin. Subclinical form of laminitis was diagnosed in seventeen of the female Esfahan mouflons (73.9%). In July 2009, the farmer added 100 g of molasses to the feed to prevent TMR sorting. Since this change in the diet of the mouflons, there have been no further cases of SL.

Clinical Presentations

The mouflons were restrained in lateral position by two assistants, and the affected limb was held up by one of the assistants and then trimmed. The herd had some cases with detectable subclinical form of

laminitis such as SH and yellow wax discoloration. Both prevalences were unexpectedly high (73.9%). Severe claw overgrowth was present in all the female mouflons (Figure 1A, B). The frequency of overgrown claws was similar in the fore and hind limbs. When their claws were trimmed, haemorrhages and yellow wax discoloration of the sole horn were revealed. Slight and moderate haemorrhage was found in the majority of positive female mouflons; solar zones were affected most frequently. Hemorrhage also was seen in the laminar region (Figure 2A, B). There were not any sign of cycling feeding pattern, epistaxis, and/or deaths within the herds. Overall, significant lameness was not observed during the period of observation. The affected cases were treated twice parenterally with selenium, vitamins A, D, and E and twice parenterally with Triplin Amine[®]; nevertheless, these treatments had little effect on the course of the disease.

Diagnostic Testing

Collected tissues were taken in 10% buffered formalin and were sent to the Baharan Pathology Laboratory at Tehran for histopathologic examination. At the laboratory, thin histologic tissue sections were prepared and stained by hematoxylin & eosin (HE) method. Histopathologically, pseudo-carcinomatous epidermal hyperplasia which is characterized by extreme proliferation of epithelial cells with large amounts of whorl-like structures (squamous eddies) that show no specific pattern, like dyskeratosis or central keratinization. The dermis was composed of large fibroblasts that surrounded by very broad bands of pale eosinophilic collagenous connective tissue fibers (keloid-like lesion) (Figure 3). The border between epidermis and dermal layer was well-demarcated, although the basal cells were not evident.

Assessments

The true mechanistic causes of laminitis in domestic cattle are poorly understood, and are assumed to be multi-factorial (Nocek, 1997; Nordlund et al., 2004; Cook and Nordlund, 2009). Three main trigger factors for claw lesion development have emerged. First, nutrition remains



Figure 1. A) dorsopalmar view of cross claw in a female Esfahan mouflon. B) palmodorsal view of overgrown claw in a female Esfahan mouflon.

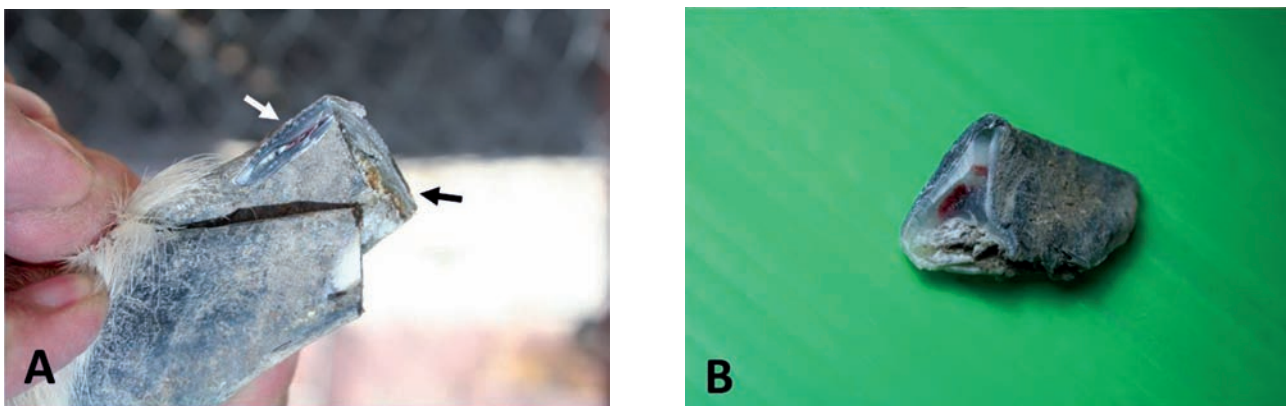


Figure 2. A) oblique view of affected claw with hemorrhage (white arrow) in the lamellar region and yellow discoloration (black arrow) after trimming in a female Esfahan mouflon. B) caudal cross section of the tip of the claw capsule in a female Esfahan mouflon.

