An Unusual Widespread Outbreak of Blindness Caused by *Mycoplasma conjunctivae* on a Large Dairy Goat Farm

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ABSTRACT

Infectious keratoconjunctivitis (IKC) is a highly contagious ocular inflammation frequently reported in domestic sheep and goats and in wild *Caprinae*. An unusually widespread outbreak of blindness is reported on a large dairy goat farm caused by *Mycoplasma conjunctivae*. Two stress factors were present prior to the onset of blindness: Firstly, Peste des Petits Ruminants (PPR) was diagnosed in several milking does three weeks prior to the first symptoms, and secondly, the entire herd was vaccinated against PPR to restrain the further propagation of the virus, which was considered a stressor for the lactating stock. Sixty-four per cent of the milking does (320/500) became temporarily or permanently blind. Subsequently, most of the stock aged 6 months or less, more than 250 kids, exhibited keratoconjunctivitis (KC). A month later clinical improvement of at least one eye was reported in 130 lactating does.

Keywords: Goat, *Mycoplasma conjunctivae*, blindness.

INTRODUCTION

Infectious keratoconjunctivitis (IKC) is a highly contagious ocular infection, frequently reported in domestic sheep and goats (1-10) and in wild *Caprinae* (11, 12). *Mycoplasma conjunctivae*, first identified by Barile *et al.* (1), is considered a primary pathogen causing ocular inflammation in both domestic and wild *Caprinae*, without the auxiliary involvement of other microorganisms (6, 7). Other *Mycoplasma* species, especially respiratory pathogens or saprophytes, may also be isolated from the corneal sac of either diseased or clinically healthy animals (2, 5, 8, 13). Mechanical or environmental/climatic factors may exacerbate infection and thus establish an ocular inflammation, such as conjunctivitis that may be transient or could progress to keratoconjunctivitis (KC). At this stage, when the cornea is involved, the disease may further progress towards corneal ulceration with temporary or permanent blindness.

The importance of *M. conjunctivae* in the etiology of IKC is supported by experimental infection studies (4, 13). However, under field conditions, other microorganisms, usually *Moraxella* (formerly *Branhamella*) *ovis* or *M. bovis* (14, 15) are frequently concomitantly isolated, and *Chlamydophila* spp. may also occasionally be present (16, 10).

In domestic sheep the role of *Chlamydia pecorum* as a major etiological agent of IKC is well established (16). Åkerstedt and Hofshage reported that in a survey of ocular pathogens of small ruminants in KC-affected herds in Norway, *M. conjunctivae* was isolated from 37% of animals

with clinical signs and from 7% of those without clinical signs (8). In farms without clinical signs of KC, *M. conjunctivae* were isolated from 8% of the animals.

In recent years, interest in *M. conjunctivae* infection has increased markedly because of the impact of the disease on free-ranging ibex (*Capra ibex*) and chamois (*Rupicapra rupicapra*) populations in the Alps and other mountain ranges in Europe (11, 12). The wild *caprinae* usually develop clinical IKC with resultant blindness, resulting in death under natural conditions.

This communication reports an unusual episode of conjunctivitis and IKC caused by *M. conjunctivae*, which led to extensive blindness in adult milking does. The predisposing factors in this outbreak may be attributed to natural and iatrogenic stressors that were present on the infected farm.

CASE HISTORY

The first clinical cases related to PPR outbreak were note on the 15th September 2011, in a dairy goat farm with 500 milking does, located in the Upper Galilee region of Israel. This farm is annually vaccinated against PPR. No specific PPR-related lesions were observed and therefore PPR was not yet suspected. However, the classical mucosal necrotic lesions appeared 4 days thereafter and ten days later PPR was confirmed on suspected material sent to Kimron Veterinary Institute (KVI), by the local practitioner. Mortality was reported especially in the young stock, 4-6 month of age, where 81 of 250 (30.4%) died. The adult stock suffered only 6 diseased animals of the 500 milking does (1%). The last clinical PPR-related manifestations were reported on the 10th October 2011. Only the animals which exhibited clinical manifestations died. PPR vaccination was started immediately and all the animals on the farm were vaccinated with the Pestevac PPR- vaccine (Jovac, Jordan Bio-industries, Jordan).

