Onderstepoort J. vet. Res., 57, 89–93 (1990)

EPIDEMIOLOGY OF WILDEBEEST-DERIVED MALIGNANT CATARRHAL FEVER IN SOUTH AFRICA: INABILITY TO TRANSFER THE DISEASE WITH AN AFRICAN FACE FLY *MUSCA XANTHOMELAS* (DIPTERA: MUSCIDAE)

B. J. H. BARNARD[1], R. G. BENGIS[2] and S. F. VOGES[1]

ABSTRACT

BARNARD, B. J. H., BENGIS, R. G. & VOGES, S. F., 1990. Epidemiology of wildebeest-derived malignant catarrhal fever in South Africa: Inability to transfer the disease with an African face fly *Musca xanthomelas* (Diptera: Muscidae). *Onderstepoort Journal of Veterinary Research*, 57, 89–93 (1990)

Under experimental conditions an African face fly *(Musca xanthomelas)* preferred to feed on cattle dung when provided with a choice of 3 different meals namely sucrose, cattle dung and blood. Flies starved overnight fed well on the eyes of cattle and rabbits, but were reluctant to feed again within 2 h after being allowed to feed on cell culture medium or on the eyes of wildebeest, and when they did feed, they preferred to feed on the external side of the eyelids and on the coagulated material in the medial canthus of the eye. Under field conditions flies were rarely seen to feed on the eyes of immobilized wildebeest.

Although *M. xanthomelas* became infected with Alcelaphinae herpes virus 1 (AHV-1) when they fed on infective wildebeest tears or cell culture medium, they lost the virus within 5 h, and recovery of infective AHV-1 particles from regurgitated cell culture medium was limited to the first 30 min after feeding. AHV-1 could not be transmitted by flies to cattle or rabbits.

The failure to transfer the virus with flies can be ascribed to their reluctance to feed on cattle or rabbits shortly after they have consumed a protein rich meal, the rapid inactivation of ingested virus and the relatively high titre of virus necessary to infect cattle via the ocular route. Furthermore, it is believed that under natural conditions flies that have emerged from cattle dung will be inclined to stay with cattle where food is freely available. Therefore, should they become infected by feeding on wildebeest tears, they probably will not feed on the eyes of cattle soon enough to transfer the virus effectively.

INTRODUCTION

Close association of cattle with wildebeest is generally regarded as necessary for transmission of wildebeest-derived malignant catarrhal fever (W-DMCF), but the occurrence of the disease across fences and even at distances of up to several hundred metres is indicative of an as yet unknown mode of transfer (Barnard, 1984; Barnard & Van de Pypekamp, 1988; Barnard, Van de Pypekamp & Griessel, 1989).

The route of entry of virus is probably the nasopharynx, as it has been shown that intra-nasal instillation of an infectious cell-free suspension of AHV-1 can cause infection in cattle and rabbits (Mushi & Wafula, 1983). Hence transfer of virus by air currents seems likely. However, it is unlikely that aerosol infection can take place over long distances. In such cases, intermediate hosts whether by mechanical or biological means may be involved. Flies (*Musca* species) have been mentioned as possible vectors of the virus (Mushi, Karstad & Jesset, 1980; Barnard & Van de Pypekamp, 1988; Rweyemamu, Karstad, Mushi, Otema, Jesset, Rowe, Drevemo & Grootenhuis, 1974).

In an investigation carried out on farms with a known high incidence of W-DMCF, *Musca lusoria, M. confiscata, M. nevilli* and *M. xanthomelas* were indentified as possible vectors that could qualify for the mechanical transmission of AHV-1 (E. M. Nevill, VRI, unpublished data, 1987).

In this study *M. xanthomelas* was used as a model to determine the possibility of mechanical transmission of AHV-1 by African face flies.

MATERIALS AND METHOD

Virus

Alcelaphine herpesvirus-1, isolated from the ocular fluid of wildebeest calves in the Kruger National Park (KNP) was used (Barnard, Bengis, Griessel & De Vos, 1989).

