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REVIEW

THE ROLE OF DIPTEROUS INSECTS IN THE MECHANICAL TRANSMISSION OF ANIMAL VIRUSES

V. M. CARN

Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking GU24 0NF, Surrey, UK

SUMMARY

Animal viruses may be transmitted by arthropods in two ways, either biologically or mechanically. Many different species of Diptera are implicated in mechanical transmission, but haematophagous species are the most important. The insects become contaminated with virus during normal feeding behaviour, and virus persists on their mouthparts or body until the next feed. Some viruses are inactivated rapidly on mouthparts, whereas others survive for many days or weeks, prolonging the potential period of transmission. Some viruses produce high titres in the skin of the infected vertebrate host, which facilitates transmission, whereas other viruses are transmitted even during relatively low levels of viraemia. Mechanical transmission by arthropods is important in the epidemiology of many animal diseases, and may be the major mode of horizontal transmission. In other instances vector spread is merely incidental.

KEYWORDS: Mechanical; transmission; arthropods; animal viruses; insects.

INTRODUCTION

Animal viruses may be transmitted by vectors in two ways, either biologically or mechanically. Biologically transmission is characterized by multiplication of virus in both vertebrate and invertebrate hosts, there being a short latent phase in the invertebrate before transmission can occur, equating to the incubatory phase in the former. Mechanical transmission is analogous to transmission by a 'flying pin', and essentially involves the invertebrate as a vehicle for inoculating virus picked up from one animal into another. Many of the Diptera (true flies and keds) are obligate haematophagous parasites, and have therefore developed a unique role in the transmission of animal viruses. Their predatory behaviour, often coupled with low host fidelity, ensures that, when sufficient virus is available, they are effective vectors.

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For some viruses, mechanical transmission by insects is merely incidental, and of negligible epidemiological importance, such as the transmission of rinderpest virus by biting flies. Other viruses are primarily transmitted without the involvement of insects, but vectors may be important under certain conditions. Many virus diseases have an epidemiology that is inextricably linked to the natural history of the appropriate vectors, and in some instances horizontal transmission of the virus in the absence of the vector does not occur.

DIPTERA AS MECHANICAL VECTORS

The obligate association between haematophagous species of Diptera and vertebrates has led to an incidental association of these species and vertebrate viruses. Many viruses are reliant on insects for transmission between vertebrate hosts, either biologically, or mechanically through contaminated mouthparts (Table I). Relative to biological transmission there is a much lower degree of specificity in the vector–virus relationship for mechanically transmitted viruses. Theoretically, under favourable conditions, any insect feeding on or associating with a vertebrate or their excretion or secretion could transmit a virus. In practice, however, although non-haematophagous flies could, through grazing on exudates, introduce viruses through cuts or abrasions, the most important vectors within the Diptera are those species that are skin piercers or scarifiers, and therefore essentially haematophagous.

Virus transmission rate is inversely proportional to the interval between vector feeds, therefore Diptera with an interrupted or repeated feeding pattern and those with low host fidelity are the most efficient mechanical vectors of viruses, as the virus needs to survive for only short intervals on the fly. Those flies with painful bites are most likely to be dislodged and then to seek a new host immediately. Larger flies with coarse mouthparts taking big meals relative to their body size cause significant lesions and ensure heavy contamination of their bodies (Krinsky, 1976). They have a greater chance of contacting pathogens in the skin and blood, and have more opportunity to transmit an infective dose to the next host.

The order Diptera

Diptera is an order of the class Insecta. The Diptera are the true flies, and include typical flies, mosquitoes and the specialized louseflies (keds). They have only one pair of wings, the hinge wings being modified into the halteres that provide equilibrium and neuromuscular co-ordination during flight. Controlled flight is of particular importance to a predatory insect and the blood-sucking Diptera therefore have a unique role in the transmission of pathogens and parasites of vertebrates. Behaviourally, blood-feeding flies are predators, with alternate long periods of rest and brief raids on the host species, whereas ecologically they are parasites.

