

# EFFECTS OF THE PATHOGENESIS OF ARBOVIRUS INFECTIONS IN CATTLE ON THE EFFICIENCIES OF VECTORS

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In the last 50 years the number of arboviruses isolated from particular insect species has reached many hundreds. If the insect has digested its blood meal, this is good evidence that the virus isolate has multiplied in the insect. Of course, PCR can find virus remnants in insect species that have taken a blood meal but the viruses may be incapable of replication within the insect tissues. There are other barriers to the insect being a valid vector. After initial replication the virus must reach the saliva, and be delivered with the saliva into the relevant part of the host to which it is adapted, to multiply. Usually it has a phase where it is in the bloodstream to be taken in a blood meal for the next round of the biological cycle. There are exceptions where viruses multiply only in surface tissues. The logistics of proving a vector are formidable. Knowledge of the behaviour of the virus within a vertebrate may assist the process and enable the investigator to focus limited resources more precisely on few species.

## METHOD OF FEEDING

The first problem the insect faces is to penetrate the epidermis. The mouthparts manage this action very quickly for the flying insect's brief visit and more slowly for tick's longer contact with a vertebrate. *Culicoides* midges, sandflies and ticks produce a wound through the epidermis and into the dermis. Lacerated capillaries ooze blood into the small wound for the pool feeders to ingest. Ticks cement their mouthparts into the wound they have produced in a fashion that varies with genera (Obenchain and Galun 1993). This prolonged contact allows for deposition of saliva and intake of blood over a much longer period, perhaps negating the requirement for multiplication (Jones et al. 1992) and giving an opportunity for viruses to be transmitted or acquired for days. In contrast, mosquitoes probe for a small blood vessel and insert their mouthparts into a small blood vessel together with much of the saliva. The mosquito does deposit some of the saliva in the dermis outside the venule in the probing process.

The differences of feeding technique affect the fate of the arbovirus. When the virus contaminated saliva is deposited extravascularly as with a midge or a tick it may multiply to some extent in adjacent tissue cells or those that are attracted as part of the inflammatory response. Lateral cell to cell spread does occur with certain viruses, for example Vesicular stomatitis virus (VSV). However, to generalise within the vertebrate, it must travel via the lymphatics to reach the bloodstream. The virus that the mosquito delivers into the bloodstream reaches its target tissue probably well within a minute. The fate of a rhabdovirus and an orbivirus are contrasted for illustration.

Bovine ephemeral fever virus (BEF) does not traverse the lymphatic system, at least in the first 2 d after viraemia commences (St.George 1993). The experiment which showed this was not continued long enough to determine how long the lymph was free of BEF or infected neutrophils that are so characteristic of ephemeral fever. The viraemia in experimental cases persists for 2-5 d. This is consistent with experimental findings that the intravenous route is the only way to produce disease experimentally (Mackerras et al. 1940). Also, live virus given intramuscularly or subcutaneously as a vaccine multiplies locally but does not generalise (Tanaka and Inaba 1986).

In contrast, bluetongue virus (BLU) undergoes first stage multiplication within the lymph system. After the first stage of replication an increased amount of virus reaches the bloodstream and is carried to the spleen for secondary cycle (Barratt-Boyes and MacLachlan 1994). Thus BLU is in the bloodstream from day 1 or 2 after transmission and thus available to a new vector. BLU remains in the bloodstream for some weeks though not at a titre high enough to infect a new vector via a blood meal after 2 weeks. Under experimental conditions BLU can be injected subcutaneously, intramuscularly or intravenously and multiply satisfactorily. This applies also to other orbiviruses which are injected experimentally (T.D. St.George unpublished data).

## SIMULTANEOUS INFECTIONS IN CATTLE WITH ARBOVIRUSES

There are many possible combinations of simultaneous infections. The discussion here will be limited to a few examples. If closely related viruses infect a cow at the same time this gives a chance for exchange of genetic information.

For example, Akabane and Aino viruses have been isolated from a single blood sample from a bull (Cybinski and Zakrzewski 1983). When two viruses of very different families enter a cow from vectors one may block the other. St. George (1985) bled 22 cattle daily during a 3 week period during an ephemeral fever epidemic. Six arboviruses, besides BEF, infected one or more of the group in the observational period. Only six of 22 fully susceptible cattle developed clinical ephemeral fever during this time. These were also the only cattle from which BEF was isolated and which seroconverted to BEF. There were no subclinical infections. Four of the seven had pre-existing immunity to CSIRO Village virus (CV), an orbivirus which produces a subclinical infection in cattle. In the other three cattle with ephemeral fever, where the viraemia with CV began shortly after BEF infection, the duration of viraemia with CV appeared to be shortened.

