



REVIEW ARTICLE

Meibomian Gland Demodicosis in Cattle: The Clinical Disease and Diagnosis

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ABSTRACT

Bovine demodicosis was surveyed in five States of the Sudan during vaccination campaigns and in abattoirs. Among the total number of cattle surveyed (48,000), 16,608 cattle had skin lesions of demodectic mange. Among those infected, 8,012 cattle (48.2%) had simultaneous skin and meibomian gland demodicosis. Meibomian gland demodicosis was characterized by lacrimation, hyperaemia and congestion of the mucous membranes, and in extreme cases, by purulent exudation, swelling and closure of the eyelids. Both eyelids showed 2-4 purulent nodules of 3-4 mm in diameter each, arranged in a linear fashion. *Demodex ghanensis* and primary pathogenic bacteria (*Moraxella bovis* and *Staphylococcus aureus*) were isolated from the infected material expressed from meibomian gland lesions. The histopathological changes seen were compatible with cell-mediated immunity. The destruction caused by the mites and associated bacteria resulted in typical granulomatous reaction. The central core of infection in the glandular acini composed of mites, bacteria and purulent exudate was infiltrated by neutrophils and a few eosinophils; surrounded by lymphocytes, plasma cells, macrophages, epithelioid, giant cells and proliferation of connective tissue.

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INTRODUCTION

Meibomian glands are found deep in the eyelids. They are independent of the hair follicle (Getty, 1975; Dellman and Eurell, 1998; Yuksel *et al.*, 2005; Cheriyan *et al.*, 2011; Rezaie *et al.*, 2012; Aydoğan *et al.*, 2012; Choi *et al.*, 2013; Ngo *et al.*, 2013). They assist in lubricating the cornea and the margins of the eyelids. The meibomian gland is composed of connective tissue of dense collagenous fibers. They are branched tubular cords. Each gland opens by a separate duct. There are a number of sebaceous nodules around each duct. The ducts are composed of stratified squamous epithelium, open collectively into a collecting tubule at the margins of the eyelids and are surrounded by stratified muscles.

Meibomian glands demodicosis received the attention of only a few workers (Kirkwood and Kendall, 1966; Baker and Fisher, 1969; Oppong, 1970; Baker, 1973; Oppong *et al.*, 1975; Himonas *et al.*, 1975; Rak and Rhagozar, 1975; Nutting *et al.*, 1975; Gearhart *et al.*, 1981; Bukva, 1986; Fantahun *et al.*, 2012). However, in Ethiopia meibomian glands and eye infection with

demodectic mange in cattle were associated with skin lesions of the disease and were never observed in cattle without skin lesions (Fantahun *et al.*, 2012). In the same study the authors found 112 out of 384 cattle with different degrees of swelling of the eyelids and 49 with lacrimation, in addition to hyperaemic and congested mucous membranes. Kirkwood and Kendall (1966) reported that the eyelids of cattle appeared normal but in histological sections, *Demodex* mites were seen in the collecting tubules of the meibomian glands with their gnathosoma directed towards the sebaceous gland. Nutting *et al.* (1975) reported that the eyelids of cattle showed 2-6 small demodectic mange lesions of 2-4 mm in diameter and 0.5 cm from the margin of the eyelids and aligned parallel to them. Esuruoso (1977) and Fantahun *et al.* (2012) reported that the infested eyelids of cattle became thickened and might result in blindness of the animal due to their physical closure. Gearhart *et al.* (1981) and Fantahun *et al.* (2012) described a bilateral palpebral demodicosis in which the lesions were in the form of firm swellings in both eyelids. The histopathological changes comprised dermal and subcutaneous necrosis and a

pyogranulomatous inflammatory reaction in the subcutis and cavitation of the centre of the lesion (Gearhart *et al.* 1981). The whole area became surrounded by a thick zone of epithelioid cells, numerous eosinophils, and a few neutrophils and foreign body giant cells (Fantahun *et al.* 2012).

In the Sudan many studies focused on investigating infectious diseases, their prevalences, distribution and other aspects, while bovine demodicosis in spite of its significance, was completely neglected, and reports on the disease were not encountered at any length (Annual Reports of the Sudan Veterinary Services, 1922- 1974) and thereafter. Research on the clinical, epidemiological and economic aspects of bovine demodicosis seems to be very essential for upgrading the general health of cattle in the Sudan and to improve the quality of the hides. The objective of this study is to look into one aspect of this disease; the clinical picture and diagnosis of meibomian gland demodicosis.