The Diptera are divided into three sub-orders, the Cyclorrhapha, the Brachycera and the Nematocera. They have a wide range of anatomical, physiological and ecological adaptations for feeding on vertebrates.

Cyclorrhapha. The Cyclorrhapha are characterized by their general stoutness,

Table I
Classification of important Dipterous vectors of animal viruses

(Sub)family or order Specific name	*Common name*	*Feeding technique*	*Mechanical transmission of Animal viruses (Other important disease agents transmitted by these Diptera)*	*References*
Cyclorapha				
Musca domestica	House flies	"Sponging" secretions	Hog cholera virus	Dorset *et al.*, 1919
			(Poliovirus)	
Glossina spp.	Tsetse flies	Excoriation, blood feeding females only	RVFV	Hoch *et al.*, 1985
			(Trypanosomes)	
Stomoxys calcitrans	Stable flies	Excoriation, Blood feeding both sexes	BHMV	Gibbs *et al.*, 1973a
			ASFV	Mellor *et al.*, 1987
			VSV	Ferris *et al.*, 1955
			CPV	Kitching & Mellor, 1986
			Friend leukaemia virus	Fisher *et al.*, 1973
			RVFV	Hoch *et al.*, 1985
			EIAV	Issel & Foil, 1984
			(*Anaplasma* and *Trypanosoma evansi*)	
Brachycera				
Tabanids	Horse flies, clegs	Large painful lesions females only	EIAV	Hawkins *et al.*, 1973, 1976
			Myxoma virus	Day, 1955
			RPV	Bhatia, 1935
			BLV	Foil *et al.*, 1988a
			HCV	Tidwell *et al.*, 1972
			WEE	Goldfield *et al.*, 1968
			(*Trypanosoma evansi*)	
Nematocera				
Simuliidae	Black or Buffalo flies	Pool feeders females only	(Protozoa)	
			(River blindness)	
Phlebotaminae	Sand flies	Pool feeders	RVFV	Hoch *et al.*, 1985
Ceratopogonidae	Midges	Pool feeders	Fowl pox virus	Fukuda *et al.*, 1979
			RVFV	Hoch *et al.*, 1985
Culicidae	Mosquitoes	Capillary feeders	Myxoma virus	Fenner *et al.*, 1952
			Shope fibroma virus	Kilham & Dalmat, 1955
			Tana pox virus	Tripathy *et al.*, 1981

and their typically specialized sponging mouthparts, designed for mopping up liquids on damp surfaces. A good example of this is the housefly, *Musca domestica*. Houseflies feed on excrement, and may also feed on ocular or nasal secretions, and on exudating wounds. During disease animals will usually have increased ocular and nasal secretions, and these will often be rich sources of virus. Many different pathogens may be mechanically transmitted, due to contamination of the flies' feet, body hairs and mouthparts. In addition, houseflies often vomit and defaecate during feeding, increasing the likelihood of transmission (Service, 1986). Adaptations to the sponging mouthparts have occurred, with the evolution of certain species from feeding on excrement and secretions to wound feeding, and thence to blood feeding. These adaptations include small teeth for excoriation, enabling the fly to feed on small amounts of blood, to the piercing mouthparts of the obligate bloodsuckers.

Species within the genera *Glossina* and *Stomoxys* are adapted to blood feeding, and both have the capacity for trapping blood between the food canal and the labium (the structure enclosing both the hypopharynx and the food canal), which are inserted into the host tissues as the insect feeds.

Stomoxys calcitrans, the widely distributed stable fly, is superficially similar to the house-fly, but both sexes are predatory, feeding 2–3 times a day, generally around the legs of cattle and horses. They also feed on poultry, but are of a particular nuisance in housed cattle, where it has been estimated that 25 flies per cow can reduce milk yields by 40–60% (Walker, 1990). Eggs are laid in dung and damp vegetation and the fly tends to rest in the shade near its hosts. These flies are incriminated in the mechanical transmission of *Anaplasma* spp. and *Trypanosoma evansi*. The mechanical transmission of vesicular stomatitis virus, Rift Valley fever virus, bovine herpes mammillitis virus and African swine fever virus have all been shown (see Table I). Lumpy skin disease virus (LSDV) has been isolated from *Stomoxys calcitrans* caught feeding on infected cattle, and sheep and goat pox has been successfully transmitted experimentally by the same species (Kitching & Mellor, 1986).