On 20 October 2011 blindness was reported on this goat farm with 500 milking does. In this region, in mid-October, days are warm and sunny, but at night the temperature can be as low as 5 °C. The first animals to exhibit IKC were adult lactating does, and 320 animals (64%) were affected within 7 days (Figure 1).

A few days later the younger stock of animals exhibited ocular inflammation and lacrimation. No new cases were noted from the end of November onwards, however 4 months after the onset 190 does remained completely blind.

MATERIALS AND METHODS

Bacteriology

Eight eye swabs were submitted for laboratory examination. Routine bacteriological assays for the isolation of the ocular flora are described elsewhere (14, 15).

For isolation of mycoplasma, ocular swabs submitted for routine diagnosis were inoculated into modified Friis mycoplasma broth and agar (17). The cultures were propagated at 37°C in a humidified atmosphere with 5% carbon

Figure 1: Ocular globe in 2-year-old doe: with keratoconjunctivitis.

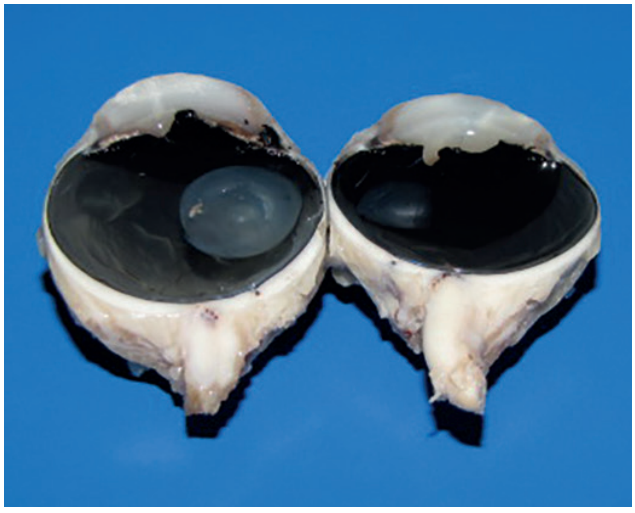

Figure 2: Gross pathology of the eyes: Note the severe thickening of the corneas.

dioxide. *M. conjunctivae* colonies were identified by indirect immunofluorescence (IMF) (18, 19).

Conjunctival swabs were rolled onto microscope slides, which were then air-dried, fixed with cold acetone for 10 min and kept at –20ºC pending examination. These samples were tested for *Chlamydophila* spp. by direct IMF with a monoclonal chlamydial group-specific fluorescein conjugate (Chlamydia cell, Brook vale, Australia) according to the manufacturer's instructions. Samples were considered positive if 10 or more fluorescent elements with characteristic morphology were seen per high power field.

PCR for detection of *Mycoplasma* spp.

Genomic DNA was extracted from ocular swabs with the Maxwell DNA Isolation Kit for Cell/Tissue and the Maxwell 16 apparatus (Promega, Madison, USA), according to the manufacturer's instructions. The DNA was amplified with the aid of the universal primers JGMF-1 (5'-ACACCATGGGAGCTGGTAAT-3') and JGMR-1 (5'-CCTCATCGACTTTCAGACCCAAGGCAT-3'), as previously described (18, 20). The nucleotide sequences of the resulting amplicons, complementary to the intragenic 16-23S rRNA spacer (ITS) of *Mycoplasma* species, were compared with data deposited in GenBank.

Gross pathology and microscopic examination

Tissue samples, including lung, heart, muscles, spleen, liver, kidney, udder, eye, and eyelids from a lactating doe with severe ocular lesions, which died on the farm, were sampled in 10% neutral buffered formalin for histological examination. The tissues were then embedded in paraffin wax, sectioned at 3-4 µm, and stained with hematoxin & eosin (H&E).

