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# A STUDY UPON CARRIERSHIP DURATION OF *MORAXELLA BOVIS* IN FACE FLIES

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## Summary

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The carriership duration of *Moraxella bovis* on body surface and in the alimentary tract of face flies was studied in experimental conditions. The flies were infected via feeding a broth culture of the referent Epp63 strain and an additional pulverization with a cultural spray.

Samples from infected flies were daily examined microbiologically for detection of *M. bovis*.

It was found out that the bacteria was isolated for 4 days in the viscera of flies (alimentary tract) whiel it persisted for a week on body surfaces of flies. The role of flies as mechanical vectors in the mechanism of transmission of bovine infectious keratoconjunctivitis is discussed.

**Key words:** face fly, keratoconjunctivitis, *Moraxella bovis, Musca autumnalis*

## INTRODUCTION

Infectious bovine keratoconjunctivitis (IBK) is an acute contagious disease, widely distributed worldwide, including our country. It is found out in both stable and pasture housing of cattle.

Clinically the disease is manifested by increased lacrimal secretion, hyperemia and oedema of conjunctives, blepharospasm, photophobia and various corneal lesions (Punch and Slatter, 1984; Jayappa and Lehr, 1986; Rogers et al., 1987).

The studies of various reseराchers evidence that the primary etiological factor is *Moraxella bovis* (Pedersen, 1970; Punch and Slatter, 1984; Lyutskanov and Bonev, 1993). The virulent genera of this species adhere to conjunctival and corneal mucosa via superficially located pili (fimbriae). The local inflammatory process is provoked by the structural proteins or other extracellular products (haemolysins, cytotoxins, enzymes etc.) of the bacterial agent.

Some authors reported the possibility of transmission of the infection by a direct contact. They however emphasize the role of domestic flies and particularly of several face flies as the commonest vectors in the mechanism of transmission. The *Musca autumnalis* species is considered as the most permanent carrier. This fly, being faculative haematrophic, prefers substrates rich in proteins (like lacrimal secretions) apart blood (Brown and Atkins, 1972; Luvchiev and Yovtchev, 1978; Gerdhardt et al., 1982; Kopecky et al., 1986).

Our study aimed to determine the duration of survival of *M. bovis* on body surfaces of flies-carriers and to follow out the duration of survival of the causative agent in the alimentary tract of flies with regard to the physiological regurgitation.

## MATERIALS AND METHODS

The time of survival of *M. bovis* on the body surface and alimentary tract of flies was determined by experimental contamination of 150 flies that were not species-affiliated. They were collected from cattle, originating from a farm, unaffected by IBK. Flies were put into a sterile glass bank, covered with a mesh. Within the bank, small Petri dishes with a sponge impregnated with a 24-hour broth culture (tripticase-soy-been broth – TSB) of the referent haemolytical and pilated *M. bovis* Epp63 strain were put. Then we added 0.02 ml egg white, 0.4 normal equine serum and 0.5 ml pasteurized milk per 1 ml broth. The extermal contamination of flies was performed by a single spraying of a 24-our broth culture of the same strain suspended in sterile TSB with a sprayer.

After a 6-hour stay in the common bank, flies were divided into 15 smaller mesh-covered banks, containing 10 flies each (=1 sample). For 15 consecutive days, we analysed one sample daily, transferring the flies every day in new sterile banks with sterile broth medium. Each sample of insects was examined for the presence of *M. bovis* on body surfaces and then, for presence of the bacteria in the viscera.

The occurrence of *M. bovis* on body surfaces of flies was proved by immersing the insects in sterile TSB. After a 2-hour stay, the flies were removed and the broth – incubated at 37 $^{o}$C for 24 hours. Then, we inoculated broth specimens on blood agar with cloxacillin according to Weber et al. (1982) and on Tween-80 agar according to Mattinson and Cox (1982).

The identification of *M. bovis* was performed by morphological and biochemical tests of cultures using an schedule for identification and differentiation (Pugh et al., 1966; Fraser and Gilmour, 1979; Bovre, 1984; Lyutskanov, 1997).

The duration of the carriership in the alimentary tract was performed similarly after triple washing of dead flies in sterile saline combined with a triple stay in 70$^{o}$ ethanol for 10, 20 and 30 min respectively. Afterwards the dead insects were stirred in a sterile mortar and cultivated in TSB. After 24-hour incubation, the forementioned procedure of isolation and identification of the agent was repeated.