The genus *Glossina* consists of the tsetse flies (*Glossina* spp.). These are responsible for the biological transmission of human and livestock trypanosomes. Species of *Glossina* also mechanically transmit certain species of trypanosome, and the mechanical transmission of Rift Valley fever virus by *Glossina morsitans* has also been demonstrated (Hoch *et al.*, 1985). They tend to have a relatively low population density, due to their low reproductivity rate, producing only one larva every 10–12 days, and are not considered a serious biting pest, despite the blood feeding of both males and females. They are very dependent on suitable vegetation for reproduction and control measures are directed towards the destruction of such habitats.

Brachycera. The *Tabanidae*, which contains the clegs and horseflies, are the most important haematophagous family within this suborder. The mouthparts are adapted for cutting through hides, forming a large lesion. Large quantities of saliva are required to maintain blood flow. Female tabanids are predatory, with good vision and flight. Although their population generally remains small they are persistent predators and follow their hosts. Cattle and sheep are distressed by the painful biting behaviour and group together closely to avoid attack, producing 'fly syndrome' which interrupts normal grazing and drinking patterns. Between 500–1000 flies may feed on a cow a day, resulting in blood loss of up to 200 ml (Walker, 1990).

The painful bites and persistent feeding behaviour of tabanids lead to vertebrate host evasion, which, coupled with the amount of blood left on their large mouthparts, favours mechanical transmission. Tabanids are important as mechanical vectors of many pathogens (Krinsky, 1976) including bacteria, trypanosomes (*Trypanosoma evansi*) and viruses: equine infectious anaemia virus was first shown to be transmitted by a tabanid in 1917 (Scott, 1917). Other animal viruses shown to be transmitted include myxoma virus, rinderpest virus, bovine leukaemia virus, and hog cholera virus (Table I). Animals showing very evasive behaviour, such as foals, suffer fewer bites than more tolerant animals, such as mares, and subsequently are at lower risk, of contracting virus infections which are mechanically transmitted by tabanids (Foil, 1989).

Nematocera. This sub-order of the Diptera contains many species implicated in the mechanical transmission of viruses, and the greatest number of species involved in the transmission of arboviruses.

The Nematocera tend to be small, fragile insects with long slender antennae, from which they derive their name (Gr. *nema*, thread; Gr. *keras*, horn.). The family Culicidae, the mosquitoes, are distinguished from the other families by their very long flexible mouthparts that allow the penetration and cannulation of individual host blood vessels. This method of blood sucking is known as capillary feeding or solenophagy. The other families are characterized by pool feeding (telemophagy), which involves the excavation of a sump or pit with the mouthparts, and then imbibing the blood that accumulates at the site.

The Simuliidae, the black or buffalo flies, include important vectors of protozoa (e.g. the avian parasite *Leucocytozoon*) and nematodes (especially *Onchocerca volvulus* the causative organism of river blindness in man), and have been recently shown biologically to transmit VSV, the first report of transmission of an arbovirus by a member of the Simuliidae (Cupp *et al.*, 1992). Despite their large numbers, and haematophagous nature of the females, little work has been done on their role in mechanical transmission. They have coarse saw-like mouthparts and are pool feeders. These bites are painful, and a large amount of saliva is required to keep up the blood flow often leading to a hypersensitivity response. Because of the painful nature of these bites and the amount of blood lost, cattle can suffer severe biting stress and anaemia, which may on occasion lead to shock and death. The problem is exacerbated by the fact that in temperate climates the flies are highly seasonal and tend to occur in huge swarms. Studies of attack rates have shown that on average one cow can be bitten 25 000 times a day (Walker, 1990).

