THE EFFECT OF ENVIRONMENTAL INSULT ON PERMEABILITY BARRIER OF THE CLAW HORN OF CATTLE

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ABSTRACT

When cattle are housed or are in enclosures the claw horn is exposed to various environmental insults that are thought to predispose to lesions which may have some detrimental effects on claw horn function. The effects of pyroleum pini (Stockholm tar) 15% and 25% soap solutions and water on the permeability barrier function of the bovine claw was studied using tracer techniques and studied by both light and electron microscope. Horse radish peroxidose and 3% Lanthanum nitrate were used as tracers for light microscopy and electron microscopy (TEM) respectively. Blocks of clinically normal claw horn were immersed in the test solutions. The blocks were left for 48 hours, 4 days and 2 weeks in each test solution. Regions observed were the wall, the white line and sole in the distal toe of the claw and the palmar plantar surface of the heel. All the claws used in the experiments were clinically normal. The effect on the permeability barrier of all treatments (pyroleum pini soap solution and water) increased with time, with the maximum effect being seen at two weeks. Changes in the permeability barrier with treatment were greatest in the heel and least in the wall. Areas of damage or microcracks in the wall allowed greater penetration of tracer than non-damaged wall horn. Water particularly affected the laminar horn leaflets of the white line, reducing the permeability barrier and allowing greater penetration of the tracer. Observations in the TEM showed a significant effect on the intercellular lipids following treatment in a 25% soap solution. The cell membranes were also altered to allow the lanthanum nitrate to enter the corneocytes. In conclusion, pyroleum pini caused minimal damage to the permeability barrier of the horn, soap solutions should be used with caution and it is inadvisable to leave cattle standing in water for long periods of time.

INTRODUCTION

Lameness in cattle is well recognised as a serious clinical, economic and welfare problem (Webster, 1987; Esselmont, 1990). Several risk factors have been identified as contributing to the aetiology of lameness in diary cattle (Collick, 1997). Environmental insults have been observed to predispose cattle to lesions of the claw that result into lameness (David, 1987; Faye and Lescorret, 1989; Mgasa, 1987; Mgasa and Arnbjerg, 1993). The constant exposure of claws to wet environment, urine and slurry can be detrimental to the claw structure. Heel horn erosions and whiteline disease that are conditions of the claw horn are common problems in cattle kept under these conditions (Mgasa and Arnbjerg, 1992).

In general it has been observed that cattle kept in moist and dirty environment are often affected by lameness. The majorities of lesions that result in lameness occur in the claws and are manifested as disruption of the normal clawhorn growth. The function of the claw capsule is to provide protection to the bones, joints and soft tissues contained within from both physical, chemical and other environmental insults. A second function of the claw capsule is to permit the transfer of weight from the bon column to the ground.

The bovine claw capsule (stratum corneum) is a thick layer composed of completely keratinized epithelial cells, the corneocytes. The claw horn is considered to be a two-phase structure consisting of corneocytes which are separated by a thin layer of lipids occupying the intercellular spaces. In human skin, the stratum corneum has been likened to a brick wall, with the bricks representing the corneocytes and the mortar represented by the highly specialized intercellular lipids (Chandrasekeran and Shaw, 1978; Michael and others, 1975; Elias, 1983). The cells provide stability to the horny layer, while the intercellular lipids have a dual function (Landmann, 1988) of maintaining the structural integrity of the tissue. First is their significance in their contribution of cell to cell attachment, which is vital for maintaining a structural barrier. Second, the intercellular lipids play an essential role in forming the permeability barrier of skin (Landmann, 1988; Prosksch and others, 1990) and horn (Kempson and Campbell, 1998). In skin, the permeability barrier is responsible for controlling the movement of water into and out of the tissue (Landmann, 1988). Disruption in the structure of the intercellular lipids is related to claw horn lesion in first-calving heifers (Logue and others, 1993). Substances which are capable of removing intercellular lipids, can cause disruption of the permeability barrier (Menon and others, 1985; Proksch and others, 1990) and allow penetration of noxious agents from the environment.