## RESULTS AND DISCUSSION

Table 1 presents the data for the duration of *M. bovis* survival. In the first two days following the contamination, *M. bovis* was isolated from body surfaces of all exam-

**Table 1.** Duration of *M. bovis* carriership in flies, contaminated both externally and via the alimentary tract

| | Days after contamination | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| On body surface | | | | | | | | | | | | | | | |
| Total number | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Infected flies | 10 | 8 | 6 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Non-infected flies | 0 | 2 | 4 | 6 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| In viscera | | | | | | | | | | | | | | | |
| Total number | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Infected flies | 9 | 5 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Non-infected flies | 1 | 5 | 8 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

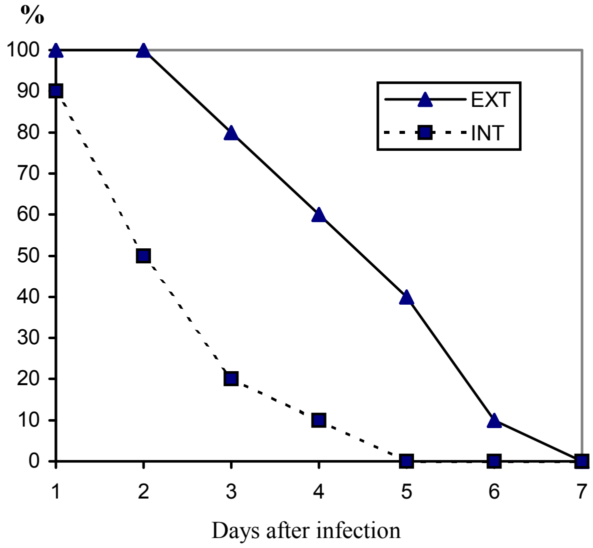

**Fig. 1.** Persistence time of *Moraxella bovis* on body surfaces (EXT) and within the alimentary tract (INT) of face flies.

ined flies (100% carriership, Fig. 1). At day 3 and 4, this percentage decreased to 80% and 60% respectively. Six days after the contamination, *M. bovis* was present only in 10% of flies while after the day 7 the IBK agent was isolated in none of insects.

Data about the persistence of bacteria in the alimentary tract show that face flies were more rapidly freed from *M. bovis*. As early as the second day after the contamination, the microbial agent was present only in half of all insects. After another 24 hours, only 20% of flies were M. bovis carriers and at day 5 the bacteria was no more isolated.

Those results showed that flies were mechanical carriers of the IBK agent and that the carriership duration was 4-7 days. According to our data, the superficial carriership was more prolonged. The persistence of the agent within the body, especially in the alimentary tract was shorter. Most probably, moraxellae are retained only in the forepart of the alimentary tract in the so-called stomodeum. Here are the openings of the saliva glands, whose secretion contains lysozyme and proteolytic enzymes. We assume that both ways of bacteria conservation (on body surfaces and in alimentary tract) are however important for the transmission of the agent from diseased to healthy animals. It could be realized by contamination of bovine conjunctive and cornea from the body surface of flies. Similar is the opinion of Cheng (1967) and Brown and Adkins (1972). They state that the survival of *M. bovis* on body surfaces of flies was no longer than 72 hours.

The second mechanism for infection of the conjunctival mucosa according to Glass and Gerhardt (1984) is by the regurgitation during the stay of flies on the conjunctiva for ingestion of lacrimal secretion. Moraxellae do not reproduce in the alimentary tract and more particularly in fly stomodeum, but survive and could be deposited by regurgitation for 4-6 days (Glass et al., 1982; Glass and Gerhardt, 1983; Coleman and Gerhardt, 1987). Our data confirm that phenomenon too. The persistence period of 4-5 days after alimentary infection found out in our studies is shorter than that of 11 days, reported by Brown and Adkins (1972). Despite the different times of carriership, this way of contaminaton could not be rejected; moreover, during their occurrence on bovine conjuncitas and corneas, flies cause microtraumas that mediate the adhesion of pathogenic varieties of *M. bovis* on those surfaces – an opinion, supported by Gerhardt et al. (1982).

Data of our and other's studies show that all measures, intended for decrease of face flies population in IBK affected herds through desinsection and desifection, are a crucial element of the general antiepizootic measures against this disease.

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