The family Ceratopogonidae consists of the midges. The important bloodsucking varieties are confined to the genera *Culicoides* and *Leptoconops.* Flight is limited but they may travel long distances with the prevailing wind. Feeding is largely restricted to the night and, being pool feeders, the bites are painful. Nocturnal swarms can cause biting stress, although this has been best recorded in temperate countries. In the tropics, breeding in mud and damp vegetation tends to be continuous, and although greater numbers are seen during and after the rainy season large swarms are less common. *Culicoides* spp. are important as biological vectors of bluetongue virus and African horse sickness virus, amongst others. They have also been implicated in the mechanical transmission of both Fowl pox and Rift Valley fever (Table I).

The mosquitoes (Culicidae), of which there are nearly 3000 species, transmit malaria, yellow fever and a number of important animal viruses. The females of all species are nocturnal, solenophagic blood feeders, laying a batch of eggs near water after each meal, a blood meal being required for the maturation of the eggs. Under favourable conditions there may be a population explosion, and the subsequent swarms of insects can induce serious clinical signs in domestic species, including loss of production. Severe swarms tend to be limited by both season and geography. Over 150 viruses have been isolated from 180 species of mosquito, the majority of these being arboviruses. Other viruses are mechanically transmitted: both the rabbit myxoma virus and birdpox virus can be transmitted for long intervals after ingestion. Mosquitoes are epidemiologically associated with the epidemics of lumpy skin disease and recent research suggests that intravenous inoculation of lumpy skin disease virus leads to a more severe clinical syndrome in cattle than seen following intradermal or intranasal inoculation (Carn & Kitching, 1995).

More species of viruses have been isolated from *Culex* spp. than from *Aedes* spp., of mosquito despite the greater number in the latter genus. Both genera of mosquito mechanically transmit myxoma virus and the epidemiology of myxomatosis is greatly influenced by the vector species (Fenner & Ratcliffe, 1965). Spread along watercourses and across flood plains is due to activity of the *Culex* spp. vectors and through semi-arid country by *Anopheles* spp. The *Culex* spp. have the advantage of more ornithophily and greater host plasticity than *Aedes* spp. They require permanent bodies of water for breeding, and have subsequently developed a degree of pollution resistance. *Aedes* spp., in contrast, often breed in temporary water, and have the added feature of producing highly drought resistant eggs, enabling them to exploit a wide range of habitat, from grasslands in all parts of the world to arctic tundra. *Aedes* spp. are vectors of Rift Valley fever virus, which they transmit transovarially (Fenner *et al.*, 1993). Mechanically they transmit Shope fibroma virus and rabbit papilloma virus.

VERTEBRATE VIRUSES TRANSMITTED BY DIPTERA

The laboratory criteria for mechanical transmission are: (1) interrupted feeding followed by sustained transmission rates in the few days immediately following the infected meal (when biologically transmitted viruses are in their extrinsic incubation period); and (2) a decrease in transmission over longer periods of time, and with the number of probes. Some arboviruses are also mechanically transmitted, so the latter is of great importance.

For mechanical transmission to occur, virus must be accessible to the vector's mouthparts, and reach high titres in the skin or the blood of the vertebrate host. Because of the requirement for presenting a high virus titre to the vector, many of those viruses most reliant on mechanical transmission produce skin tumours or nodules. Few of these viruses are reliant solely on vector transmission for spread, but biting flies may have a large influence on the disease pattern in the natural state. Equine infectious anaemia (EIA) virus is one of the few examples of a mechanically transmitted virus that appears to be completely reliant on an appropriate vector for horizontal transmission.

A wide range of viruses are transmitted by the Diptera. For a virus to be mechanically transmitted, a threshold titre in skin or blood must be reached and the virus must be resistant to desiccation and pH change. The mechanically transmitted virus must be resistant to inactivation and survive exposure on the insect's mouthparts until it next feeds. Both DNA and RNA viruses can fulfil these conditions, and there seems to be little vector specificity. Biological transmission requires little or no ability to survive adverse environmental conditions, but the virus must be adapted to replication in two hosts from different phyla, and therefore be able to circumvent two entirely different immune systems and multiply at widely different temperatures. Biological vectors transmit predominantly RNA viruses.

