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KERATITIS IN REINDEER

INVESTIGATIONS OF MYCOPLASMA, RICKETTSIA, RICKETTSIA-LIKE ORGANISMS AND VIRUS*

By

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REHBINDER, CLAES: *Keratitis in reindeer. Investigations of mycoplasma, rickettsia, rickettsia-like organisms and virus.* Acta vet. scand. 1977, *18*, 65—74. — Six investigations were performed in order to explore the possibility that mycoplasma, rickettsia, rickettsia-like organisms or virus can be connected with outbreaks of keratitis in reindeer. It has not been possible in the investigations undertaken to confirm the presence of any of these organisms, indicating that they do not act as etiological agents in keratitis in reindeer.

reindeer; keratitis; mycoplasma; rickettsia; RLO; virus.

In some respects the clinical picture of keratitis in reindeer resembles that of infectious kerato-conjunctivitis in domestic ruminants (*Bergman* 1912, *Wilcox* 1968, *Surman* 1968, *Rehbinder* 1970, *McCauley et al.* 1971, *Winqvist & Rehbinder* 1973, *Pedersen* 1973).

Many organisms have been associated with the etiology of kerato-conjunctivitis in domestic ruminants, including mycoplasma, rickettsia-like organisms (RLO) and virus.

Mycoplasma is stated to be a frequent etiological agent in bovine and caprine kerato-conjunctivitis (*McCauley et al., Barile et al.* 1972, *Surman* 1973). Mycoplasma has also been recovered in calves with kerato-conjunctivitis (*Langford & Dorward* 1969, *Nicolet & Buttiker* 1974). These studies suggest that the severity of the ocular lesions may be related to a mixed infection of mycoplasma and Moraxella bovis. Rickettsia-like organisms (RLO) have been stated to be etiological agents in ovine

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kerato-conjunctivitis (*Coles* 1931, *Hofland et al.* 1969) as well as bovine kerato-conjunctivitis (*Blakemore* 1947). *Surman* (1973), however, showed through comparative and morphological studies by light and electron microscopy that RLO and mycoplasma were identical.

Mycoplasmic and RLO infections are always reported in connection with intra- and extracellular pleomorph organisms (*Coles, Blakemore, Surman* 1968, *Hofland et al.* 1969).

Eneqvist (1956) suggests rickettsia as a possible etiological agent in keratitis in reindeer yet without having demonstrated the organisms. Infectious bovine rhinotracheitis (IBR) virus may be associated with outbreaks of kerato-conjunctivitis in cattle (*Hughes et al.* 1964). *Pugh et al.* (1970) stated, however, that IBR virus was not the primary etiological agent of infectious bovine kerato-conjunctivitis, but that IBR virus was an enhancing factor toward the pathogenic effects of Moraxella bovis as it creates a more suitable environment for bacteria. *Gray* (1966) verifies this interaction between bacteria and virus. *Sykes et al.* (1962) reproduced kerato-conjunctivitis in cattle, using viruses isolated from cases of IBR and bovine "cancer-eye" lesions (a probable DNA-virus) as well as various strains of IBR virus. Recently *Kummeneje* (1976) isolated Neisseria ovis and Colesiota conjunctivae-like microorganisms from an outbreak of keratitis in a Norwegian reindeer herd. *Wilcox* (1970) produced kerato-conjunctivitis in calves with adenovirus. However, only 2 out of 22 calves developed keratitis.

The purpose of this investigation was to explore whether mycoplasma, rickettsia, RLO or virus can be connected with outbreaks of keratitis in reindeer.

MATERIAL AND METHODS

Clinically normal and naturally affected reindeer calves showing clinical symptoms of various stages of conjunctival and corneal changes were selected from Lappland herds assembled for marking of the calves by the owners. The selected animals were examined with a lamp before and after fluorescein staining, and the macroscopical picture of each eye was described.

Investigation I

Smears from the conjunctival sacs of 139 eyes were prepared as shown in Table 1. Five of these smears were taken from calves

in which larvae of the fly Cephenomyia trompe L had been instilled in the conjunctival sac. The smears were left to dry and then stained according to May-Grünwald-Giemsa. A differential count of 200 leukocytes was performed on each slide when possible. A thorough search for organisms according to *Coles* (1931) and *Surman* (1968, 1973) was undertaken.

Investigation II

Eight calves exhibiting slight to severe keratitis, 5 of which bilaterally affected, were slaughtered. The affected corneas were immediately removed and submerged into liquid nitrogen. Prior to the slaughter, blood samples (20 ml) had been taken from the jugular vein and centrifugated. The plasma was removed, and the samples were stored in a thermoscontainer and sent to the laboratory together with the corneas.

The corneas submerged in liquid nitrogen for virus isolation were sent to the laboratory of the National Veterinary Institute (NVI). Suspensions were prepared by grinding the corneas with sterile sand using mortar and pestle. After centrifugation of the suspensions at 300 r.p.m. for 10 min, 100 i.u./ml penicillin, 100 μg/ml streptomycin and 50 μg/ml mycostatin were added to one part of the supernatant, while 1000 μg/ml streptomycin and 50 μg/ml mycostatin were added to the other part. The first part of the suspension was used for virus isolation studies in monolayer cell cultures of reindeer calf kidney. The cell cultures were made from the kidneys of a clinically healthy reindeer calf born in Stockholm and never in contact with the disease.