Of the remainder in the group CV established a viraemia of 12 to 17 d which appeared to block BEF infection in 10 of the other 15 cattle and other arboviruses (Akabane, Aino, Peaton, Douglas, Tinaroo and Kimberley viruses) in the remaining 5. All the cattle that did not develop BEF infection and clinical ephemeral fever, because of the interference by the other arboviruses, were susceptible in the next epidemic 2 years later (St. George 1985).

It appears that the first virus to establish and multiply has successfully blocked the other. In this instance, the outbreak of ephemeral fever was halted while susceptible animals remained. The rhabdovirus BEF which goes directly into the bloodstream is competing with an orbivirus (CV virus) which presumably replicates in the lymph system first in a similar fashion to BLU. Both viruses are from types which induce interferon (interleukins) production by the host tissue (Eksteen and Huismans 1972, St. George et al. 1986, Uren and Zakrzewski 1989). Interleukins, being soluble, would be in the lymph as well as in the plasma.

In the outbreak of Tammar Wallaby Disease (Kirkland et al. 2000), there was a high mortality in wallabies. However, no homologous antibody was found in wallabies that did not die. It is possible that infection by the newly identified orbivirus was blocked by one or other of the arboviruses that are also carried by *Culicoides* vectors (for example, Wallal or Warrego virus).

## TARGETING VECTORS

How can knowledge of route of infection help to narrow the search for a vector? In the case of BLU, 52 species of insect were listed by Gibbs and Greiner (1988) as potential vectors. As these were all *Culicoides* species there is no advantage from knowledge of the pathogenesis within the vertebrate host. However, in the case of the rhabdovirus, BEF has been isolated from mosquitoes and *Culicoides* (Table 1). If the insects that rely on pool feeding are eliminated, the three *Culicoides* species, from which BEF has been isolated, are eliminated. This leaves the mosquitoes. Both Culicine and Anopheline mosquitoes are represented in Table 1 as sources of BEF. This means that all mosquito species biting cattle are suspect. Additional species of mosquito must be found to cover the vast range of Africa, Asia and Australia that ephemeral fever is found, beyond the range of these species. However, the false trail which has wasted effort by processing hundreds of thousands of *Culicoides* species for BEF, can be avoided in the future.

**Table** Which are the vectors?

Mosquito and <i>Culicoides</i> species		
Vector		Virus
Pool of Culicines	<i>Cx. orbostiensis</i>	BEF
	<i>Ur. nivipe</i>	
	<i>Ur. albescens</i>	
	<i>Ae. carmentis</i>	
	<i>An. bancroftii</i>	
	<i>Cx. annulirostris</i>	KIM
<i>Culicoides</i>	<i>C. brevitarsis</i>	BEF
	<i>C. imicola</i>	
	<i>C. coarctatus</i>	
	<i>C. brevitarsis</i>	KIM

There is other evidence that can be advanced to support the evidence from pathogenesis studies that mosquitoes are vectors of ephemeral fever and not *Culicoides*. Experimental ephemeral fever is produced reliably only by the intravenous injection of virulent virus (Mackerras et al. 1940).

The epidemiology of ephemeral fever in Australia is not consistent with *C. brevitarsis* Kieffer being a vector (St. George 1993, Kirkland 1993). After experimental infection of *C. brevitarsis* and *Culex annulirostris* Skuse with BEF, only the mosquito excreted BEF in saliva (Muller 1987, Muller pers comm). KIM virus that is closely related to BEF has been isolated from *Cx. annulirostris* as well as *C. brevitarsis*. Thus, in Australia, *Cx. annulirostris* should be a prime target in the search for vectors of ephemeral fever using techniques not available before the 1990's with considerable economy of effort. Strategies for control of vectors can also be more rationally based on the natural history of mosquitoes rather than *Culicoides*.