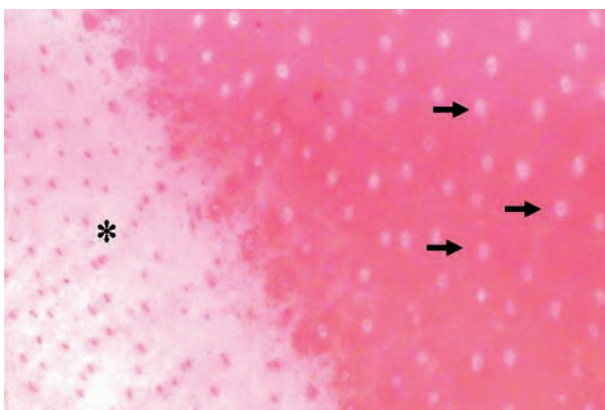


Figure 3. Pseudoepitheliomatous is showing acanthocytosis with eddy-like structures (black arrows). Keloid-like lesion in the dermis: large fibroblasts, which parallel with collagen fibers (asterisk).

a significant item, whether it is through the maintenance of horn quality, through trace elements and

vitamins such as biotin (Tomlinson et al., 2004), or through an association with subacute ruminal acidosis (Thoefner et al., 2004; Marie Krause et al., 2006; Enemark, 2009; Plaizier et al., 2009). Second, hormonal changes at parturient time appear to be associated with non-inflammatory changes in the connective tissue of the corium that impair the resilience of the feet to external stresses, mediated through the activation of certain gelatinoproteases (Tarlton et al., 2002; Knott et al., 2007). External trauma is a third trigger factor and can result in traumatic injuries to the claw (Chesterton et al., 1989). This study indicates that indeed these kinds of factors might exist in the development of SH in captive mouflons, trauma being the most important one for several reasons. First, the most-frequently affected zones (solar and lamellar region) are most heavily loaded. Second, SH is more prevalent in older

mouflons which may be a result of increasing body weight in captivity, which increases the pressure on the claws. Also, feed changes might be more frequently experienced by older mouflons. Third, these female mouflon had been kept in a concrete-floored yard suggesting that mouflons have not adapted well to man-made housing systems.

This herd has had facilities problems that would force mouflons to stand on concrete for long periods of time. Veterinarians have recognized for years that exposure to hard surfaces is a contributing factor in laminitis (Bradley et al., 1989; Greenough and Vermunt, 1991, 1996; Frankena et al., 1992; Bergsten and Frank, 1996; Sogstad et al., 2005). Nordlund et al. (2004) believe that 'excess standing time on concrete' as a factor of equal importance to ruminal acidosis in laminitis problem herds. As a consequence of the high mechanical load of sole and heel, the initial alterations following circulatory disturbances are not only restricted to the laminar region but also occur as primary lesions in the tissue at the ground surface (Greenough, 2007). The impact of flooring on lameness may be mediated through the duration of contact, the distance walked on the surface, and through properties of the surface which may cause trauma to the claw (Cook and Nordlund, 2009).

Concrete is a far from ideal surface for cows to walk and stand on (Phillips and Morris, 2000). It is clear that concrete floors do not provide enough friction to allow natural locomotion behavior (Cook and Nordlund, 2009). Slippery or traumatic concrete surfaces may result in an increased tendency for SH, and sand flooring may provide a reasonable solution to the problem. Sand, because of its ability to supply cushion and traction, allows mouflons to perform the processes of jumping and running more easily, without fear of slipping.

The pathological changes in the claws confirmed the diagnosis of SL, and they were similar to the changes described by other studies: Lesions that have been considered by researchers to be associated with SL include flaky or chalky white powdery sole (Greenough and Vermunt, 1991; Enevoldsen and Crohn, 1991), yellow discolored sole and hemorrhage of the sole (Bradley et al., 1989; Kempson et al., 1993; Greenough and Vermunt, 1991; Enevoldsen and Crohn, 1991), deformed claws (Bradley et al., 1989; Livesy and Fleming, 1984), and overgrown

claw (Bradley et al., 1989; Enevoldsen and Crohn, 1991; Livesy and Fleming, 1984). Lesions observed in SL result from impaired production of claw horn. The underlying events in the process are vascular. Disturbances in dermal microvasculature could result from a variety of causes ranging from circulating vasoactive substances to mechanical trauma (Chesterton et al., 1989; Boosman et al., 1989; Vermunt and Leach, 1992). Histological studies carried out on samples of horn from the mouflons examined in this study showed that sole haemorrhages were practically always accompanied by histological and morpho-logical changes in the laminar region where the horn of the white line is generated (Leach et al., 1997).