When cattle are housed their claws and the horn are exposed to slurry, a mixture of urine and faeces, or to manure, a mixture of urine, faeces and straw. The damaging effect of slurry is well recognised in the development of heel erosion (*Erosio ungulae*), as it is commonly known as slurry heel (Bergsten, 1995). The overall aim of this study was to investigate the effect of environmental insults, such as water and chemicals and their effects on permeability barrier. The materials studied were (I) *pyroleum pini*, a pine tar derivative, also known as Stockholm tar, a treatment for claw horn lesions; (ii) soap, which in solution is used in foot baths and cleansing; and (ii) water, which is common in the environment, often in excess. The effect of these materials on the permeability barrier of claw horn was studied using tracer techniques in both the light and electron microscopes.

MATERIALS AND METHODS

Feet, distal to the carpus or tarsus were obtained from the abattoir immediately after death. After washing, the claws were examined and only those that were clinically normal were chosen for the experiments. Claws with gross horn defects or massive heel erosions were rejected. In total, feet from 11 different cattle were used in the study, four feet for experiments 1 and 2 and three for experiment 3. Blocks of horn 1cm^3 were removed from the ground surface of the heel and the distal ground surface of the dorsal wall at the toes of both medial and lateral claws. The distal wall samples included the white line and the sole (Diagram 1) and will be referred to as the toe sample. The blocks of tissue were removed using a vibrating circular saw and gross dissection.

Permeability studies

In order to study the permeability barrier in the light microscope, horseradish peroxidase (HRP) was used as a tracer as it is soluble in water, of low molecular weight and reacts with 3'3-diaminobenzene tetrachloride (DAB) to produce a brown-black precipitate (reaction product) which could be easily seen in the light microscope. The permeability of tissues was assessed on the penetration of the reaction product in cellular layers.

For transmission electronic microscopy (TEM), lanthanum nitrate was used as a tracer to confirm the observations seen in the light microscope. Lanthanum nitrate is water soluble, like HRP, electron dense and therefore easily visualized in the electron microscope. As lanthanum nitrate is a much smaller molecule than HRP it has a greater potential for deeper penetration into the tissue.

Experiment I

Blocks of heel and toe were divided into two, one half was the test block and the other was the control. Feet from four different cattle were used in this experiment. The control blocks were washed in distilled water and further sub-divided into two; one half, for permeability study, was placed in 3% HRP in 0.9M saline for 48 hours, washed in distilled water and then sectioned on a cryostat. Sections 12µm thick were then placed in DAB for 40 minutes. After washing, the sections were mounted in water and viewed immediately in a photomicroscope. The other half of the control block was sectioned, without further treatment, on a cryostat and sections 10µm thick were stained with Nile blue in order to differentiate the lipids within the tissue.

The test blocks were sub-divided into three and immersed in *pyroleum pini* for 24 hours, 48 hours and 2 weeks respectively. The blocks were washed and then immersed in HRP for permeability studies as above (Diagram 2).

Experiment 2

In this experiment blocks of toe and heel were taken as described above. The blocks were divided into three, one part was treated with tap water, another with 15% soap solution (teepol) and the third with 25% soap solution (teepol). In order to examine the effect of time, each of the blocks was further divided into three. Tissue blocks were prepared for permeability studies using HRP as described above.

Experiment 3

Claws from three cattle were used in this experiment. Blocks of tissue from the toe and heel of one foot were used as a control. These blocks were immersed in 3% lanthanumnitrate in distilled water for 24 hours. The samples were washed in distilled water and slivers no more than 1mm in one dimension were taken from the centre of the bock and fixed in 3% glutaraldihyde for 24 hours. After washing, the slivers were post-fixed in 1% osmium tetroxide in distilled water. Following dehydration in acetone the tissues were embedded in araldite. Thin sections, 50-60nm thick, were stained in 50% uranyl acetate and Reynold's citrate and viewed in a Philips EM400. Each of the test blocks from the sole and toe were divided into three. The blocks were immersed for two weeks in the following; (i) tap water; (ii) 15% soap solution; and (iii) 25% soapy solution. The blocks were washed and placed in 3% lanthanum nitrate for 24 hours and processed for electron microscopy as described above.

Results

Light microscopy

The extent of penetration of HRP could be seen by the presence of the dense reaction product following immension of the sections in DAB.