The inoculated culture tubes were incubated at 37°C in a roller drum for 7 days. The fluid from the cell cultures was passaged twice. The culture tubes were microscopically inspected daily.

The second part of the suspension was inoculated into the yolk sac of embryonated hens' eggs in order to test for rickettsia. The inoculated eggs were incubated at 38°C and candled daily for 7 days.

Investigation III

Two calves exhibiting slight to severe degree of keratitis on both eyes were brought to the NVI where they were slaughtered. Material was prepared for inoculation into reindeer kidney cell culture and embryonated hens' eggs as previously described. The

same suspension was instilled into the lower conjunctival sac of 3 clinically healthy reindeer calves (1 ml/eye) born in Stockholm and never in contact with the disease. The corneas were not scarified.

Investigation IV

One hundred captured females of the fly Cephenomyia trompe L were sent to NVI. They were prepared for inoculation into cell culture and embryonated hens' eggs as previously described in reference to the corneas.

Investigation V

Three calves with bilateral conjunctivitis and erosions on the corneal surface (detected by fluorescein staining), 2 calves with focal opacity in the center of the cornea, and 2 calves with normal eyes were selected.

Blood samples from the jugular vein were taken and treated as described in Investigation II. The calves were slaughtered and 4 corneas with erosions, 2 with focal opacity and 3 from normal calves were rapidly removed and submerged in liquid nitrogen. After preparation as previously described, the material was inoculated into reindeer calf kidney cell culture. From the first and second cell culture passage, cells were prepared for electron microscopy. The 2 corneas with erosions, the cornea with focal opacity and the normal cornea were fixed immediately at varying temperature in 3 % glutaraldehyde solution buffered to pH 7.4 with cacodylate. The tissue culture specimens were processed in the same manner. Before embedding, small pieces were postfixed in 2.67 % OsO_4 buffered with s-collidine. After embedding in Epon, 1 µ thick sections were cut and stained with toluidine blue for light microscopy. Thin sections were prepared on an LKB ultratome, picked up on uncoated copper grids, and stained with uranyl acetate. They were then examined in a Philips EM 201 at 60 kw with magnifications varying between 1,800 and 26,000 times for corneas and 1,800 and 38,000 times for the tissue culture specimens.

Investigation VI

Six reindeer calves, 3 of which were sheltered from the sun and the remaining 3 kept in an open corral, were injected intravenously with 0.5 ml blood taken from the jugular vein from

reindeer calves in early stages of keratitis. The treatment was repeated daily for 3 days, and the animals were carefully examined every morning and evening for 13 days.

RESULTS

Investigation I

The results of the cell counts are presented in Table 1. Statistical calculations were according to Fisher's exact test.

A significant difference ($P < 0.001$) was observed between the normal group and 3 of the 4 groups with larvae present, i.e. larvae and conjunctivitis, larvae erosions and conjunctivitis, and larvae inoculated into the conjunctival sac. These 3 groups exhibited a higher proportion of eosinophilic granulocytes and a lower proportion of neutrophilic granulocytes than those found in the normal group. This was, however, not the case in the group with larvae and kerato-conjunctivitis. All the smears from these groups exhibited a predominance of neutrophils, a low number of lymphocytes. All groups contained a markedly low level of lymphocytes. No plasma cells were found in any of the smears.

Table 1. Cell counts from conjunctival smears.

Groups of animals	Number of eyes	Neutrophils		Eosinophils		Lymphocytes	
Normal	44*	97	(92—99)	0.5	(0—1.5)	2.5	(1—7)
Conjunctivitis	35	97.7	(93—100)	0.4	(0—5)	1.9	(0—6.5)
Keratitis and conjunctivitis	11	98	(95—100)	0.1	(0—5)	1.9	(0—3.5)
Erosions and conjunctivitis	14	97	(95—99.5)	0.3	(0—1.5)	2.7	(0.5—4.5)
Larvae and conjunctivitis	5	93.5	(91—98.5)	4	(0—8)	2.5	(1—5)
Larvae, erosions and conjunctivitis	6	93	(77.5—97)	4	(0—20)	3	(2.5—4)
Larvae, keratitis and conjunctivitis	9	96.8	(92—99)	0.2	(0—1)	3	(0.5—7)
Inoculated larvae	5	91.3	(87.5—95)	7	(4—9)	1.6	(0—5)

* Smears from 54 eyes were prepared but in 10 of these less than 100 leukocytes were present.

No total counts could be performed, but it was noted that 10 out of 54 smears, of the normal group, contained less than 100 white cells and had to be discarded.

No pleomorphic organisms similar to those described by *Coles* (1931) and *Surman* (1968) were detected in the smears, nor were any inclusion bodies present in the epithelial cells.