MATERIALS AND METHODS

Survey

In the Sudan States of Northern, Southern and Western Kordofan, East Equatoria and Bahr El-Jebel, 48,000 cattle were surveyed for demodectic mange during routine vaccination campaigns and in abattoirs. Animals with skin lesions and eye infection suggestive of demodectic mange (16,608) were clinically inspected. The distribution of skin lesions over the animals' bodies was recorded and tabulated. Also the clinical picture of meibomian gland lesions was described.

The eyes and eyelids of infected and control non-infected cattle were carefully examined using a magnifying lens. Purulent infected material was expressed from the lesions using sterile techniques. Each sample of infected material was divided in two parts. The first part of each specimen was kept in sterile Bijou bottles and refrigerated for bacteriological investigations and second part containing equal volumes of glycerol and ethanol for parasitological investigations. Two sets of impression smears and swabs were also collected from the eyes of non-infected cattle and refrigerated.

After being slaughtered, the eyelids from 25 cattle with eye infection and from 10 non-infected ones were excised and removed. The biopsy and necropsy specimens were fixed in 10 percent formal saline.

Laboratory Investigations

Parasitological examination

A small piece from each specimen of the infected purulent material was crushed between two microscope slides. Another piece from each specimen was placed in the middle of a microscope slide, a drop of 20 percent potassium hydroxide was added and the preparations were gently heated and covered with coverslips. The two preparations were examined under the microscope Abu-Samra *et al.* (1984).

Bacteriological Investigations

Two milliliters of sterile nutrient broth were added to the refrigerated infected material in each bottle. The contents of the bottles were thoroughly mixed using a

mechanical shaker. The procedures adopted for the preparation of culture media and media for biochemical tests were according to standard methods and techniques (Barrow and Feltham, 1993).

Each of the specimens was cultured under aerobic, anaerobic and increased carbon dioxide conditions at 37°C for 24-48 hours on the following media: nutrient agar (Oxoid, 1998) 5 percent sheep, bovine or horse blood enriched agar prepared from blood agar, McConkey's agar and nutrient broth (Oxoid). Moreover, one set of the seeded blood enriched agar was incubated at 33 °C in a humid chamber. Pure cultures were obtained through serial subcultures. The pure isolates were biochemically tested.

Histopathological Investigations

The necropsy specimens were processed, embedded in paraffin wax and sectioned at 5 µm prior to staining with haematoxylin and eosin stain and examined as indicated by Bancroft and Harry (1994).

RESULTS

Among the total number (48,000) of animals surveyed during vaccination campaigns and examined in abattoirs; 16,608 had skin lesions. Obvious clinical lesions suggestive of demodectic mange were observed among 14,562 (87.7%) of the infected animals, while the remaining 2,046 cattle (12.3%) had subclinical lesions that were detected after careful palpation of the skin. Simultaneous skin and meibomian gland demodicosis was encountered in 8,012 of the infected animals (48.2%). None of the infected cattle had only gland demodicosis. The predilection sites of the lesions were the neck, withers, shoulders and forequarters (Table 1). As the disease progressed, the lesions spread until the whole body became involved.

Five forms of skin lesions were recognized. They were papules, nodules and papules, nodules and few pustules, pustules and few nodules (Fig. 1) or pustules and crust-covered lesions. The lesions were confined to certain parts of the body or were generalized involving the whole body (Table 1).

Among the 300 cattle, 218 animals had simultaneous skin and meibomian gland lesions (72.7%). The remaining 82 (27.3%) cattle had skin but no eye infection, and their eyes were free of any clinically detectable abnormality.

Meibomian gland demodicosis was characterized by swelling of the eyelids, lacrimation, hyperaemia and congestion of the conjunctival mucous membranes, and in extreme cases by purulent exudation, swelling and closure of the eyelids (Fig. 2). The infected animals showed bilateral palpebral demodicosis. Each eyelid showed 2-4 purulent nodules of 3-4 mm in diameter arranged in a linear fashion (Fig. 3). Inspection of the eyes was much resented by the animals. In the majority of animals, the lower eyelids were more affected and disfigured than the upper ones. Most of the animals with eye infection preferred shaded areas, had pruritus and were rubbing, gnawing and scratching their body against trees and objects, and their heads against their body.