Controls

Blocks of heel horn immersed in HRP but with no other treatment showed reaction product had penetrated the outer 2-4µm of the tissue (figure 3). The horn was of relatively good quality with no surface cracks or defects. Some of the tubule cores did contain the dark deposits of reaction

product (Figure 3). However, other control blocks placed in 0.9N saline alone, minus HRP, also showed the same reaction in the tubule cores indicating the presence of endogenous peroxidase. This peroxidase is most likely to have come from microhaemorrhage into the tubule cores.

The control sample (Figure 4) incorporating the distal wall, white line and sole showed a similar degree of penetration with HRP into the outer surface of the wall of 2-4µm. cracks in the ground surface of the white line showed a greater penetration of the tracer, but only of the order of the 6-8µm. A similar penetration was seen in the sole.

The arrangement of the laminar horn leaflets interspersed with interdigital horn made up the structure of the white line (Figure 4). The structures seen in the interdigitating horn of the white line (Figure 4) were a pale brown colour when viewed in the light microscope; This staining reaction was different to the reddish brown colour of the reaction of DAB and HRP. Further observations of cryosections stained in floxine tartrazine confirmed the observation that these tubule like structures consisted of loosely arranged keratinized squames. The different reaction to the stain indicates a variation in the type of keratinization. The horn tubule cores in the wall showed a similar reaction (Figure 4). Occasionally the tubule-like structures in the wall stained reddish brown showing the presence of blood.

Treatments

Following treatment in *pyroleum pini,* water, 15% soap solution and 25% soap solution, the change in the permeability increased up to two weeks. There was some difference after 24 hours, but the maximum effect was seen after two weeks. The micrographs described all show sections of blocks of horn left in the respective solutions for two weeks.

Pyroleum pini (Stockholm tar)

The blocks heel horn left in *pyroleum pini* for two weeks showed an increase in staining at the outer surface to approximately 80µm (Figure 5). In areas of weakness or damage in the horn the penetration of the tracer increased in focal areas to approximately 300µm (Figure 5). In these regions the reaction product was seen in microcracks which were orientated parallel to the outer surface (Figure 5).

The distal wall sample (Figure 6) showed penetration of the tracer in cracks in the distal wall to a depth of 70-80µm and in the wall between the cracks to a depth of approximately 15µm. This section (Figure 6) showed reaction production in the laminar leaflets of the white line but not the interdigitating horn.

Reaction product had dispersed from a defect in the sole horn away from the palmar/plantar surface towards the soft tissues for a depth of 440µm (figure 6). The dispersal of stain from the large crack towards the ground surface was on average 30µm (figure 6). The penetration of tracer deep into the sole and the laminar leaflets was thought to have occurred from tracer entering the cut edge of the block rather than the ground surface.

Water

Following treatment for two weeks in water, penetration of the tracer in the heel horn as seen by the reaction product through the outer surface ranged from 300 to 400µm (figure 7). There was a definite gradient of stain intensity which decreased from the surface (Figure 7). The surface of

the heel horn in this sample was smooth with no significant cracks (Figure 7). The reaction product present in the horn tubule cores, deep within the sample was due to haemorrhage (Figure 7).

The interesting observation in the block of tissue from the distal toe, placed in water for two weeks, was the spread of reaction product from the cracks at the ground surface through the laminar horn leaflets (Figure 80. This was particularly obvious from the ground surface cracks of the white line. The HRP visualized by reaction to DAB had penetrated the laminar horn leaflets for a depth of 1-3mm (Figure 8). There was no reaction product in the interdigitating horn (Figure 8). The penetration of the tracer through the outer surface of the sole (Figure 8) was in the order of 15µm

Soap Solutions

Leaving blocks of heel horn in a solution of soap (teepol) for two weeks dramatically affected the degree of penetration of the tracer as seen by the presence of reaction product (figure 9 and 10). However, the stronger solution, 25% soap, created the greatest difference with the presence of reaction product in heel horn to a depth of 1-1mm from the outer surface, compared to 170-180µm for 15% soap solution (Figures 9 and 10).

