EXPERIMENTAL LARVAL INFESTATION OF ZEBU CATTLE AND ITS CROSSES: EFFECT OF ANTI-HISTAMINE ALONE OR IN COMBINED WITH DEXAME-THASONE TREATMENT ON THE ENGORGEMENT AND OVIPOSITION ON TICK FEEDING LESION

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ABSTRACT: Four calves from each group of purebred Kedah kelantan (KK), halfbred KK X Friesian, and quarterbred KK X Friesian were experimentally infested with *Boophilus microplus* larvae. Two calves from each genotype were injected, intramuscularly with antihistamine while the remaining two animals in each genotype received the same dose of antihistamine and dexamethasone. Dexamethasone combined anti-histamine treatment suppress tick resistance as manifested by the production of higher number of engorged female ticks, higher mean weight of replete ticks, mean weight of eggs and mean number of larvae hatched from 1 g of eggs. In anti-histamine treated animals there was no reduction of resistant in all animals as manifested by a few ticks were able to feed successfully. At 1, 2, and 3 hours post-larval attachment in anti-histamine and dexamethasone treated cattle there was complete ablation of the cellular infiltration in the dermis beneath the tick mouthparts, especially eosinophil and basophils. There was little destruction of tissue. However, in anti-histamine t reated cattle there were more cellular infiltration and degranulation in the dermis. The cells infiltrating the dermis were mainly eosinophils followed by neutrophs, mast cells and basophils and some of these cells showed sign of degranulation. At 24 hours post-larval attachment, animals treated with anti-histamine and dexamethasone showed reduction of, cellular infiltration, degranulation, size of the epidermal lesion and tissue damage. The neutrophils were the predominant cells within the epidermal lesions. However, animals in anti-histamine treatment showed edema, more cellular infiltration and degranulation, and destruction of tissues as compared to antihistamine and dexamethasone treated animals. In anti-histamine treated cattle the epidermal lesions were obviously larger and the percentage of eosinophils and basophils were higher than those of antihistamine and dexamethasone treated animals. *(JUMMEC 2002; 2:135 - 141)*

KEYWORDS: Kedah-Kelantan cattle, KK X Friesian cattle, *B. microplus*, dexamethasone, antihistamine, cellular response.

Introduction

The Zebus and its crosses are relatively resistant to the cattle tick, *B. microplus*, as compared to pure European type cattle (1,2,3). Many previous studies have shown that the acquisition of resistance to ticks has an immunological basis (4,5,6)

Previous studies have shown that the granulocytes especially eosinophils and basophils may play a major role in the rejection of ticks in the early stages of infestation (7,8,9). Brown et al., (7) used highly specific antisera and demonstrate that basophils and to a lesser extent eosinophils, were responsible for the expression of resistance in guinea pigs infested with *A. americanum*. This finding strongly supported the effecter role of these cells in the expression of immunity against ticks. Resistance of tick infestation is a tissue response (1,10,11). The ability of certain breeds of cattle to resist infesta-

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tion by ticks was greatly influenced by the ability of the skin to mount a cellular response.

The role of histamine in the expression of host resistance to ticks has been suggested (12, 13, 14,15). Elevated histamine levels were found in blood (12) and skin (16) of cattle resistant to B. microplus. Histamine is a potent vasodilator, released at the site of injury (15,17). It is also increases permeability of the capillaries and thus serves to recruit blood cells to the area in response to injury, which may either be caused by trauma or invading organisms. It has been shown that histamine is chemotactic to eosinophils (18). The accumulation of eosinophils, among other cells, at the site of tick infestation may be a consequence of this effect (7). In most cases, the net effect is the manifestation of hypersensitivity reactions in the affected tissue (19)

The abrogation of acquired resistance to nematode infection in rodents and sheep by immunosuppressive glucocorticoids has been well documented (20,21). In sheep this was associated with a reduction in the numbers of circulating leucocytes (21) and in rats, with reduced number of intestinal intraepithelial mast cells and eosinophils (20). Both cortisone and corticotropin treatments reduced the number of globule leucocytes in the rat mucosal tissue (22). Corticosteroid drugs have been widely used in several host species to modify both natural resistance and acquired immunity to nematode parasites. By their use it is possible to achieve maturity of parasites in otherwise unsuitable hosts (23), to increase natural susceptibility to infection (24), to suppress the development of immunity during a primary infection and to prevent the action of an established immune response upon a challenge infection (25).