There are other instances where rhabdoviruses have been isolated from both mosquitoes and *Culicoides* midges. Fukuoka virus has been isolated in Japan from *Cx. tritaeniorhynchus* Giles and *C. punctatus* (Kaneko et al. 1986). Oakvale virus has been isolated from *Cx. edwardsi* and *C. austropalpalis* Lee and Reye, both bird feeding insects in Australia. If there is a need to determine the vectors of these viruses initial effort should be focussed on the mosquito species.

## AN HYPOTHESIS WITH WIDER IMPLICATIONS

The Rhabdoviridae are a diverse group of viruses. Not all are arboviruses. Rabies virus, the type species, is spread by the bite of a mammal. The pathogenesis is very well understood. This virus and its close relatives traverse the neural system and then reach the salivary glands of the vertebrate host and not the lymphatics to complete their life cycle. Vesicular stomatitis virus spreads laterally in skin tissue and does not generalise from a local infection (Wilks 1994, TM Monath pers comm). To these two very different rhabdoviruses, BEF can be added. Thus, within the rhabdoviridae, it may be a characteristic of the family that multiplication with the lymph system is not a part of their natural history. They have exploited different pathways in their successful biological cycles.

## REFERENCES

- Barratt-Boyes SM and MacLachlan NJ (1994) Dynamics of viral spread in bluetongue virus infected calves. *Vet Microbiol* 40: 361-371.
- Cybinski DH and Zakrzewski H (1983) A dual infection with Akabane and Aino viruses. *Aust Vet J* 60: 283.
- Eksteen PAL and Huismans H (1972) Interferon induction by bluetongue virus and bluetongue virus ribonucleic acid. *Onderstepoort J Vet Res* 39: 125-132.
- Gibbs EPJ and Greiner EC (1988) Bluetongue and epizootic hemorrhagic disease. In: *The arboviruses: epidemiology and ecology*. Vol. 11 (Monath TP, Ed) CRC Press, Boca Raton. pp. 39-70.
- Jones LD, Kaufman DK and Nuttall PA (1992) Modification of the skin feeding site by tick saliva mediates virus transmission. *Experientia* 48: 779-782.
- Kaneko N, Inaba Y, Akashi H, Muira Y, Shorthose J and Kurashige K (1986) Isolation of a new bovine ephemeral fever virus. *Aust Vet J* 63: 29.
- Kirkland PD, Davis RJ, Rose K, Cooper D and Blumstein D (2000) Isolation of orbiviruses during an epizootic of sudden death in Tammar wallabies (*Macropus eugenii*). *Arbovirus Res Aust* 8:
- Kirkland PD (1993) The epidemiology of bovine ephemeral fever in south-eastern Australia: evidence for a mosquito vector. *Bovine ephemeral fever and related rhabdoviruses*. ACIAR Proc. No 44. (St. George TD, Uren MF, Young PL and Hoffmann D, Eds) pp. 33-37.
- Mackerras IM, Mackerras MJ and Burnet FM (1940) Experimental studies of ephemeral fever in Australian cattle. CSIRO Melbourne Australia No. 136 (120 pages).
- Muller MJ (1987) Transmission and *in vitro* excretion of bluetongue virus serotype 1 by inoculated *Culicoides brevitarsis* (Diptera: Ceratopogonidae). *J Med Ent* 24: 206-211.
- Obenchain FD and Galun R (1993) In *Physiology of Ticks*. Pergamon Press, Oxford. 509 pages.
- St. George TD (1985) Studies on the pathogenesis of bovine ephemeral fever. *Virology and Serology*. *Vet Microbiol* 10: 493-504.
- St. George TD (1993) The natural history of ephemeral fever of cattle. *Bovine ephemeral fever and related rhabdoviruses*. ACIAR Proc. No 44. (St. George TD, Uren MF, Young PL and Hoffmann D, Eds) pp. 13-19.
- St. George TD, Uren MF and Zakrzewski H (1986) The pathogenesis and treatment of bovine ephemeral fever. *Arbovirus Res Aust* 4: 303-307.
- Tanaka Y and Inaba Y (1986) Live/killed ephemeral fever vaccine in Japan. *Arbovirus Res Aust* 4: 319-323.
- Uren MF and Zakrzewski H (1989) Mechanisms of immunity to BEF. *Arbovirus Res Aust* 5: 274-276.
- Wilks CR (1994) Vesicular stomatitis and other vesiculovirus infections. *Infectious diseases of livestock with special reference to Southern Africa*. (Coetzer JAW, Coetzer GR and Tustin RC, Eds). Oxford University Press, Capetown.