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Histopathological Investigation of Skin and Hides Damage of Small and Large Ruminants due to Naturally Infested Ticks

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Abstract. Ticks are important ectoparasites which transmit many disease pathogen to animals; these are labelled tick borne diseases (TBD). Tick induced damage to skin and hides has not received attention. Skin and hides are important for the leather product industry, particularly in Pakistan. Due to economic importance and financial loss by ticks in leather industry, the present study was designed to investigate skin and hides damage due to ticks at microscopic level. Naturally tick infested tissue samples of hides and skin were collected from slaughter houses. Primary lesions at tick feeding sites showed epidermal edema with adjacent dermal edema. Histopathological examination revealed degeneration of epidermal layer down to the basal layer. Epidermal and sub dermal layers often displayed focal necrosis infiltrated with neutrophils and mononuclear cells at tick bite sites. Hyperplasia of keratinocytes was also seen at sites of ruptured epidermis. Quality of leather depends upon the grain (Outer) surface skin/hides. Ticks infestation damages the outer surface, due to bites, inflammatory responses, and secondary bacterial infections that often become established at feeding sites. Control of ticks should be given consideration to reduce infestation induced losses in the leather industry in Pakistan.

INTRODUCTION

Ticks belong to Arachnida class, and are divided into two families, *Ixodidae* and *Argasidae*. These are important vectors of viral, bacterial and protozoal diseases collectively known as tick-borne diseases (TBD) (Shemshad *et al*., 2012). Ticks attach firmly to the hosts and bite at the site of attachment for blood feeding. During feeding tick transmit TBDs to their host. TBDs include Babesiosis, theileriosis, Anaplasmosis, Q fever, Lyme disease and Louping ill (Jubb and Kennedy, 2016). Ticks are seen as an important vector of disease transmission but in literature little attention has been given to their role in skin and hide damage and their impact on the leather industry. Leather products are important throughout the world. In Pakistan, however, this industry occupies a central role; more than 800 tanneries employ more than 0.5 million people and contribute 6.56% of export and 6.15% manufacturing GDP (Chaudhary *et al*., 2011). In domestic ruminants most of the skin and hides damage is due to hard ticks (*Ixodidae*). Hard ticks pierce the hide and skin of its host by inserting its chelicerate with hypostome (Gashaw and Mersha, 2013). In Pakistan overall damaged skin and hides represented 33.88% of the total hide count; ticks damage is reported for 3.08% of hides sampled (Chaudhary *et al*., 2011). Skin damage is initiated after biting, leading to inflammatory responses, hypersensitivity, necrosis, collagen degeneration, hyperkeratosis, sloughing of epidermal layer, papules, wheals, irritation, wounds, subsequent ulceration and scar formation (Gbolagunte *et al*., 2009). These complication may lead to

secondary bacterial infection and fly myiasis. TBDs and tick bite result in poor quality of skin and hide (Minjauw and Mcleod, 2003). Heavy infestation of ticks on the flank and back region of hosts produce small pin point scars (Fentahun *et al*., 2012). Skin and hides are used in leather industry which is an important source of income. Damage by ticks to skin can result in significant reduction of income, or even losses in the leather agronomy (Sertse and Wossene, 2007). Tick bite scars in tanning industry appear as pin point pores after the leather bluing process that reduce the market price of leather (Chaudhary *et al.*, 2011). Due to the economic importance of leather industry, the objective of present study is to investigate the damage at microscopic level to confirm the damaging effects of ticks on skin and hides. Educating farmers and butchers as to the costs of tick infestations will help in adopting possible control and preventive measures.

MATERIALS AND METHODS

Sampling

Tissue samples of skin and hides infested with ticks were collected from slaughter houses of Lahore (31.5204° N, 74.3587° E). 25 naturally acquired tick infected cattle, 25 buffalo, 25 sheep and 25 goats were identified, tagged prior to slaughter; host skin tick bite sites were marked on host animals during tagging. After slaughter, tissue samples of skin and hide were cut from infected area of dewlap, axillary and perineal regions. Tissue samples were preserved in 10% buffered formalin. The labeled tissue samples were transported to histopathology lab of University of Veterinary and Animal Sciences, Lahore.

Paraffin embedding of skin and hide tissue samples

Tissue samples including skin and hide infected with ticks were fixed for 24 hour in 10% buffered formalin solution, dehydrated, embedded in paraffin wax and sectioned. After fixation with formalin, all samples were placed under running tap water for hydration purpose for 26h. Then samples were dehydrated with ascending concentration of alcohol. Alcohol from tissue samples were removed by xylene. Then samples were infiltrated with paraffin wax. After embedding, blocks of tissue samples were made, sectioned at 4µm size and mounted on glass slide. These glass slides were placed in oven at 56°C for 2h (Kingston *et al.*, 2007).