The animal viruses that are mechanically transmitted with no evidence of biological transmission belong to the Poxviridae, Herpesviridae and Papovaviridae, all of which are DNA viruses, and also to the Retroviridae, which are RNA viruses. Some viruses are transmitted both mechanically and biologically, occasionally by the same vector, and these include bluetongue virus, African swine fever virus, Rift Valley fever virus and vesicular stomatitis virus (Table II).

Incidental association of virus and insect

Some viruses are primarily transmitted by contact or aerosol, but may under certain conditions be transmitted by arthropods. For example, foot-and-mouth disease virus (FMDV), which is primarily spread by contact and well known for its ability to spread by aerosol, may be transmitted by flies contaminated by the virus-rich secretions produced by the infected animal (Hyslop, 1970) or by tsetse flies during feeding (Webb, 1990). However, vectors are of negligible importance in the epidemiology of the disease. Rinderpest virus is also primarily spread by contact, but may be spread by biting flies (Bhatia, 1935): again, the importance of vectors in the natural history of the disease is minimal. Bluetongue virus, which is biologically transmitted by *Culicoides imicola* may also be mechanically transmitted by the sheep ked, *Melophagus ovinus* (Luedke *et al.*, 1965), the ked's role in transmission is largely incidental.

Feline leukaemia virus (FeLV) is known to transmit horizontally in experimental cat colonies, and feline lymphosarcoma occurs in clusters in multi-cat households and breeding colonies, probably due largely to transmission of the virus in the saliva during mutual grooming or feeding behaviour. FeLV infected lymphocytes were detected in a mosquito that had previously fed on a FeLV positive cat suffering from lymphosarcoma (Hardy *et al.*, 1975). It is therefore possible that haematophagous ectoparasites could transmit FeLV. Cat fleas, *Ctenocephalus* spp., mosquitoes and other biting flies may therefore have a role (Hardy *et al.*, 1975).

Rift Valley fever (RVF) virus is, like bluetongue virus, also predominantly transmitted biologically, but mosquitoes are the principle vector. However, it has been

Table II
Animal viruses mechanically transmitted by Diptera

Virus family	*Virus genus*	*Virus species*	*Principal mode of horizontal transmission*	*Mechanical vector*	*References*
Poxviridae	Avipoxvirus	Fowl pox virus	Mechanical/contact	Mosquitoes	Brody, 1936
				Midges	Fukuda *et al.*, 1979
	Capripoxvirus	Sheep pox virus	Contact	*Stomoxys*	Kitching & Mellor, 1986
		Lumpy skin disease virus	Mechanical	Mosquitoes	Carn & Kitching, 1995
	Leporipoxvirus	Myxoma virus	Mechanical/contact	Mosquitoes	Fenner *et al.*, 1952
				Tabanids	Day, 1955
		Shope fibroma virus	Mechanical	Mosquitoes	Kilham & Dalmat, 1955
	Unclassified	Squirrel pox virus	Mechanical	Mosquitoes	Tripathy *et al.*, 1981
Unclassified	African swine fever virus	African swine fever virus	Biological/contact/fomites	*Stomoxys*	Mellor *et al.*, 1987
Retroviridae	Lentivirus	Equine infectious anaemia virus	Mechanical	Tabanids, mosquitoes, *Stomoxys*	Hawkins *et al.*, 1973 Issel & Foil, 1984
		Bovine leukaemia virus	Mechanical	Tabanids	Foil *et al.*, 1988a Ohshima *et al.*, 1981
		Feline leukaemia virus	Contact	Tabanids	Hardy *et al.*, 1975
		Friend leukaemia virus		*Stomoxys*, mosquitoes	Fisher *et al.*, 1973
Paramyxoviridae	Morbillivirus	Rinderpest virus	Contact	Tabanids	Bhatia, 1935
Reoviridae	Orbivirus	Bluetongue virus	Biological	Midges, keds	Luedke *et al.*, 1965
Togaviridae	Pestivirus	Bovine viral diarrhoea virus	Contact	Tabanids and *Stomoxys*	Tarry *et al.*, 1991
		Hog cholera virus	Contact/fomites	Tabanids	Tidwell *et al.*, 1972
		Western equine encephalitis	Biological	Tabanids	Goldfield *et al.*, 1968
Papovaviridae	Papillomavirus	Rabbit papilloma virus	Contact	Mosquitoes	Dalmat, 1957
Bunyaviridae	Phleboviridae	Rift Valley fever virus	Biological	Tsetse, *Stomoxys*, sandflies, midges	Hoch *et al.*, 1985
Rhabdoviridae	Vesiculovirus	Vesicular stomatitis virus	Biological, mechanical	Tabanids, *Stomoxys*, Mosquitoes	Ferris *et al.*, 1955
Herpesviridae	Herpesvirus	Bovine herpes mammillitis virus	Mechanical	*Stomoxys*	Gibbs *et al.*, 1973a
Picornaviridae	Aphthovirus	Foot-and-mouth disease virus	Contact	Biting flies	Hyslop, 1970