Virus assay

Foetal lamb kidney cells (FLK) cultivated in Eagle's medium containing 5 % bovine serum, benzyl penicillin (500 iu/mℓ), streptomycin sulphate (200 µg/mℓ), amphotericin B (2,5 µg/mℓ) and vancomycin hydrochloride (0,5 mg/mℓ), were used for virus assay. Cells sufficient to form a monolayer within 24 h, together with the material to be assayed were inoculated simultaneously into 25 mℓ plastic cell culture flasks. The flasks were incubated at 33 °C–35 °C and examined regularly for the appearance of cytopathic foci (CPF). Three days after the first appearance of CPF they were counted microscopically and the titre of virus determined.

Ten flies or portions thereof were macerated and used for assay of virus. All titres of virus given are the averages of at least 3 repeat tests and are expressed as CPF/mℓ or CPF/10 flies.

Experimental animals

Locally bred albino rabbits, Bonsmara-type cattle, and free-living wildebeest calves in the KNP and 14 wildebeest calves in captivity, were used.

Flies

M. xanthomelas from a colony maintained by the Section of Entomology VRI, Onderstepoort were used. For virological work pupae, on the point of eclosing were cleaned by immersion in 0,5 % formalin for 5 min and then allowed to eclose in sterilized cages at 22 °C. Until used they were provided with a sterile solution of 5 % sucrose in tapwater supplemented with benzyl penicillin, (500 iu/mℓ), streptomycin sulphate (400 µg/mℓ) and amphotericin B (25 µg/mℓ).

INFECTION OF FLIES

Flies were starved for several hours, usually overnight, before they were infected by feeding them on AHV-1-infected cell culture material or directly on

[1] Veterinary Research Institute, Onderstepoort 0110
[2] P.O. Box 12, Skukuza 1330
Received 25 October 1989—Editor

the eyes of wildebeest calves likely to be excreting virus in their ocular fluid. The flies were allowed to feed till they lost interest (10–20 min). All feeding experiments were performed at approximately 24 °C. To facilitate the feeding process on the eyes of rabbits, cattle or wildebeest, circular feeding cages were constructed (Fig. 1). At 1 end of the cage, transparent perspex allowed a clear vision and at the other end a circular of foam rubber provided a close fit on the head of the animal to prevent flies from escaping during feeding. Flies to be fed on animals were immobilized by cooling in a refrigerator, then transferred to the cage and left for at least 1 h to adapt. The cage was then positioned over the eye of an immobilized wildebeest and the slidingdoor of the cage removed. After feeding, the flies moved to the upper end of the cage and the slidingdoor could be replaced. Flies could then be used for virus assay or for ocular infection of rabbits or cattle.

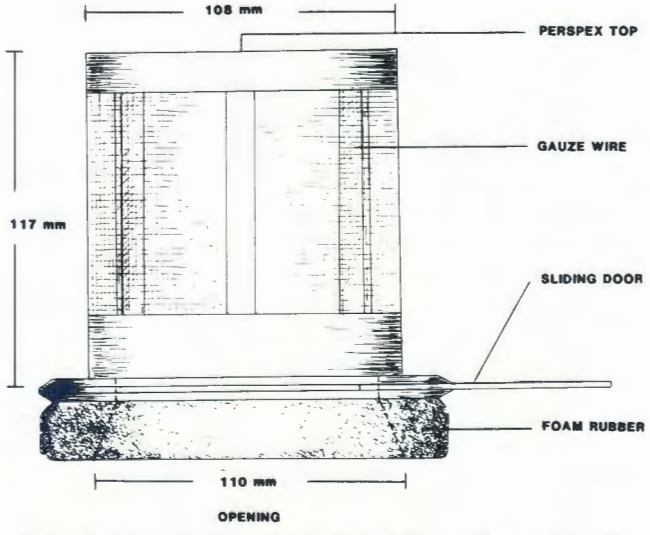

FIG. 1 Cage to facilitate the feeding of flies on the eyes of cattle, wildebeest and rabbits

Infectivity of cell-free and cell-associate AHV-1 for flies

AHV-1 infected FLK cell cultures were harvested when 75 % of cells showed cytopathic (CP) changes. A portion of the culture was centrifuged for 10 min at 2000 *g* to separate the cells. The sediment and supernatant were assayed separately for virus. The rest of the culture was divided into 3 portions, the first of which was treated ultrasonically until most cells had ruptured, the 2nd portion was exposed to 3 cycles of freezing and thawing and the 3rd portion was used as an untreated control. Flies fed on the differently treated cultures were assayed for virus 30 min after feeding was stopped.