The macroscopic appearance of the eyes of normal non-infected cattle, showed no detectable abnormality and

Table 1: Distribution of demodectic mange lesions over the animals' bodies in 16,608 cattle*

Lesions	Number of animals	Location of lesions										Degree of infection
		Head	Neck●	Brisket	Axillae	Withers	Shoulders	Forelegs□	Sides	Hind legs◆	Generalized	
Papules	1230	-	+	+	+	-	+	-	+	-	-	Moderate
	816	-	-	+	+	-	-	-	-	-	-	Light
Nodules and Papules	4130	+	+	+	+	+	+	+	+	+	+	Severe
	4023	-	+	+	+	+	+	-	+	+	+	Moderate
Nodules and few pustules	1070	+	+	+	+	-	+	-	-	-	-	Light
	1748	+	+	+	+	+	+	+	+	+	+	Severe
Pustules and few nodules	805	-	+	+	+	+	+	-	+	-	+	Moderate
	460	+	+	+	-	-	-	-	-	-	-	Light
Pustules and crust-covered Lesions	412	+	+	+	+	+	+	+	+	+	+	Severe
	418	+	+	-	-	-	+	-	+	+	-	Moderate
Pustules and crust-covered Lesions	372	+	+	-	-	-	-	-	-	-	-	Light
	334	+	+	+	+	+	-	+	+	+	+	Severe
Lesions	422	+	+	-	-	-	-	-	+	-	-	Moderate
	332	+	+	-	-	+	-	-	-	-	-	Light

* 8012 of which had also meibomian gland lesions; □ To knee joint; ● Including dewlap; ◆ To hock joint



Fig. 1: Simultaneous skin and meibomian gland demodicosis in a bull. Note swelling of the eyelids and nodules and pustules especially on the head, neck, dewlap and shoulder.



Fig. 2: Skin and meibomian gland demodicosis in a cow and calf. Note scattered nodules on the skin of both cow and calf, swelling of the eyelids of the cow, and exudation of the eye and swelling and closure of the eyelids of the calf.



Fig. 3: Simultaneous skin and meibomian gland demodicosis. Note swelling of the eyelids and nodules on the upper eyelid arranged in a linear fashion.

examination of the eyelids using a magnifying lens revealed tiny clear openings of the meibomian glands arranged in a linear fashion at the margins of the eyelids (Fig. 4).

Demodex bovis was isolated from purulent material expelled from skin lesions (Fig. 5). Examination of the crushed infected material from meibomian gland lesions showed numerous different developmental stages of *Demodex mites*, pus and breakdown products of the lacrimal apparatus. The mites were isolated and identified as *Demodex ghanensis* mites (Fig. 6).

Culture of the 218 specimens of infected material from meibomian gland lesions revealed growth of organisms from 128 (58.7%) specimens, and no growth was obtained from the remaining 90 specimens (41.3%). *Moraxella bovis* was isolated from 102 specimens, and mixed *Moraxella bovis* and *Staphylococcus aureus* were isolated from 26 specimens.

Gram's stained impression smears from the eyes of non-infected cattle showed insignificant numbers of microorganisms, and no growth was obtained from swab cultures.

Histopathological examination of the eyelids of cattle with meibomian gland lesions showed blepharitis and severe pathological changes compared by non-infected cattle. *Demodex ghanensis* invaded the meibomian glands through the orifices of the main collecting tubules. A few mites were seen migrating towards the ducts of the gland, causing slight dilatation of the main collecting tubules (Fig. 7). There was infiltration of lymphocytes and eosinophils outside. The mites caused severe irritation by their movement, invasion of the ducts and continuous feeding on the glandular tissue. Many glandular acini showed parts or remnants of the mite, associated bacteria and cell debris amongst a homogenous mass of exudates and degenerate leucocytes, and were surrounded by dense connective tissue (Fig. 8). There was atrophy of the epithelial lining and marked dilatation of the main collecting tubule and ducts of the meibomian glands. Damage of the wall of the main collecting tubule and/ or ducts of the gland, and the seeping-out of their contents in the surrounding connective tissue, caused haemorrhage, infiltration by eosinophils and neutrophils and evolved 'high-turnover' granulomas with influx of macrophages and lymphocytes (Fig. 9). In some sections, there was