The sections of the wall, white line and sole from blocks placed in soap (teepol) solution also showed increased penetration of the tracer which was concentration dependent. In a 25% solution of soap the reaction product was seen at focal points to a depth of 1-5mm into the wall from the ground surface (Figure 12). As with the samples placed in water, the reaction product was present within the laminar leaflets (Figure 12) with both concentrations of soap. The reaction product in the sole was of greater density and depth for both 15% and 25% soap solutions (Figure 11). The change in the permeability characteristics was more uniform in the sole (Figure 11) compared to the wall, where the changes were more localized in focal points (Figure 11). The greatest intensity of reaction product was seen at the ground surface and in particular in the surface cracks (Figures 11 and 12).

The use of lanthanum nitrate as a tracer illustrated the effect of a 25% soap solution on the wall horn. The control samples placed in lanthanum nitrate showed minimal penetration from the surface via the intercellular spaces. The structure of the control corneocytes was good as they contained well-defined bundles of keratin filaments interspersed with matrix. Following treatment with 25% soap solution, lanthanum nitrate penetrated via the intercellular spaces for several cell layers from the outer surface of the wall. The tracer appeared as an electron dense granular or crystalline deposit at the surface of the wall and within the intercellular spaces. Lanthanum nitrate was also found in the interfilamentous matrix within the corneocytes. This had occurred not only within the surface corneocytes, where membrane damage was expected, but also into corneocytes at a distance from the surface. This effect was not seen in the normal control specimens.

DISCUSSION

Horseradish peroxidase (HRP) has been used extensively to study the permeability of a number of covering and lining epithelia (Landmann, 1988). The small molecular size, high solubility in aqueous solution and ease of detection on the light microscope make HRP ideal for studying the permeability barrier (Squier, 1973). The advantages of lanthanum nitrate are its low molecular

weight (lower than HRP), high solubility in water and electron density when is viewed in the transmission electron microscope (TEM). A major disadvantage of lanthanum nitrate is the lack of easy detection in the light microscope. TEM sections are by their nature of a very small selected area and this can confound the overall picture unless adequate control studies are performed. The TEM did provide valuable information as to the cellular reaction to the treatment. Using HRP as a tracer enabled an overall picture to be established for each of the regions of the horn. Lanthanum nitrate enabled the authors to study the effects of a soap solution at a cellular level.

One of the protective functions of the claw capsule is derived from its low permeability to water soluble substances. The bovine claw horn is exposed to a large variety of environmental insults and without an effective barrier to the loss and penetration of water and water-soluble materials, the claw horn structure and function would soon be compromised. There are two possible routes for penetration of water and the tracers across the horn. The first is via the transcellular spaces and the second is by crossing the cell membrane and entering the cell, a transcellular route (Squier, 1991). It has been suggested (Chandrasekaran and Shaw, 1978; Landmann, 1988) that substances with different chemical properties traverse the barrier region by different routes, some by the transcellular route and others by the intercellular route. The evidence from this study has shown that soap solution had the highest effect on intercellular lipids and the cell membranes allowing increased (tracer) movement between and through the cells. Water similarly had more or less a similar effect. *Pyroleum pini* had minimal effects compared to the other treatments.

As was expected, the effect of a soap solution on the intecellular lipids and the permeability barrier increased with concentration. Soap is frequently used in claw health. However, the effects observed here indicate that it can be detrimental to the permeability barrier function if high concentrations are used. A field trial is required to establish the balance of concentration needed for improved claw hygiene cleanliness with the necessity of keeping permeability barrier damage to a minimum. Observations in the TEM showed that the soap solution not only damaged the intercellular layers, but also the cell membranes, allowing the lanthanum nitrate to enter the corneocytes themselves. It is not clear why the lanthanum nitrate was confined to the interfilamentous matrix, other than the region of the cells in which bound water resides (Forslind and others, 1980).