Location of AHV-1 in flies

Flies fed on AHV-1 infected cultures were dissected into four different portions namely wings and legs, heads, thoraces and abdomens. The structures were assayed separately for the presence of virus.

Persistence of AHV-1 in flies

Flies fed on infective AHV-1 cell cultures were assayed after 0,5 h; 1 h; 3 h; 4 h and 5 h, for the presence of virus. The progeny of infected flies were also assayed for virus, and the presence of AHV-1 in regurgitated crop contents was also determined. Flies were fed and then allowed to regurgitate into plastic cell culture flasks moistened with cell culture medium. The flies were kept in the flasks for 15 min and immediately after their removal LFK cells suspended in culture medium were added to the flasks. The flasks were shaken lightly to wash down the regurgitated material, and incubated, as described.

Ocular infection of cattle and rabbits

Five oxen and 16 rabbits were infected by instilling infective cell culture material inside the eyelids. Ox 1 received 1 mℓ of culture material once only: Ox 2 was infected with 0,2 mℓ on 5 consecutive days; Ox 3 received 0,1 mℓ on 10 consecutive days; Ox 4 was infected with 0,05 mℓ on 15 consecutive days and Ox 5 on 20 consecutive days with 0,02 mℓ. Rabbits were likewise infected with volumes of cell culture material varying from 0,01 mℓ–0,5 mℓ and also infected intra-ocularly with fluid, collected 1 h previously from the eyes of wildebeest calves 2–3 months of age.

Infection of cattle and rabbits by flies was also attempted. Feeding-cages containing 50 flies fed, 0,5 h–1 h previously on infective culture material, were positioned over the eyes of cattle and rabbits. The cages were held in position for at least 30 m. For comparison, 1 ox and 1 rabbit were infected parenterally with a similar culture. All animals were observed daily and their temperatures were recorded for at least 90 days or until death.

Meal preference of M. xanthomelas

Fifty flies were placed in a 2 ℓ rectangular perspex cage for experimentation. This was repeated 3 times. Flies at different stages of physiological development were starved and fed in such a way so as to mimic situations possibly occurring in nature. Fresh heparinized cattle blood was used as a source of protein and a 5 % solution of sucrose in tap-water provided energy. Freshly collected cattle dung was used for comparison. Pledgelets of absorbent paper were soaked in blood and sucrose solution. The pledgelets and the dung were supplied to the flies in identical flat dishes with a diameter of 30 mm. During feeding the position of the 3 dishes were occasionally alternated. The flies were observed for a period of 39 min. Every 3 min the flies feeding at each dish were counted and the meal preference determined by calculating the percentage of flies feeding at any 1 dish.

Behaviour and feeding of free-living flies on wildebeest

The behaviour and feeding of free-living flies on wildebeest was observed on 14 wildebeest calves held in captivity near Sentrum on a farm 3 km distant from a herd of cattle (Barnard, Van de Pypekamp & Griessel, 1989). The calves were immobilized at monthly intervals from April to October.

RESULTS

Infectivity for M. xanthomelas *of cell-free and cell-associated virus*

The titres of virus in the cellular fraction and in the supernatant of cell cultures were $3,7 \times 10^5$ cpf/mℓ and $3,7 \times 10^3$ cpf/mℓ respectively (Table 1). The titre of virus was not markedly affected by sonication or 3 cycles of freeze thawing, and was very similar to that of untreated cultures. The treatment did not affect the infectivity of the virus for flies. In all further experiments, untreated cultures were used for infection of flies and experimental animals.