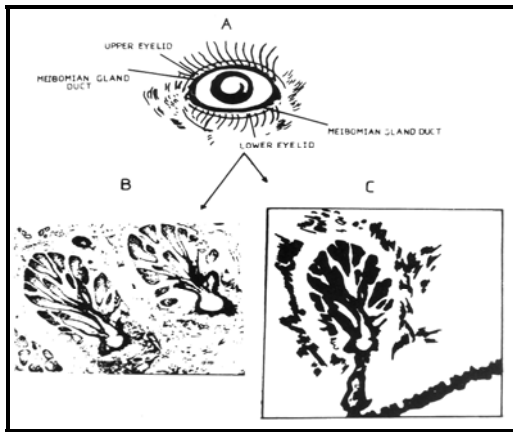


Fig. 4: Illustrated diagram of the macroscopic structure of the normal bovine eye (A), histological (B) and diagrammatic (C) appearance of the glandular tissue and collecting ducts of the meibomian gland.

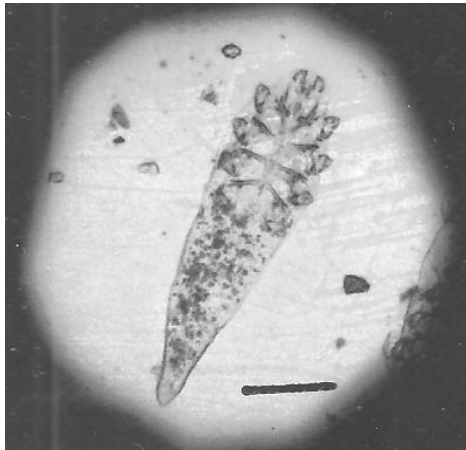


Fig. 5: *Demodex bovis* mite isolated from infected material expressed from skin lesions of demodectic mange in a bull. Scale bar: 60 μ m.

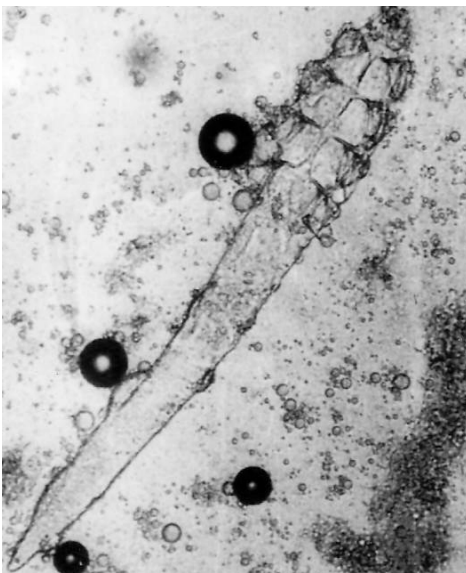


Fig. 6: *Demodex ghanensis* mite in infected material expressed from meibomian gland lesions of demodectic mange in a cow. 20% potassium hydroxide solution. Scale bar : 50 μ m.

abscessation and cavitation of the central core of the glandular tissue (Fig. 10). The degenerated mites and associated bacteria were engulfed and digested by the giant cells, resulting in healing of the lesions as judged by the progressive proliferation of connective tissue and degeneration of the granulomatous reaction in different areas of the same section or in different sections.

DISCUSSION

In spite of the prevalence of bovine demodicosis in the Sudan it was interesting to report that no mention of the disease was encountered in the Annual Reports of the Sudan Veterinary Services (1922-1974), and no explanation could be given to disease. However, the first authentic report was made by Abu-Samra (1974). The severe simultaneous skin and meibomian gland lesions that were encountered in 8,012 cattle (48.2 %) were probably the first report in the available literature. In Ethiopia the percentage of animals infected with meibomian gland and eye demodicosis was low (Yacob *et al.*, 2008a, 2008b; Fantahun *et al.*, 2012). However, Matthes and Bukva (1993) reported a percentage of 94% in Mongolia which was higher than the percentage observed in the present study. Moreover, the high incidence of meibomian gland demodicosis proved that the gland was quite susceptible to infection with *D. ghanensis* as it was the only mite isolated from the purulent infected material expressed from the lesions.