The objectives of this study were to determine the effect of antihistamine alone and in combination with dexamethasone on, the engorgement and oviposition of

B. microplus, the cellular infiltration at tick-attachment sites in pure local Zebu cattle and its European crosses.

Material and methods

Ticks

Twenty days old B. microplus larvae were used for infestation. The adult female ticks were those collected from the University Putra Malaysia dairy herd (Friesian-Sahiwal) and the engorged adults, eggs and the larvae maintained in an incubator 28 °C at approximately 80% relative humidity. Two susceptible cattle were regularly used for the engorged tick production

Experimental animals

A total of 12 six-month old healthy calves that have been grazed in B. microplus-infested pasture were used in the study. The calves included four purebred Kedah-Kelanatn (KK) cattle, four 50% KK and 50% Friesian, and four 25% KK and 75% Friesian cattle.

Infestation

The animals were infested and biopsies of tick feeding sites taken according to the technique described by Amin-Babjee and Riek (26). All twelve experimental steers were infested with 20,000 of B microplus larvae each on the same day before the first injection of dexamethasone and antihistamine.

For infestation, larvae were placed along the vertebral column of each animal. Small batches of larvae were placed within aluminum rings cemented (Selly's adhesive) to the previously shaved areas of the skin of the lumber region of each animal. The larvae were confined within the rings by fine-meshed cloth secured with rubber bands. Biopsies of skin within the rings were taken at 1, 2, 3, and 24 hours after infestation using a 0.05 mm trephine, under local anesthesia (2 ml of 2% xylocaine).

Two animals from each genotype were injected, intramuscularly with 200 mg (25 mg/ml) of tripelenamine hydrochloride (anti-histamine) daily for five days (Treatment 1) while the remaining two animals in each genotype received the same dose of the tripelenamine hydrochloride and 16 mg (2 mg/ml) dexamethasone intramuscularly daily for five days (Treatment 2).

Collection and weighing of engorged female ticks

The numbers and weight of engorged females from each animal were recorded daily and the mean numbers and weight of all ticks engorged on individual animal was calculated according to the technique described by Amin-Babjee and Riek (26).

Measurement of weight of eggs

The masses of egg produced by engorged ticks from each animal were mixed, weighed in the same manner as described by Amin-Babjee and Riek (26)

Measurement of larval production from 1 g of eggs

The counting of larvae was done two weeks after hatching (active larvae) according to the technique described by Amin-Babjee and Riek (26).

Process of skin biopsies with larval attachment for light microscopy study. Skin biopsies with attached larvae were taken from each animal at 1, 2, 3, and 24 hours after larval infestations. Processing the skin biopsies from fixation, trimming, dehydration, clearing, infiltration, embedding and staining were as described by Amin-Babjee and Riek, (26).

Analysis of the cellular response

Serial sections containing attachment sites (cement substance plus mouthparts of the larvae) with maximum cellular reactions were selected and analyzed for cellular response. Eosinophils, neutrophils, basophils mononuclear cells and mast cells were counted using an oil immersion (1000x) objective in 0.15 mm^2 areas, covering the center of the lesion and areas adjacent on both sides. Analyses were performed on three different feeding sites for each animal. The results were expressed as the mean cell count.