Investigation II

Cytopathic effect in cell culture, one criterium of virus growth, was not observed. Although the material prepared for inoculation was sterile when tested on serum agar plates, the second suspension inoculated into the yolk sac of embryonated hens' eggs killed the eggs by contamination with not specified bacteria. After streptomycin and mycostatin were added to the contaminated ground material, the material was centrifugated at 300 r.p.m. The supernatant exhibited no growth when tested on serum agar plates. The material was passaged twice on embryonated hens' eggs. Since the eggs continually became contaminated, bacterial infection from the original material probably was not completely irradicated by the antibiotics added.

Investigations III and IV

Three passages of the material were done in cell culture and embryonated eggs with negative results. No bacterial contamination developed in the inoculated eggs.

Investigation V

No cytopathic effect was observed in the inoculated cell culture. The ultrastructural investigation of cells did not reveal any virus or changes due to virus.

In the ultrastructural investigation on eyes with focal opacity and erosions, virus, rickettsia, RLO and mycoplasma were not demonstrable. The case of focal opacity corresponded with the picture earlier described by *Winqvist & Rehbinder* (1973).

The cases of erosion appeared to be deep, extending almost to the basal layer. They showed signs of beginning inflammation with invading neutrophilic granulocytes congregating in the stroma and the epithelium in the vicinity of the erosion. The ultrastructure of the epithelium surrounding the erosion and the underlying stroma showed striking changes consistent with

the description earlier given of cases of general strong opacity by *Winqvist & Rehbinder.*

However, only cells in the vicinity of the erosion appeared to be altered. The deeper parts of the stroma, the posterior limiting lamina, and the endothelium appeared unchanged.

Investigation VI

The calves injected with blood from diseased calves remained healthy and did not exhibit any visible ocular changes.

DISCUSSION

There were no differences in the differential counts of the conjunctival smears of normal and diseased animals with the exception of the 3 groups with larvae present. No smears investigated showed an increasing amount of lymphocytes or the presence of plasma cells as described by *Surman* (1968, 1973) and *McCauley et al.* (1971) in connection with mycoplasma infections. No mononuclear cell reaction was observed as may be the case in ocular manifestations of viral systemic diseases (*Bistner* 1973). In the cases with larvae present the ocular smears from cases of manifested keratitis did not differ from the groups without larvae, while larvae in the 3 groups with less severe lesions evidently caused an increase of the eosinophilic granulocytes. No total counts could be performed, but it was noted that in 10 out of 54 smears of normal eyes there were less than 100 white cells present. This indicates that the only difference between diseased and normal cases was a greater amount of leukocytes in diseased eyes. Apparently, the normal bacterial flora of the conjunctival sac, in reindeer as well as in other animals (*Bistner et al.* 1969, *Ojo et al.* 1972, *Rehbinder & Tschäppät* 1974, *Young & Hill* 1974) and in man (*Cason & Winkler* 1954) will maintain a continuous migration of leukocytes into the conjunctival sac. The outflow appears to increase in cases of inflammation while the composition will remain almost unchanged.

Examination of the conjunctival smears has not revealed any pleomorph organisms similar to those described in connection with mycoplasma infections (*Coles* 1931, *Blakemore* 1947, *Surman* 1968, 1973, *Hofland et al.* 1969, *McCauley et al.*).

Inclusion bodies as described in connection with keratitis caused by viral infections (*Wilcox* 1969, 1970, *Bistner*) were not present in any of the smears.

It was not possible to draw any conclusions concerning virus, mycoplasma, rickettsia or RLO from the contaminated material of Investigation II. No bacterial contamination occurred when the procedure was repeated in Investigation III. The investigation did not reveal any virus, rickettsia, RLO or mycoplasma nor was this the case with Cephenomyia trompe flies and larvae. Furthermore, it was not possible to transmit the disease through suspensions prepared from affected corneas.

The ultrastructural investigations of tissue cultures in attempts to detect virus were futile.

The ultrastructure of eyes with erosions exhibited an appearance similar to earlier description of keratitis in reindeer (*Winqvist & Rehbinder* 1973), having no specific features indicating a viral etiology. No findings or structural changes indicate the presence of mycoplasma or RLO.

Attempts to transmit the disease through intravenously injecting blood from diseased animals into healthy calves were also negative. Limited emphasis should be given to the negative result of attempts to isolate rickettsia, partly because it is considered difficult and tedious to isolate rickettsia (*Smadel & Jackson* 1964) and partly because it was considered necessary to add antibiotics to prevent bacterial contamination.

None of the 6 investigations performed resulted in positive findings concerning mycoplasma, RLO or virus. This seems to indicate that these organisms do not act as etiological agents in keratitis in reindeer.

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SAMMANFATTNING

Keratit hos ren. Undersökningar avseende mycoplasmer, rickettsia, rickettsia-liknande organismer och virus.

Sex olika undersökningar har företagits i avsikt att utröna huruvida mycoplasma, rickettsia, rickettsia-liknande organismer eller virus är förbundna med utbrott av keratit hos ren. Ingen av dessa organismer har kunnat påvisas i någon av de genomförda undersökningarna tydande på att dessa organismer inte är att betrakta som orsak till keratit hos ren.

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