observed that disease spreads very rapidly in some flocks, implying that mechanical transmission may also be important (Jupp *et al.*, 1984). Mechanical transmission of RVF virus to laboratory animals and lambs has been shown to occur following the feeding of many different Diptera on infected hamsters (Hoch *et al.*, 1985). *Glossina morstitans*, several mosquito species, *Stomoxys calcitrans*, *Lutomyzia longipalpis* and *Culicoides variipennis* were all competent vectors. The probability of transmission was inversely proportional to the time delay before second probing, and proportional to the initial viraemia titre.

Arthropods do not seem to be of great importance in the transmission of sheep and goat pox (capripox), despite the usual association between pox viruses and vectors (see next section). Natural capripox infections occur throughout the endemic area as a result of contact between diseased and susceptible animals (Encyclopaedia Britannica, 1810; Davies, 1976; Murty & Singh, 1971). Transmission of capripoxvirus by *Stomoxys calcitrans* in two out of three attempts between sheep has been shown (Kitching & Mellor, 1986). In the same experiment, transmission by biting lice (*Mallophaga* spp.), sucking lice (*Damalina* spp.), sheep head flies (*Hydrotea irritans*) and midges (*Culicoides nubeculosus*) was unsuccessful, although virus was isolated from *Hydrotea irritans* post feeding. From the large number of papules that developed on susceptible sheep following the feeding of infected *Stomoxys calcitrans*, it was estimated that 10% of the flies transmitted virus. Experimentally animals may be infected intradermally, intravenously, subcutaneously or by aerosol (Kitching & Taylor, 1985).

Stomoxys calcitrans has also been shown to be an experimentally competent mechanical vector of African swine fever virus (ASVF). African swine fever is an important disease of domestic pigs causing pyrexia, severe internal haemorrhages and high mortality. Infected animals have high viraemias. The transmission of the virus is usually biological, *Ornithordoros* spp. being the natural soft tick host. Outside the endemic area of the biological vector, transmission occurs through the ingestion of infected feed and through contact. *Stomoxys calcitrans* has been shown to transmit ASFV over a period of 24 h after feeding on an infected pig, and may be relevant to the control measures to be used during an outbreak (Mellor *et al.*, 1987).

African swine fever is the most important differential diagnosis for hog cholera (classical swine fever). This disease, caused by the Pestivirus hog cholera virus (HCV), manifests itself as an acute haemorrhagic disorder, or when piglets are infected *in utero* as weak and deformed litters, or abortion. The most important means of transmission for this virus is across the placenta or through contact. Infected feed is a common source of a new outbreak, but some epidemiological evidence indicates that flies may be another means of dissemination (Tidwell *et al.*, 1972). Muscid flies have been shown to transmit the virus following exposure to ocular secretions, and *Stomoxys calcitrans* through biting within 24 h of feeding (Dorset *et al.*, 1919). Trials conducted by Tidwell *et al.* (1972) showed the potential for two *Tabanus* spp. to transmit HCV, and implicated three others. Transmission by mosquitoes was not demonstrated, but numbers were limited.