The isolation of only *D. bovis* from skin lesions and only *D. ghanensis* from meibomian gland lesions of the same animals was of interest, different from all previously published work, and was subject to speculation. The most probable explanation to this finding was that each species of mite is morphologically adapted to suit the confines of its habitat, and possessed distinct anatomical structures that enabled them to pave their way through their habitat and become well established and reproduce. This finding was contrary to the findings of Oppong (1970) and Slingenbergh *et al.* (1980) who isolated *D. ghanensis*, *D. bovis* and a demodicid shorter than *D. bovis* from the meibomian glands of cattle and Baker (1973) who reported the existence of both *D. ghanensis* and *D. bovis* in the meibomian glands of cattle.

The bacteria involved in demodectic mange lesions were critically investigated and identified for the first time. *Moraxella bovis* and *Staphylococcus aureus* are primary pathogens which caused allergic reactions and produced toxins and enzymes which aggravated the lesions produced by *D. ghanensis* mites, resulting in severe damage of the glands. However, these organisms could not be demonstrated in impression smears or isolated in swab cultures from the eyes of non-infected cattle. This proved that the two organisms did not exist as natural inhabitants of the eyes of non-infected cattle. *Moraxella bovis* might have been acquired from the animals' surroundings being contaminated by ocular discharges from cattle infected with infectious keratitis, while *Staphylococcus aureus* might have been acquired from the skin when the animals scratched or rubbed their irritated eyes against their bodies. *Moraxella bovis* was reported to be of high morbidity (80%) reaching epizootic

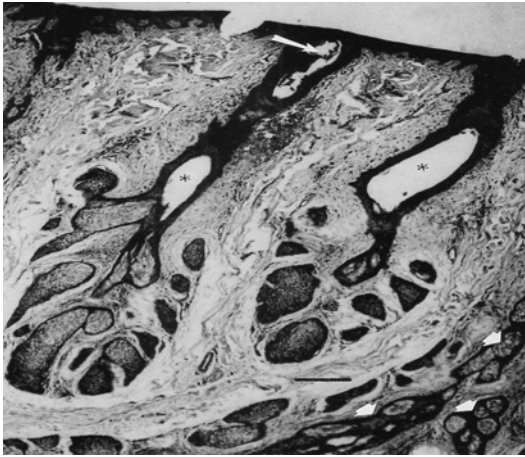


Fig. 7: Sections from the eyelid of a cow, showing meibomian glands infected with demodectic mange. Note dilatation of the main collecting tubules (asterisks) and *Demodex ghanensis* mites in the main collecting tubules, ducts and glandular acini (arrows and arrow heads). Haematoxylin and eosin. Scale bar: 120 μ m.



Fig. 8: Section from an infected meibomian gland, showing *D. ghanensis* mites (asterisks) in the glandular acini, exudate (EX), hemorrhage, cellular infiltration, granulomatous reaction surrounded by dense fibrous tissue proliferation (arrows). Haematoxylin and eosin. Scale bar: 200 μ m.

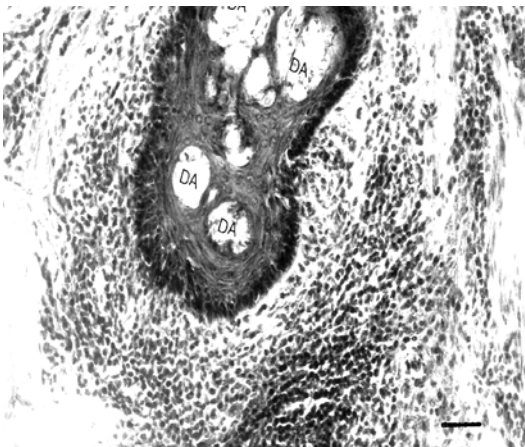


Fig. 9: Meibomian gland demodicosis showing a typical granuloma. Note damaged acini (DA) surrounded by reparative and degenerative dominant connective tissue proliferation, giant and epithelioid cells in the inner layers and macrophages, lymphocytes plasma cells and eosinophils in the outer layers. Haematoxylin and eosin. Scale bar : 100 μ m.