It has been proposed that yellow discoloration of the sole is the result of intercellular material escaping from inflamed corium or dermis (Kempson et al., 1993). Microscopically, evidence of blood and serum leakage across the basement membrane between the dermis and epidermis has been observed. These ultrastructural changes are observed before visible hemorrhage of the sole becomes apparent (Kempson et al., 1993). It is also speculated that yellow discoloration of the sole may be a more sensitive indicator of SL than SH (Smilie et al., 1996).

Overgrown claws showed the highest frequency of occurrence and were mostly bilateral. This observation agrees with previous reports (Mohammed et al., 1996; Bokko et al., 2003). The frequency of overgrown claws was similar in the fore and hind limbs. This disagrees with the findings of Paul-Bokko and Chaudhari (2001). In captive ruminants housed in small enclosures, characteristic hypertrophy of the outer claws of the hind limbs is often observed (Fowler, 1980; Keller et al., 2009). In small enclosures, decreasing mobility and activity do not provide the abrasion that minimizes claw overgrowth. The mouflons with overgrown claw were predisposed to chronic overload and subsequently claw lesion under current housing conditions. The factors which predispose sheep to lameness range from hard environmental terrain, unkempt and overgrown claws, and traumatic injuries (Paul-Bokko and Chaudhari, 2001; Bokko et al., 2003). No significant lameness during the period of observation was observed. This lack of significant lameness was not surprising because the goal of this

study was to observe occurrence of SL. By definition, SL does not cause clinical lameness.

Some of the greatest advances during the last 25 years have been associated with a shift to disease prevention, rather than treatment, and the increasing focus on herds (LeBlanc et al., 2006). SARA is so closely linked to feeding conditions that correction of feed rations and/or feed management is essential to solve the problem (Marie Krause et al., 2006; Enemark, 2009). The addition of molasses to the feed because of high viscosity prevent of TMR sorting. Thus, mouflons will not be able to consume more fermentable carbohydrates after feed delivery. However, further studies are needed to assess the possible roles played by the feeding of concentrates feeds, in the aetiology of laminitis in mouflons.

Our findings are in line with the findings of the researchers who have suggested that subclinical laminitis is a multifactorial disease that involves a complex interaction between nutrition and management (Frankena et al., 1992; Bergsten and Frank, 1996; Smilie et al., 1999; Nordlund et al., 2004; Donovan et al., 2004; Cook and Nordlund, 2009). Feeding management, proper design of facilities, and routine trimming seems to prevent subclinical laminitis is predisposed for more serious lesions in captive Esfahan mouflons.

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التهاب تحت بالینی نسج مورق در میش‌های ماده وحشی اصفهان (*Ovis orientalis isphahanica*): مطالعه پاتولوژیک ماکروسکوپی و میکروسکوپی

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چکیده

گله شامل ۲۳ میش و ۲ قوچ به اسارت گرفته شده بود که به همراه بره‌های خود نگهداری می‌شدند. دام‌ها وحشی بوده و از زادگاه طبیعی خود (استان اصفهان) آورده شده بودند. دام‌ها در یک محوطه کوچک با کفی از جنس بتون نگهداری می‌شدند. جیره شامل یونجه، سیلوی ذرت و یک کنسانتره تجاری بود. کپسول شاخی انگشت رشد بیش از اندازه‌ای داشت و نشانه‌های التهاب تحت بالینی نسج مورق همچون خونریزی کف و زردشدگی کنسانتره نسج شاخی قابل تأیید بود. هر دو نشانه به شکل غیرقابل انتظاری میزان شیوعی برابر ۷۳/۹٪ را از خود نشان دادند. نمونه‌های مورد مطالعه در طول معاینات انجام شده طی ۴ ماه بر روی یک گله میش وحشی بدست آمده بودند و از نظر بالینی و پاتولوژی مورد ارزیابی قرار گرفتند. هیپرپلازی اپیدرمی پزودوکارسینوماتوزی که با تزاید بیش از اندازه سلول‌های اپیتلیالی با ساختارهای مارپیچی فاقد الگوی خاص مانند دیس کراتوزیس یا شاخی شدن مرکزی مشخص می‌شود، مشاهده شد. یافته‌های این مطالعه با نتایج سایر تحقیقات که التهاب تحت بالینی نسج مورق را بیماری چند عاملی دانسته و با عواملی همچون تغذیه و مدیریت که در تقابل با یکدیگر هستند، همسو است. مدیریت جیره، طراحی مناسب محل نگهداری و سم‌چینی منظم به نظر می‌رسد در پیشگیری از التهاب نسج مورق که عاملی مستعدکننده در بروز ضایعات شدیدتر در سم است، موثر باشد.

واژه‌های کلیدی: میش غیر اهلی، پودودرماتیت، خونریزی کف، لامینایتیس تحت بالینی

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