Staining of paraffin embedded tissues

Glass slides mounted with tissue sections were subjected to staining protocol. Before staining the paraffin wax was removed by xylene and xylene was removed by descending concentration of alcohol. Alcohol from tissues were removed by water. After washing all the slides were subjected to basic stain (hematoxylin) for 15 minutes. Stained slides were washed with water, acid alcohol and ammonia alcohol. After washing slides were counter stained with eosin for 3 minutes. Theses slides were washed with alcohol and xylene. In last step all the slide tissues were covered with coverslip. Then slides were observed under microscope preinstalled with camera and display monitor screen.

RESULTS

Gross examination of skin and hides was performed. Hides of cattle and buffalo showed mild swelling, red papule and erythema at tick bite sites. In some tissue samples vesicles and some ulceration was seen. After the pickling and bluing process, tick infested hides display marked ticks bite lesions. These lesions were small pin point pores in the hide, which can be seen in Figure 1. Hyperkeratosis and widening of intracellular spaces with degeneration of epidermal layer from basal lamina was seen in hide of infested bovine tissue samples. Tick feeding sites also showed damage from focal area necrosis, collagen degeneration, spatial disorientation in the grain and dermal layer infiltrated with neutrophils, basophils, eosinophils and mononuclear cells. Hyperplasia of keratinocytes was observed at side of ruptured epidermis. These histopathological changes can be seen in Figure 2. The primary lesion found in skin of both sheep and goats

Figure 1. Hide of bovine showing marked ticks bite lesions with small pin point pores after pickling and bluing process of leather tanning process.

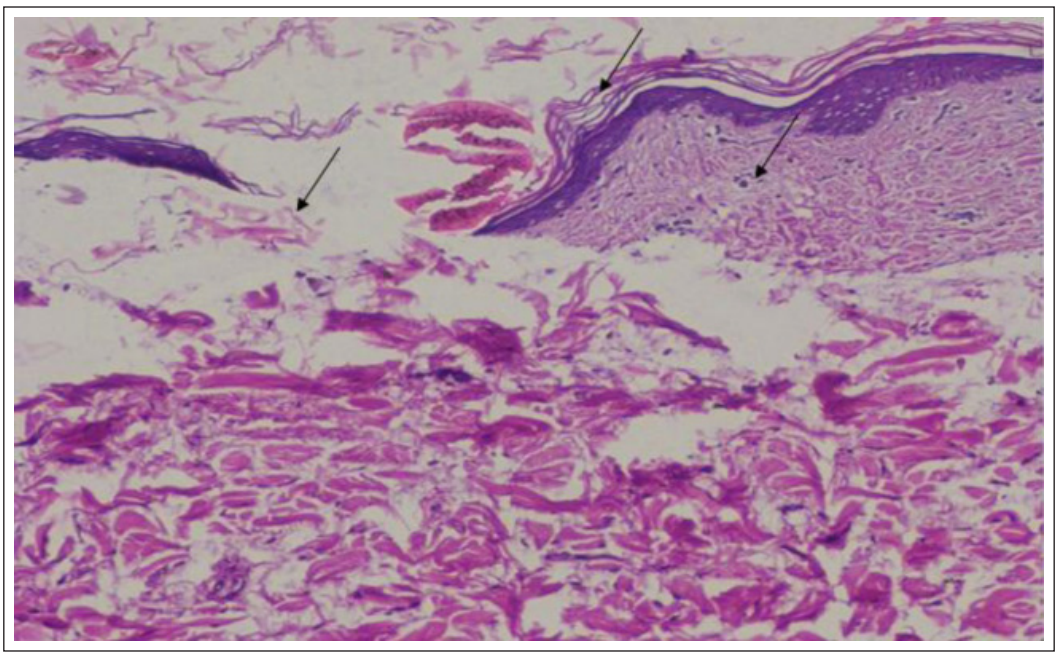

Figure 2. Tissue slide of bovine hide. Epidermal hyperkeratosis and degeneration from basal lamina is seen. Collagen degeneration, necrosis and presence of some mononuclear cells in the dermis are also seen.