TABLE 1 Infectivity of cell-free and cell associated AHV-1 for the African face fly *M. xanthomelas*

Treatment of AHV-1 Infected cell cultures	Titre of virus in:	
	Cell culture[1]	Flies fed on a Similar culture[2]
Cell fraction	$3,7 \times 10^5$	Not tested
Supernatant	$3,7 \times 10^3$	Not tested
Freezing and thawing	$7,6 \times 10^5$	4×10^3
Sonication	$7,3 \times 10^5$	$4,4 \times 10^3$
Untreated control	$7,9 \times 10^5$	$4,3 \times 10^3$

[1] Titre of virus expressed as cytopathic foci/mℓ
[2] Titre expressed as cytopathic foci/10 flies

Location of virus in flies

No virus was detected in cultures inoculated with the legs and wings of infected flies (Table 2), while most virus was recovered from the abdomen which contained at least 99 % of the recovered virus. In subsequent experiments whole flies were used for assay of virus.

TABLE 2 Isolation of AHV-1 from different anatomical structures of the African face fly *M. xanthomelas* 15 min after experimental infection

Anatomical structure	Virus isolated Cytopathic foci/10 flies
Legs and wings	None
Head	$1,2 \times 10^1$
Thorax	4×10^1
Abdomen	$2,8 \times 10^4$

Persistence of AHV-1 in flies

Virus could be recovered only from flies fed less than 5 h previously on cultures containing 1×10^5 cpf/mℓ but not from flies fed on cultures containing 6×10^3 cpf/mℓ. Thirty min after feeding, 4×10^2 cpf/10 flies and at 5 h only 6×10^1 cpf/10 flies could be recovered (Fig. 2). The titre of virus recovered from regurgitated crop contents of flies fed 30 min previously varied from 0 cpf/10 flies–8 cpf/10 flies. No virus was recovered from flies that had eclosed from the pupae of infected flies.

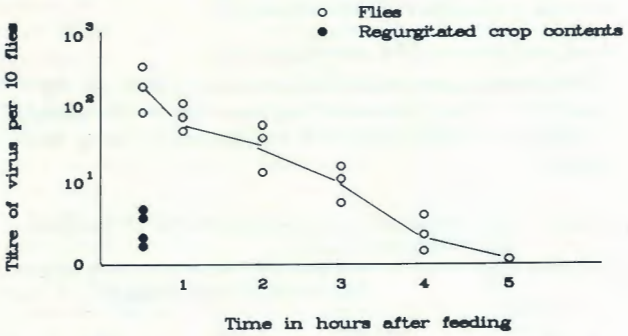

FIG. 2 Recovery of AHV-1 from *M. xanthomelas* fed on cell culture material containing 10^5 cytopathic forming foci per mℓ

Ocular infection of cattle and rabbits

Infection by ocular instillation of cell culture material was accomplished in cattle but not in rabbits (Table 3). An ox infected on 15 consecutive days with $6,1 \times 10^3$ cpf developed typical W-DMCF 42 days after the last instillation but another ox exposed to $6,1 \times 10^4$ on 10 consecutive days did not develop signs of W-DMCF. The incubation period in an ox and rabbit infected parenterally with a similar amount of virus was 24 days and 25 days respectively.

Infection of rabbits by ocular instillation of wildebeest tears containing $2,8 \times 10^4$ cpf was unsuccessful, nor could rabbits or cattle be infected by exposure to 50 flies which had fed on the eyes of wildebeest calves known to excrete virus in their tears or on cell cultures containing 1×10^5 cpf/mℓ–$6,1 \times 10^5$ cpf/mℓ.

TABLE 3 Infection of cattle and rabbits by various routes with AHV-1

Virus and exposure					Reaction	
Animals	Source	Route[1]	Titre of virus	Number of exposures[2]	First rise in temperature on day	Death on day
Cattle						
1	Culture	io	$6,1 \times 10^5$	1	35	42
2	Culture	io	$1,2 \times 10^5$	5	44	50
3	Culture	io	$6,1 \times 10^4$	10	NR[3]	NR
4	Culture	io	$6,1 \times 10^3$	15	42	52
5	Culture	io	$6,1 \times 10^2$	20	NR	NR
6	Culture	iv	$6,1 \times 10^3$	1	24	29
7	Wildebeest tears	io	1×10^5	50 flies	NR	NR
Rabbits						
1–2	Culture	io	$1,2 \times 10^5$	1	NR	NR
3–4	Culture	io	$6,1 \times 10^4$	5	NR	NR
5–6	Culture	io	$3,1 \times 10^4$	10	NR	NR
7–8	Culture	io	$6,1 \times 10^3$	15	NR	NR
9–13	Wildebeest tears	io	$2,8 \times 10^4$	1	NR	NR
4–16	Wildebeest tears	io	$2,8 \times 10^4$	50 flies	NR	NR
17	Culture	ip	$1,2 \times 10^5$	1	25	29