Results

Tick biological parameters

Effect of antihistamine alone and combination of antihistamins and dexamethasone on the engorgement and oviposition of

B. microplus larvae in local Zebu and its crosses.

The mean numbers of engorged female ticks, their mean weights, the mean egg masses produced by each engorged female tick and the mean numbers of larvae produced per gram of eggs for each breed in antihistamine alone and in combined antihistamine and dexamethasone treatment groups are summarized in Table (1)

In general, the mean numbers of replete female ticks, mean weight of replete ticks, mean weight of egg masses and the mean numbers of larvae hatched from one gram eggs laid by replete female ticks dropped from antihistamine and dexamethasone treated breed were higher than those dropped from antihistamine alone.

Histology

Effect of antihistamine alone and combined antihistamine and dexamethasone on the cutaneous cellular responses at 1, 2, 3 and 24 hours post larval attachment sites in local Zebu and its crosses.

The temporal changes in cellular responses at tick attachment sites for the two treatments and three genotypes at 1, 2, 3, and 24 hours after larval infestation are summarized Table (2). Antihistamine and dexamethasone treatment completely ablated or reduced the cellular infiltration at tick attachment sites in all genotypes compare with antihistamine alone. Antihistamine treatment had little or no effect on the cellular infiltration.

Overall, various cell-type at tick-feeding lesions were higher in antihistamine than antihistamine and dexamethasone treatment in all genotypes (Plate 1 and 2; Table 2).

Table 1. Effect of Anti-Histamine Alone or Combined with Dexamethasone Treatment on the Engorgement and Oviposition Rates of *B. microplus* Following Experimental Infestation in Cattle

Genotype	Treatment	No. Engorg Tick	Wt. Engorg. Tick (mg)	Wt. egg/tick (mg)	No, Hatch Larvae/g
KKKK	1	3	136	73	8742
	2	28	139	74	9465
KKFF	1	78	152	77	9151
	2	265	156	79	10945
KFFF	1	172	155	77	10158
	2	435	157	80	10687

Treatment 1 = Antihistamine treatment;
Treatment 2 = Antihistamine and dexamethasone treatment
KKKK = Purebred Kedah-Kelantan (KK) cattle
KKFF = Crossbred (50% KK and 50% Friesian)
KFFF = Crossbred (25% KK and 75% Friesian)
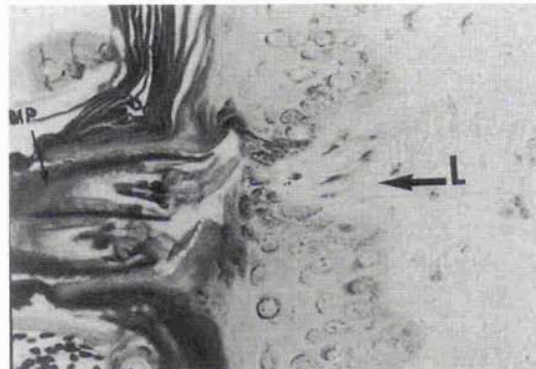For each treatment, the values are the average of two animals

Plate 1. Feeding Sites of *Boophilus microplus* Lavae (3 h Postinfection) in Anti Histamine and Dexamethasone Treated Zebu (KKKK) Cattle. The dermis is virtually ablated. (Stain with Alkaline Giemsa). As = Attachment Site; L- Few Cellular Infiltration (Primary Magnification x 40)
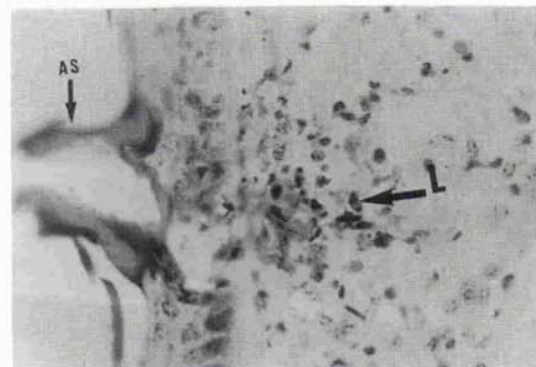

Plate 2. Feeding sites of *Boophilus miroplus* Larvae (3 h postinfestation) in Anti-histamine treated Zebu (KKKK) Cattle. Note. The moderate infiltration of celles within the dermis. (stained with Alkaline Giemsa) As = Attachment site; L=Lesion (Primary Magnification x40)

In antihistamine treatment the number of eosinophils in purebred at 3, and 24 hours post-larval attachment were higher than the crossbred, while the number of basophils at 24 hours post-larval feeding sites were higher than the crossbred.