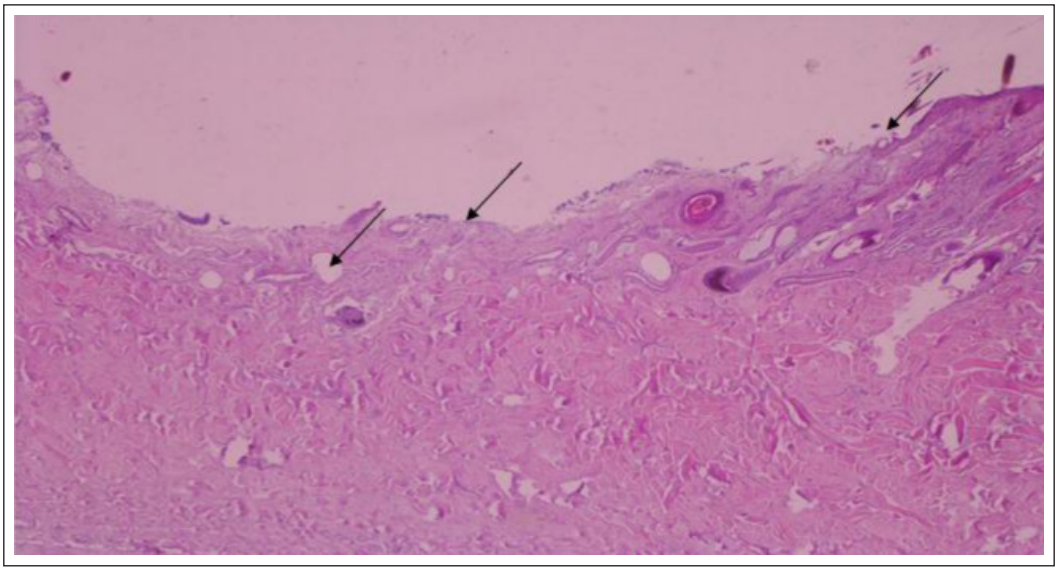

Figure 3. Tissue slide of caprine skin. Focal areas of adjacent sub epidermal edema is seen. In this area there was total sloughing of the epidermis up to stratum basale.

was focal epidermal edema with adjacent dermal edema/ sub epidermal edema. Sloughing of epidermal keratin layer was also observed. These lesions can be seen in Figure 3. Microscopic analysis of stained tick bite tissue sites revealed predominance of neutrophils over basophils, eosinophils and mononuclear cells. In stratum spinosum, marked spongiosis was observed having edematous fluid between keratinocytes with stretched desmosome accompanied by basophilic micro abscesses.

DISCUSSION

Gross lesions of papules and erythema develop due to the mouth part of tick which penetrates the epidermal layer and lodges in the dermal papillae. Mouth parts piercing the dermal blood vessels lead to hemorrhaging and collagen degeneration at the feeding site (Wall and Shearer, 2008). In present study microscopic infiltration of eosinophilic folliculitis and neutrophils was observed (Zajaz and Conboy, 2012). Tick bite lesions cause rupture and ulceration of skin which can become wounds, inviting blow or botfly infestation (myiasis). Myiasis is a frequent complication of tick infestation. Tick bite lesions resulting in wounds, providing a place for fly larvae which causes itching and irritation on skin, further inducing exudation and bacterial infection (Gashaw and Mersha, 2013). The warble fly is a chief cause of skin and hide damage in ruminants; fly infestation often follows formation of open ulcers at tick feeding sites (Wall and Hearer, 2008). Tick infestation causes damage to both skin and hide of small and large ruminants at both gross and microscopic levels, which impart economic losses to the leather industry in Pakistan (Chaudhry *et al.*, 2011). Tick bites induce localized tissue injury during feeding. Bite lesion may be vulnerable to secondary bacterial infection and fly myiasis, greatly reducing the value of skin and hide (Taylor *et al.*, 2007). The quality of leather also depends on health status of animals. Heavy infestation of ticks causes loss in live weight gain, poor production and decreases the quality of skin and hide (Kettle, 1995). Ticks not only transmit pathogens to animals but also causes skin and hide damage which ultimately leads to financial loss in livestock sector. The quality of skin and hides to large extent is related to its grain surface. The damage to skin may be due to ectoparasites, scratches on animals during handling at farm and transportation to abattoir, during flaying

process and inappropriate handling and preservation technique of skin and hides after slaughtering. Among ectoparasites, ticks are most important (Chaudhary *et al*., 2011). Several ectoparasites clades may cause integument damage but here our main focus is on ticks. Control of ticks and treatment of animals is imperative to compensate for losses due to damaged hide. Control of ticks is possible by use of acaricide spray available in market. Each animal requires acaricide treatment suspended in greasy medium to achieve long lasting effect (Rajput *et al.*, 2006). Acaricide include different chemicals like; organophosphate, carbamate, and pyrethroid. Pyrethroid is safer group of acaricide for the control of ticks (Mullen and Durden, 2002).

CONCLUSION

Tick play a significant role in skin and hide damage in small and large ruminates; tick infestations can thereby reduce or degrade the quality of animal host skin, which is used for leather products. Ticks bites are known to induce focal collagen degeneration, sloughing of epidermal layer and necrotic infiltration of neutrophils and basophils at the microscopic level. Grossly, ticks damage can be identified as pustules, crusts or scabs at the site of bite. These tick bite lesions, and the secondary complications that can occur at tick feeding sites can significantly degrade the quality of a host animal's skin; this in turn, may lead to serious losses for livestock owners involved in the leather agronomy. Hence, control of ticks should be given priority, in order to mitigate tick infestation induced losses in the domestic leather industry. Livestock farmers would certainly benefit from education concerning economic losses incurred by tick infestation in Pakistan.

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Conflict of interest

There is no conflict of authors in the publication of this paper.

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