[1] io—intraocular; iv—intravenous; ip—intraperitoneal
[2] Exposed on consecutive days
[3] NR—No reaction

Flies starved overnight fed well on cell culture fluid and on the eyes of wildebeest calves. However, when they were transferred to cattle or rabbits within the hour they were reluctant to feed. When they did, they preferred to feed on the external surface of the eye-lids or on the mucus in the medial canthus of the eye. Good feeding was only possible after starvation period of at least 4 h.

Meal preference of M. xanthomelas

Flies less than 6 h old did not feed (Table 4). At 9 h and 24 h they favoured sucrose and cattle dung, but flies 80-h-old fed well on sucrose, dung and blood.

TABLE 4 Meal preference of the African face fly *M. xanthomelas 3–80 h after eclosion*

Age in hours	Percentage of flies feeding on[1]		
	Sucrose solution	Cattle-dung	Blood
3	0	0	0
6	0	0	0
9	15,4	18,0	0
24	11,3	14,6	0
80	20,8	41,5	33,0

[1] Three repeats with 50 flies each

Under laboratory conditions the meal preference of 80-h-old flies was to a large extent determined by their previous meal (Table 5). Flies starved for 5 h after a meal of either dung or blood preferred sucrose, while flies supplied with an initial meal of sucrose preferred dung. In each case dung was just as popular as or more so than blood. After a free-choise meal of blood, sucrose and dung and after starvation periods of 1 h; 2 h and 3 h (Table 6), dung was the most popular and blood the least popular meal.

TABLE 5 Meal preference of the African face fly *M. xanthomelas* 80-h-old and starved for 5 h after a previous meal of sucrose, catte dung or blood

Previous meal	Percentage of flies[1] feeding on		
	Sucrose solution	Cattle dung	Blood
Sucrose	1,2	8,4	1,2
Cattle dung	34,2	7,3	6,2
Blood	27,7	6,3	6,3

[1] Fifty flies were used in 3 repeats

TABLE 6 Meal preference of the African face fly *M. xanthomelas* 80-h-old, 1, 2 and 3 h after a free choice meal of sucrose, cattle dung and blood

Time after previous meal	Percentage of flies feeding on[1]		
	Sucrose	Dung	Blood
1 h	40,0	60,0	30,0
2 h	40,0	55,0	20,0
3 h	55,0	75,0	20,0
Average	45,0	63,3	23,0

[1] Three repeats with 50 each

Behaviour and feeding of free-living flies on wildebeest

The external openings of the nose and the muzzle of wildebeest are dry and flies did not favour them for feeding, but the eyes of the immobilized wildebeest were visited by flies. Although the long hair of the eyebrows and eyelashes prevent a direct approach, flies used the hairless area over the infraorbital gland as a landing site from where they crawled towards the eye. However, they rarely fed on the eye itself, and preferred to feed on the external surface of the eyelids and on the partially dried tears in the medial canthus of the eye.

DISCUSSION

To fulfil the role of an effective mechanical transmitter of AHV-1 the vector must be closely associated with both wildebeest and the susceptible host. In order to transmit the virus, the vector must also be able to pick up a sufficient number of infective virus particles from the source and preserve them in such a way as to retain their infectivity until the particles can be succesfully transferred to the susceptible host.

Flies and, in particular, the house fly *(M. domestica)* have been incriminated as a vector in the spread primarily of bacteria, but are also reported to have mechanically transported some viruses (James & Haiwood, 1979). However, proof of the transmission of viral diseases by flies is lacking. This may be due to their inability to pick up an infective dose of virus, as was suggested by Gough & Jörgenson (1983).