The permeability barrier is responsible for controlling the transepiderm water flux in skin (Grubauer and others, 1978). Disruption of the permeability barrier by acetone stripping to remove lipids was followed by an immediate increase in transepidermal water loss (Grubauer and others, 1987). If the same mechanism is present in the claw horn, the claw with an intact permeability barrier is more likely to be able to withstand extremes of environmental insults than those in which the barrier is compromised. Evidence from studies on equine hoof horn has shown that the permeability barrier in horn of poor quality was weaker than in good quality horn, therefore the effect on the claw horn of the treatments used in this study would be expected to be greater if claws with disrupted or compromised claw horn had been used. This has been further verified by increased penetration of reaction product in areas of damaged horn such as microcracks.

The materials tested in this study, *pyroleum pini* and soap solutions, were used for a relatively long times and high concentration in the case of soap. In the field, *pyroleum pini* would be applied for a similar length of time as it is always painted on to the claws regularly. Inthis study, the effect on the permeability barrier did increase with time with *pyroleum pini* but there was no

major change in the structure of the horn when viewed in the light microscope. The claws used in this study were of good quality and more effect would be expected if the integrity of the horn was already compromised (Langridge and others, 1998).

Pyroleum pini is cheap and readily available in the third world and is widely used as a claw horn dressing. Its properties are thought to be antimicrobial and water repellant. The later properties are probably the most significant as *pyroleum pini* is used on claw horn defects and wounds to protect the tissue from further damage due to wet and dirty environmental conditions. The study reported here showed no major adverse effect on the claw horn of *pyroleum pini*, although long term use would best be avoided in order to preserve the permeability barrier. Further controlled field studies are required to establish the efficacy of this material in the treatment of claw horn lesions.

Footbaths are used routinely as a measure directed towards the control of infectious diseases of the digital and interdigital skin (Kloosterman, 1997). They are not a substitute for good claw hygiene but can assist in the control of the environmental bacterial burden (Kloosterman, 1997). The common foot bath chemicals used in the developed countries such as formalin, copper sulphate and zinc sulphate have their disadvantages (Kloosterman, 1997; Kempson and others, 1998). In the mid west states of the United States soap solutions are being used to good effect in foot baths to improve claw hygiene (Bungi, persona communication). Soap is cheap and readily available throughout the world. This was the reason for its choice in this study. One major property of soap is its ability to dissolve fats. As the permeability barrier in skin and horn (Kempson and Campbell, 1988; Kempson and others, 1998) resides in the intercellular lipids it was important to observe the effect of long term treatment with soap solutions on the claw horn. Disruption to the permeability barrier of the ski induces metabolic changes in the underlying epidermis which results in increased lipid synthesis (Harris and others, 1997) leading to barrier recovery. The damage to the cell membranes is more significant as there is no known mechanism for their repair in the stratum corneum. This could lead to increased loss of intracellular water and easy entry of noxious materials and microorganisms to the cells. Therefore, the excessive use of soap solutions for claw horn care is not encouraged. However, further research is required in order to determine the concentration that can be safely used for claw health.

The effect of leaving blocks of claw horn for two weeks in water was to increase the penetration of HRP as visualized by reaction to DAB, differentially through the laminar horn leaflets. The ability of water to disrupt the permeability barrier of the laminar horn leaflet component of the white line could explain why white line lesions are common in cattle kept in wet and dirty environments (Edwards, 1980; Greenough, 1997). Weakness of the laminar horn leaflet component of the whiteline will allow the penetration of foreign bodies such as grit which in turn leads to the formation of claw horn abscesses (Edwards, 1980; Collick, 1997).

It must be acknowledged that by leaving blocks of claw horn for two weeks post mortem, the tissues will have been subjected to a certain degree of putrefaction. The other material used in the study, *pyroleum pini* and soap solution will have discouraged putrefaction. Therefore extrapolation of the results of the experiments to test the effect of water to the live animal must be done with caution. The need for field experiments on live animals is therefore indicated.

The effect of the treatments on the heel horn was greater than that of the sole which in turn was more than in the wall. The wall was the most resistant with increased permeability following all treatments occurring at focal points where there were surface cracks or other defects. The heel

horn was the most vulnerable to the external environment. These variations reflect differences in the nature and extent of the permeability barrier in different regions of the claw (Langridge and others 1998).