RESULTS

Bacteriology

No pathogens other than *M. conjunctivae* were isolated from eight swabs taken from affected eyes. All eight samples were found positive by universal mycoplasma PCR. Sequence analysis of four PCR products revealed 100% (478/478; E-value 0.0) homology to *M. conjunctivae* strain Goat (Accession number FJ226571; D.V. Volokhov, direct submission).

Virology

Routine viral assays carried out on the ocular and respiratory samples yielded negative results.

Gross and histopathology

Animals clinically exhibiting various stages of the progressive lesions were suspected as blind as judged by field clinical examinations.

Sectioned eyes showed severe thickening of the corneas (Figure 2).

Histological examination revealed that the normal architecture of the cornea was completely distorted due to focally extensive erosions of the central part of the non-keratinized

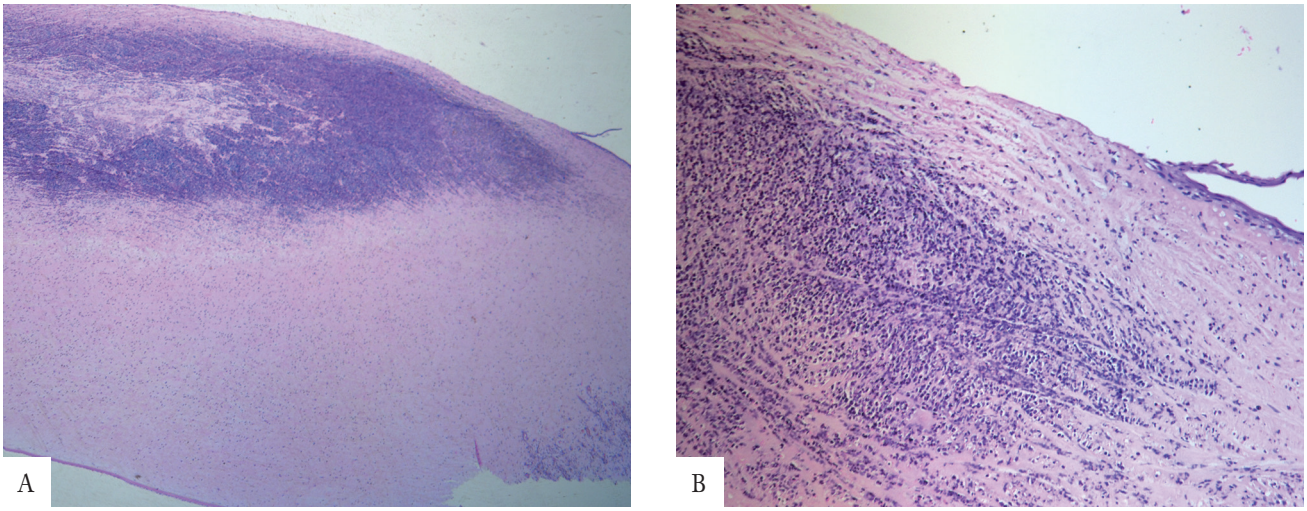

Figure 3: A. Cornea shows extensive focal erosion in the central part of the cornea. ×40. H&E. B. The underlying stroma of the cornea with severe infiltration of inflammatory cells. ×200. H&E.

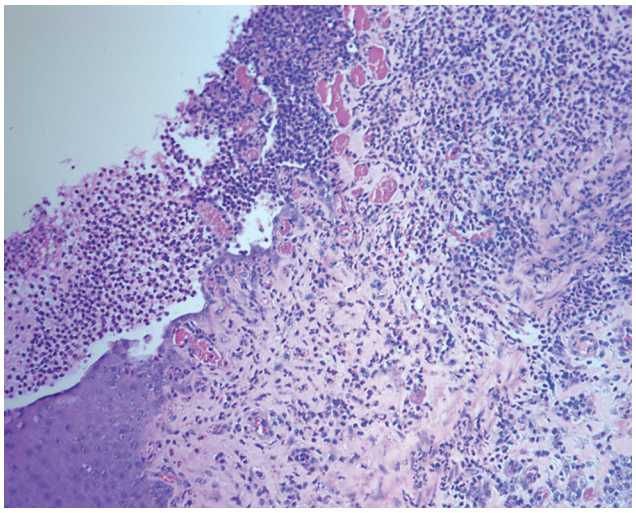

Figure 4: Eyelid of an affected goat with ulceration of the squamous epithelium and infiltration of mainly neutrophils. ×200. H&E.