In a 1-year investigation *M. xanthomelas, M. confiscata, M. nevilli* and *M. lusoria* were identified as possible vectors qualifying for the mechanical transmission of AHV-1, and *M. xanthomelas* was found to be by far the most abundant species reared from cattle dung collected in the veld next to a camp with wildebeest which were the source of several cases of W-DMCF. Only *M. tempestatum* and *M. confiscata* were commonly reared from wildebeest dung and then only in summer (E. M. Nevill, unpublished data, 1987). *Musca* spp. occur throughout the Bushveld of the Transvaal and they are often seen on wounds and around the eyes and muzzle of cattle, so they probably readily feed on ocular and nasal secretions and on blood. Flies of the genus *Musca* are associated also with wildebeest and some were caught from immobilized animals. It is therefore reasonable to assume that they could transfer AHV-1 between wildebeest and cattle. *M. tempestatum* was the only fly commonly seen in large numbers on wildebeest but was not seen on cattle.

The most likely source of AHV-1 for the infection of flies is the ocular fluid of wildebeest calves less than 4 months old and able to excrete up to 3,7 $\times$ 10^5 cpf/mℓ in their tears (Barnard, Bengis, Griessel & De Vos, 1989). Although the long eyebrows and eyelashes of wildebeest are effective obstacles to approaching flies, the flies manage to reach the eye by using the hairless area over the infraorbital gland below the eye, as a landing strip from where they crawl towards the eye. Flies feeding in the eyes of immobilized wildebeest were seldom observed but they usually fed on the moist areas on the outside of the eyelids or on mucus in the medial canthus of the eye. A similar observation has been made on cattle, and it was suggested that the face fly *M. autumnales* prefers more concentrated and partialy dried food (Van Geem & Broce, 1985).

It is unlikely that blood and nasal mucus are important sources of virus for the infection of flies, as apart from the fact that the titre of virus is lower in

these 2 sources (Barnard, Bengis, Griessel & De Vos, 1989). They are also less acceptable to flies. Like most game, wildebeest have tough skins, consequently open or bleeding wounds are usually very rare. In a large number of immobilized wildebeest no flies were seen on the muzzle or nostrils. Since these areas are dry they are unattractive to flies. Furthermore, the nasal cavity is protected by an extremely mobile nasal flap or nostril with which the nasal passage can be closed completely to prevent flies from entering the cavity to feed on the mucus membrane.

Dietary protein is critical for egg maturation in flies (Wang, 1964) and tears, nasal mucus and cattle dung can be utilized by flies to complete gonotrophic development (Van Geem & Broce, 1985). Under natural conditions, face flies breed in cattle dung, but not in wildebeest dung (E. M. Nevill, unpublished data, 1987). Should cattle be present when they eclose, they are surrounded by an abundance of food including dung, tears and saliva of cattle and blood spots caused by biting insects. Wildebeest, an alternative source of food and the reservoir of AHV-1, are mobile and may be less acceptabe to flies.

In the present investigation, under laboratory conditions, dung was preferred to blood. This preference was observed even in flies previously supplied with a free-choice meal of sucrose, blood and dung or dung only. In most instances, cattle dung was found to be 3 times more attractive than blood. As cattle and wildebeest usually do not mix or graze together and especially in the South African situation where the 2 species are often kept in separate camps, it is reasonable to be lieve that only a small number of flies will leave cattle to feed on wildebeest tears and then return to cattle for their next meal of cattle tears. Even if this does happen occasionally, to transmit the virus the flies must feed on cattle within a short time after they have left the wildebeest.

AHV-1 is relatively labile and blood specimens for the isolation of virus must be processed within hours after collection (Plowright, 1964). In the present investigation, virus could not be recovered from flies infected more than 5 h previously and the number of infective particles that could be recovered decreased from approximately 400 cpf/10 flies infected 30 min previously to less than 10 cpf/10 flies 4 h–5 h later. As no virus was recovered from the legs or wings of flies after exposure transmission of virus could only take place when infected flies regurgitate on the ocular epithelium of cattle, a tissue susceptible to infection. The number of infective virus particles recovered from the regurgitated crop contents of flies was regularly less than 8 cpf/10 flies when tested 30 min after exposure to infective cell cultures with a titre of virus equal to the highest titre encountered in wildebeest tears. It is therefore reasonable to assume that in order to infect cattle flies must reach and feed on the eye of cattle within minutes after they have consumed infective wildebeest tears. This is considered to be unlikely, as *M. xanthomelas* that had fed on the eyes of wildebeest calves were reluctant to feed on the eyes of rabbits 2 h later, and flies exposed to protein rich cell culture medium reacted in a similar way when they were offered a meal on the eyes of cattle or rabbits. In these circumstances flies may be more interested in dung than tears.