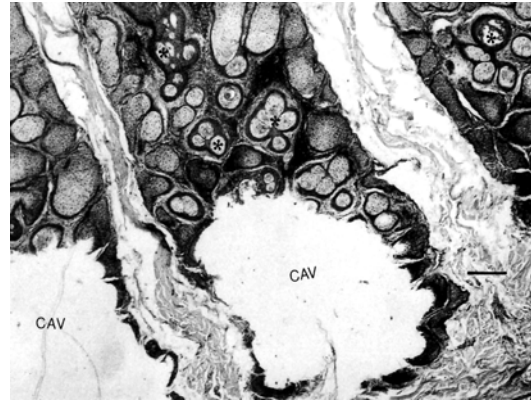


Fig. 10: Section from the eyelid of a bull infected with demodectic mange, showing cavitation of the central core of the meibomian gland (CAV) and damaged acini, hemorrhage, marked cellular infiltration, multiple granulomas (asterisks), and connective tissue proliferation. Haematoxylin and eosin. Scale bar: 120 μ m.

proportions when transmission agents (*Musca autumnalis* flies, dust and long grass contaminated by ocular discharges from infected cattle) become available (Radostits *et al.* 2007).

Failure to isolate bacteria from 90 specimens (41.3%) of purulent material expressed from meibomian gland lesions was probably due to the destruction of the bacteria by the reparative and degenerative reaction of the high turn-over granulomatous reaction (humoral and cellular responses). The isolation of the mites (*D. ghanensis*) from these specimens was probably due to their possession of a chitinous wall making them resilient and resistant and would take a longer time to be destroyed, engulfed and digested by the macrophages.

The current work proved that demodectic mange is a serious health problem in cattle. The deleterious effects of the disease can be summarized in the following: (1) the lesions produced were suppurative, (2) cattle with hide and meibomian glands demodicosis had severe pruritus that was alleviated by continuous scratching, rubbing, licking and gnawing at the affected areas, resulting in great loss of grazing time and (3) animals with severe eye infection were sensitive to bright sunlight and showed partial or complete closure of the eyelids which prevented them from proper grazing and seeking good pasture. The contributory effect of those factors would result in poor health, increased susceptibility to infection by other pathogens and would seriously affect production. Those findings authenticated the reports of Coles (1967) that most chronic infections had associated normocytic normochromic anaemia; Radostits *et al.* (2007) reported that chronic suppurative processes could cause normocytic normochromic anaemia by depressing erythropoiesis; Yeoman (1966) and Esuruoso (1977) reported that demodectic mange caused emaciation and resulted in 15 percent mortality rates among Zebu cattle.

The mites caused severe irritation by their movement and continuous gnawing and feeding on the glandular acini. Their secretions, excretions and somatic debris might have caused allergic and/ or immunologic responses. The lesions were aggravated by actively or passively introduced primary pathogenic *Moraxella bovis*

and *Staphylococcus aureus*. Those bacteria caused allergic reactions and produced toxins and enzymes which exacerbated the lesions causing severe pruritus resulting in scratching, rubbing, licking and gnawing at the affected areas. This produced more inflammation, wounds and damage of the meibomian glands (Brown *et al.*, 1998; Harriet, *et al.*; Hess and Angelos, 2006).

The damage and seepage-out of the contents of the main collecting tubules and ducts of the meibomian glands in the surrounding connective tissue of the eyelids resulted in severe histopathological changes which were highly compatible with cell-mediated immunity. This was in support of Ruffli and Mumcuoglu (1981) who reported that on the basis of histopathological investigations, an immunological response to the parasite seemed to be implied. Partial or complete damage of the wall of the main collecting tubules or ducts of the meibomian glands and liberation of their contents in the surrounding connective tissue resulted in severe histopathological changes characterized by massive 'high-turnover' granulomatous reaction with influx of macrophages and lymphocytes. This proved that *Demodex* mites and associated bacteria were both persistent and immunogenic; producing the severe and progressive disease that was encountered in natural field cases. This finding was in agreement with Thomson (1978) and Dick *et al.* (1983) who reported that when the inflammatory agent was both persistent and antigenic, a 'high-turnover' granuloma evolved with influx of macrophages and lymphocytes.

Conclusion

The above findings led to the conclusion that meibomian gland demodicosis is quite prevalent in cattle in the surveyed states of the Sudan. The lesions of meibomian gland demodicosis produced by *Demodex ghanensis* are aggravated by the primary pathogenic *Moraxella bovis* and *Staphylococcus aureus*. Moreover, the results obtained in the current investigation proved that demodectic mange is a serious health problem in cattle which may result in serious economic losses. The pathological changes produced by the disease proved that *Demodex ghanensis* mites and associated bacteria were both persistent and immunogenic; producing the severe and progressive disease that was encountered in natural field cases. Further researches to investigate meibomian gland demodicosis are warranted.

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