Discussion

The results of the current study showed that after an experimental infestation with 20,000 larvae of *B. microplus*, the mean numbers of replete female ticks, mean weight of replete ticks, mean weight of egg masses and the mean numbers of larvae hatched from one gram eggs laid by replete female ticks dropped from antihistamine and dexamethasone treated breed were higher than those dropped from antihistamine alone. Also the results of the present study indicated that the mean numbers of eosinophils and basophils infiltrating tick feeding sites were higher in antihistamine treated

than antihistamine and dexamethasone treated cattle at 1,2,3,and 24 hours post –larval attachment in all breeds. Results indicated that the injection of antihistamine did not affect the resistance level and the cellular infiltration in all three genotypes of cattle. It is possible that the intramuscular injection of antihistamine used in this study had no effect/influence on the migration and degranulation of eosinophils and basophils at attachment sites. However, several previous authors have reported that the use of antihistamine affects the expression of cattle resistance to ticks (14,16).

Antihistamine has some effect on suppression of resistance level in cattle. Riek (12) reported elevated levels of histamine in the blood of cattle resistant to *B. microplus* and stated that resistant hosts reacted more than susceptible hosts to intradermal injection of histamine. Tatchell and Bennett (27) stated that a significant increase occurred in the yield of engorged *B. microplus*

Table 2. Effect of anti-histamine alone or in combined with dexamethasone treatment on cutaneous cellular responses following experimental infestation with *B. microplus* larvae in cattle.

Genotype	Treatment	Hour	Mast cells	Eosinophils	Neutrophils	Mononuclear cells	Basophils
KKKKK	1	1	5.5	4.7	2.7	1.6	0.0
		2	6.5	8.8	5.8	1.5	2.5
		3	7.8	13.3	10.8	3.2	4.6
		24	0.0	9.3	274.3	3.3	10.4
	2	1	4.3	0.0	1.3	0.5	0.0
		2	4.5	0.0	2.7	0.8	0.0
		3	3.0	0.5	3.3	1.3	0.2
		24	0.0	2.0	235.0	3.3	2.2
KKKFF	1	1	7.8	3.2	3.2	2.3	0.0
		2	7.7	6.2	5.3	2.8	0.2
		3	6.8	7.7	8.3	2.0	4.5
		24	0.0	5.4	233.5	2.5	6.1
	2	1	4.3	0.0	0.5	1.7	0.0
		2	4.8	0.0	2.0	1.3	0.0
		3	4.8	0.0	2.2	2.2	0.0
		24	0.0	0.0	187.2	2.5	0.0
KFFF	1	1	6.7	3.5	2.2	1.8	0.0
		2	5.8	6.5	4.2	2.7	0.5
		3	7.5	6.2	6.7	2.2	1.3
		24	0.0	6.7	195.7	2.7	5.3
	2	1	4.8	0.0	0.2	0.5	0.0
		2	4.7	0.0	2.7	2.3	0.0
		3	3.2	0.0	3.3	1.8	0.0
		24	0.0	0.0	169.7	3.2	0.0

Treatment 1 Antihistamine; Treatment 2 Antihistamine and Dexamethasone; For each treatment, the values are the average of two animals.