The minimum dose of virus for the infection of cattle via the ocular route is not known. Previously it was not possible to infect cattle by ocular instillation of 10^3 cpf of AHV-1 (B. J. H. Barnard, unpublished results, 1986) and in this investigation an ox exposed to 6×10^4 cpf did not become infected but another ox died with typical W-DMCF after the instillation of $6{,}1 \times 10^3$ cpf of AHV-1 on 15 consecutive days. This is an indication that the minimum infective dose via the ocular route is approximately 10^3 cpf–10^4 cpf. At least 1 250 flies must therefore first feed on wildebeest tears, with a titre of virus of 10^5 cpf or more, and then, within minutes, return to cattle to regurgitate in their eyes. This is considered to be very unlikely. In the winter however, flies may be more dependent on the moisture and food provided from the eyes of cattle and wildebeest, which may, in part, explain the winter transmission of W-DMCF in South Africa.

REFERENCES

BARNARD, B. J. H., 1984. Transmitters and potential transmitters of malignant catarrhal fever. Proceedings XIII World Congress on Disease in Cattle. Durban South Africa. September 1984.

BARNARD, B. J. H. & VAN DE PYPEKAMP, H. E., 1988. Wildebeest derived malignant catarrhal fever: Unusual epidemiology in South Africa. *Onderstepoort Journal of Veterinary Research,* 55, 69–71.

BARNARD, B. J. H., VAN DE PYPEKAMP, H. E. & GRIESSEL, MONICA D., 1989. Epidemiology of Wildebeest derived malignant catarrhal fever in an outbreak in the North-western Transvaal: Indications of an intermediate host. *Onderstepoort Journal of Veterinary Research,* 56, 135–139.

BARNARD, B. J. H., BENGIS, R., GRIESSEL, MONICA D. & DE VOS, V., 1989. Excretion of Alcelaphine herpes virus-1 by captive and free-living wildebeest *(Connochaetes taurinus). Onderstepoort Journal of Veterinary Research,* 56, 131–134.

GOUGH, P. M. & JÖRGENSON, R. D., 1983. Identification of transmissible gastroenteritis virus in home flies *(Musca domestica* Linnaeus). *American Journal of Veterinary Research,* 44, 2078–2082.

JAMES, M. T. & HAIWOOD, R. F., 1979. The housefly and its relatives: in Herm's medical entomology, pp. 249–265, ed. London: MacMillan Co.

MUSHI, E. L. & WAFULA, J. A., 1983. Infectivity of cell-free malignant catarrhal fever virus in rabbits and cattle. *Veterinary Research Communications,* 6, 153–155.

MUSHI, E. L., KARSTAD, L. & JESSET, D. M., 1980. Isolation of bovine malignant catarrhal fever virus from ocular and nasal secretions of wildebeest calves. *Research in Veterinary Science,* 29, 161–171.

PLOWRIGHT, W., 1964. Studies on bovine malignant catarrhal fever of cattle. D.VSc. Thesis, University of Pretoria. South Africa.

RWEYEMAMU, M. M., KARSTAD, L., MUSHI, E. Z., OTEMA, J. C., JESSET, D. M., ROWE, L., DREVEMO, S. & GROOTENHUIS, J., 1974. Malignant catarrhal fever virus in nasal secretions of wildebeest: a probable mechanism for virus transmission. *Journal of Wildlife Diseases,* 10, 478–487.

VAN GEEM, T. A. & BROCE, A., 1985. Significance of cattle discharges and secretions as protein sources for ovarian development in the face fly (Diptera: Muscidae). *Environmental Entomology,* 14, 60–64.

WANG, C. M., 1964. Laboratory observations on the life history and habits of the face fly *(Musca autumnalis)* (Diptera: Muscidae). *Annales of the Entomological Society of America,* 57, 563–569.