CONCLUSION

In conclusion, the soap solution had the greatest effect on the permeability barrier of the claw horn of the distal wall, whiteline and sole in the toe region and in the heel. The effect of water was selective for the laminar horn leaflets of the white line. Changes in the permeability barrier increased with time and also, in the case of soap, with concentration. It is concluded that environmental insults can disrupt the permeability barrier of the claw horn and predispose cattle to lesions of the claw thus causing lameness.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the Association of Commonwealth Universities for providing the Commonwealth Fellowship and financial support for the studies. Thanks are also due to Mr. Colin Warwick, Mr. Gordon Goodall and Mr. Stephen Mitchell for their outstanding technical support. The skill and patience of Mrs. Anne Stirling-Whyte and Ms F. Mwingirihela in typing this manuscript is acknowledged.

REFERENCES

Bergsten, C. (1995). Digital disorders in dairy cattle with special reference to laminitis and heel erosion: The influence of housing, management and nutrition. PhD thesis, Sveriges Lantbruks Universitet, Skara, Sweden.

Chandrasekaran, S. K. and Shaw, J. E. (1978). *Current Problems in Dermatology* **7**, 142-155.

Collick, D. W. (1997). Lameness in Cattle, 3rd Edition (ed. P. R. Greenough & A. D. Weaver). W.B. Saunders Company, pp.104-106.

David, G. P. (1987). Possible behacvioural factors associated with high incidence of solar ulceration in dairy cattle. Proceedings of the British Cattle Veterinary Association, 1986-87, pp.203-208.

Elias, P. M. (1983). *Journal of Investigative Dermatology* **80,** 44-49.

Edwards, G. B. (1980). *Vet annual* **20,** 227-232.

Esslemont, R. J. (1990). The cost of lameness in dairy herds. In: Proceeedings of the 6th International Symposium of disease of the ruminant digit. British Cattle Veterinary Association, Liverpool, England, pp.237-251.

Faye, B. and Lescourret, F. (1989). *Preventive Veterinary Medicine* **7,** 267-287

Forslind, B.; Nordstrom, G.; Toijer, D. and Eriksson, K. (1980). *Acta Derm. Venereol Stock.)* 80: 217-222.

Greenough, P. R. (1997). Lameness in Cattle, 3rd edition (ed. P. R. Greenough and A. D. Weaver). W.B. Saunders Company, pp.107-109.

Harris, I. R.; Farrell, A. M.; Grunfeld, C.; Hollaeran, W. M.; Elias, P. M. and Kempson, S. A. and Logue, D. N. (1993). *Veterinary Record* 132: 499-502.

Kempson, S. A. and Campbell, E. H. (1998). *Equine Vet. J., Suppl.* 26: 15-21.

Kloosterman, P. (1997). Lameness in Cattle, 3rd edition (ed. P. R. Greenough and A. D. Weaver). W.B. Saunders Company, pp.133-135.

Landmann, L. (1988). *Anatomy and Embryology* 178: 1-13.

Langridge, A.; Kempson, S. A. and Jones, J. A. (1998). Permeability of the bovine hoof horn. In: Proceedings of the 10th International Symposium on Lameness in Ruminants (ed. Ch. J. Lisher and P. Ossent), Licerne, Switzerland.

Logue, D. N.; Offer, J. E. and Kempson, S. A. (1993): Irish Veterinary Journal

Menon, G. K.; Feingold, K. R.; Moser, A. H.; Brown, B. E.; Elias, P. M. (1985): *J. Lipind Research* 26, 418-427.

Mgasa, M. N. (1987). *Bulletin of Animal Health and Production in Africa* 37: 227-230.

Mgasa, M. N. and Arnbjerg, J. (1993). Small Ruminant Research, 10: 55-62.

Michaels, A. S; Chandrasekaran, S. K. and Shaw, X. (1975). *Aiche J.* 21: 985-996.

Proskch, E.; Elias, P. M. and Feingold, K. R. (1990). *J. Clinical Investigation* 85: 874-882.

Squier, C. A. and Rooney, S. L. (1978). *J. Ultrastructural Research* 54: 2860291.

Squier, C. A. (1991). *Rev. Oral. Biol. Med.* 2: 13-32.

Webster, A. J. F. (1986). Understanding the Dairy Cow. BSP Professional Books, Oxford