from eight out of twelve cattle (*Bos taurus* and *Bos indicus*) treated with antihistamine promethazine hydrochloride. Histamine is released by mast cells in immediate hypersensitivity reactions in bovine (28) and its presence beneath the attachment sites of young larvae causes them to be detached (14). *Bos taurus* cattle with different degrees of resistance to *B. microplus* have responses to tick allergen that correlate with their resistance level. The total amount of histamine in the skin also correlates with both resistance and the immediate hypersensitivity reactions. Treatment with anti-histamine drug mepyramine maleate suppresses the cutaneous hypersensitivity reactions. The results suggest the main pharmacologically active agent in these reactions is histamine, and that the total amount of it available locally in the skin may have a role in the resistance to this parasite (16). Circumstantial evidence suggests that the earlier detachment of *B. microplus* larvae from highly resistant *Bos taurus* cattle follows the release of histamine at attachment sites. *In vivo* and *in vitro* experiments show that a proportion of the larvae will detach following injection or infusion of histamine. Other mediators such as bradykinin, prostaglandin E2, 5-hydroxytryptamine and dopamine have little or no effect on tick behavior *in vivo*. Sensitivity to histamine declines as larval attachments stabilize, and repeated injections have no effect on the weight of the larvae after 3 days on the host (14).

Several authors have also suggested a role for histamine in the expression of resistance of laboratory animals (15, 17). Bagnall (13) showed that type-1 histamine receptor antagonist, mepyramine, reduced the expression of tick resistance and obtained a slight decrease in the rejection of ticks when administered to guinea pigs resistant to *I. holocyclus* larvae. Allen (29) demonstrated that the host skin reaction to tick-resistant guinea pigs was characterized by an increase infiltration of basophils. The accumulation of basophils provided a localized high concentration of histamine. The histamine contained in the basophil granules can be released either by antibody dependent (30) or lymphokine mediated (31) immunologica reactions, or by combination of both. Histamine at tick attachment sites may play a major role in the rejection of ticks. Basophils and mast cell granules are rich source of histamine (30). Thus the net consequence of these cellular interactions at tick feeding sites results in increased levels of histamine at tick feeding sites. Because of the chemical nature of histamine it could inhibit digestion or some other physiological process by possible altering enzyme structure or reactivity.

In other tick-host relationships, where infiltrating basophil leucocytes are more numerous in tick lesion (13,32) histamine may play a more important role in the rejection. Histamine has been shown to have a direct effect on tick attachment and feeding (14,15,17). Probably the most important action of histamine is in causing irritation, leading to removal of ticks by self-grooming in highly resistant cattle (33). If histamine is responsible for repeated detachment of larvae during the first day of infestation, this could facilitate their removal by the host (34).

In this experiment, it was shown that only dexamethasone and not antihistamine, caused cellular ablation at tick attachment sites and this correlated with the suppression of tick resistance by the increase in numbers of ticks produced in the treated animals, this is consistence with the result of Eckblad et al (35) and Amin-Babjee et al., (36). Amin-Babjee et al (36) using European breeds cattle showed that dexamethasone, a potent glucocorticoid, when administered in a therapeutic dose induced immunosuppression, as shown by increase in the number of ticks, reduction in immediate hypersensitivity reactions, and obvious reduction or complete ablation of the cellular infiltration at the feeding sites of the tick larvae. In cattle, the administration of the glucocorticoid, a-fluoroprednisolone or dexamethasone is known to have a variety of potential effects: induction of lymphopenia (37), suppression of lymphocyte blastogenesis (38) and impaired of the functions of neutrophils (39).

Several workers have used immunosuppression drugs to study their effect on other host/parasite relationship. Rats infected with *S. ratti* and treated with corticosteroids do not expel their own burdens (20). Cortosone, as an anti-inflammatory agent, is known to affect the level of both eosinophils (40) and mast cells (41). Treatment of resistant sheep with immunosuppressive drug dexamethasone abolished resistance to challenge infections with *T. colubriformis* and was associated with both the depletion of globule leucocytes, mast cells and eosinophils from the small intestinal mucosa, as well as, a reduction in mucus larval migration inhibitory activity (42).

The present results showed that eosinophils and basophils are important cellular responses at feeding sites of larval stage and this response has contributed to resistance in the anti-histamine treated groups in all genotypes. Anti-histamine has no effect on the cellular infiltration. Both these cells were depleted at 3 and 24 hours after infestation in animal treated with anti-histamine and dexamethasone.

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