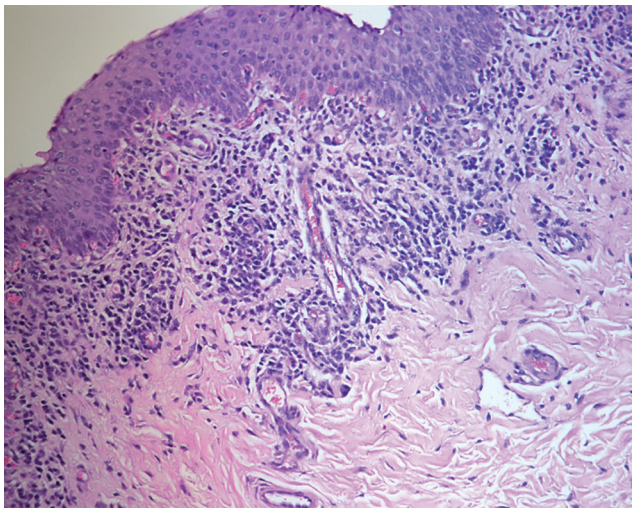

Figure 5: Eyelid of an affected goat. Hyperplasia of the squamous cell epithelium and mononuclear cell infiltration of the submucosa. ×100. H&E.

epithelium of the cornea and in addition to a diffuse infiltration of inflammatory cells – mainly neutrophils – into the underlying stroma (Figure 3). The eyelids showed multifocal ulcerations of the squamous epithelium and diffuse infiltration of inflammatory cells, mainly neutrophils (Figure 4). The conjunctival mucosa showed multifocal hyperplasia of the squamous epithelium with mononuclear cells infiltration into the sub-mucosa (Figure 5). All these lesions met criteria for a diagnosis of acute to sub-acute keratoconjunctivitis.

No pathological changes were observed in any of the other examined organs or tissues.

DISCUSSION

This is the second reported IKC outbreak in intensive farming herds of small ruminants in Israel caused by *M. conjunctivae*. In the first published case in Israel (10), only lambs were affected, in agreement with previously published findings (5, 7, 9, 13). From 2005 to the present date only two additional eye swab samples submitted to the KVI yielded *M. conjunctivae* (Lysnyansky, personal communication).

In recent years there has been increasing recognition of the prevalence and importance of disease syndromes of multifactorial etiology. It is often difficult to elucidate the role of each of the infecting microorganisms, especially because their relative importance may differ among different cases. Synergism between *Mycoplasma* spp. and other bacteria or viruses in ruminants has been described frequently. However, the organisms may act sequentially rather than in concert, and may not be present in the lesion or the affected organ at the same time (20).

The IKC episode described in the present report was not a novel occurrence in this flock. Although clinical signs were observed in the past, no nasal or ocular swabs were sent to the Kimron Veterinary Institute for bacterialogical diagnosis, therefore the possibility that the pathogen was endemic in the flock cannot be excluded.

Mycoplasma conjunctivae have been previously described as a major pathogen, capable of establishing IKC in the absence of co-infection (6, 7). However, the present outbreak exhibited several unusual characteristics. Firstly, the adult stock exhibited clinical ocular signs before the infection appeared in the younger stock. This is in contrast to the usual situation where the disease appears first in lambs. The second unusual characteristic of the present outbreak was the high proportion (64%) of affected animals in which the infection could be attributed to a previous PPR outbreak and the ensuing mass vaccinations, which might have acted as stressors.

In conclusion although being a rare episode as described in this case *M. conjunctivae* can cause a severe widespread outbreak of blindness in adult goat and not only in young kids as described elsewhere.

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