Bovine leukaemia virus (VLV), a B lymphocyte-associated retrovirus, causes two clinical syndromes in affected cattle, a chronic lymphocytosis or lymphosarcoma. The virus is predominantly transmitted vertically through ingestion in the colostrum and milk. Horizontal transmission requires the transfer of infected lymphocytes, *via* the mouthparts of insects, or as a consequence of the use of contaminated needles or veterinary instruments. Horizontal transmission occurs more frequently during the summer months, indicating the relative importance of arthropod vectors (Bech-Nielsen *et al.*, 1978). Transmission trials demonstrated the mechanical transmission of BLV to goats and sheep from a cow with a persistent lymphocytosis, but 50–100 *Tabanus fuscicostatus* were required; no transmission occurred with groups of 10 or 25 flies (Foil *et al.*, 1988a). Studies have shown that at least 10% of the blood residue on the mouthparts of this fly may be deposited during the second probe (Foil *et al.*, 1987).

Epidemiological association between virus and insect

Many viruses may be transmitted mechanically, in addition to other means, but if vector transmission is effective then the epidemiology of the disease will be significantly influenced by vector distribution and dynamics. It is notoriously difficult to implicate a vector by laboratory data derived from analysis of field catches of potential vectors during a disease outbreak: the causative virus may be isolated from all haematophagous species following feeding, regardless of their role in transmission. Consequently, an association between disease and vector is usually made after the analysis of epidemiological data. Temporal and geographical patterns of disease occurrence and spread are then associated with vector distributions.

Bovid herpes virus-2 (BHV-2) infection causes the clinical syndrome of bovine herpes mammillitis (BHM), a localized, painful condition of the teats and udder, usually seen in first calving heifers. Both BHM and pseudo-lumpy skin disease (another manifestation of BHV-2 infection) have a seasonal prevalence, and both are thought to be initiated by biting insects (Scott, 1990). BHM can only be produced experimentally when the virus is introduced below the level of the stratum germinativum of the udder or teat skin, and *Stomoxys calcitrans* has been proposed as a mechanical vector (Gibbs *et al.*, 1972, 1973a). *Stomoxys calcitrans* fed on infected blood have mechanically transmitted enough virus to infect cell monolayers, one $TCID_{50}$ being the minimal infective dose for cattle (Gibbs *et al.*, 1973b).

Arthropods have been implicated in the mechanical transmission of a large number of poxviruses (Table II), suggesting that they are a significant means of poxvirus transmission. The pathology caused by the poxviruses consists of a variety of epidermal changes, ranging from typical vesicular pock formation, such as produced by variola (smallpox) virus, to a fibromatous lesion, such as Shope fibromatosis, to subcutaneous tumour formation, such as occurs in rabbits with myxomatosis. These lesions are very rich in virus and provide attractive feeding sites for arthropods.

Tripathy *et al.* (1981) reported circumstantial or experimental evidence for arthropod transmission in Tanapox (Yaba-like disease), Shope fibromatosis, hare fibromatosis, and squirrel fibromatosis. Squirrel pox has been experimentally transmitted by mosquitoes, and the occurrence of Tanapox in children living along the Tana river in Kenya has also been linked to mosquitoes. The host–virus–vector relationships of Shope fibroma virus was elucidated by Kilham and Dalmat (1955), who showed that virus was localized in the head region of the mosquito, and that the mosquitoes remained infective for 5–6 weeks. Although there was some evidence for biological transmission, the virus titre decreased initially to rise again over several weeks, and no virus was ever isolated from the thorax, abdomen or salivary glands of infected mosquitoes. The importance of the fibroma in the disease cycle was also evident. The fibromata, as well as being particularly attractive to the mosquitoes, were also potent reservoirs of live virus. Fibromas were shown to persist over winter in a naturally infected rabbit, and to remain infective for 10 months experimentally, despite high antibody level. High virus concentrations were still detectable in experimentally induced lesions 1 year after inoculation.

The importance of mechanical transmission by vectors in the epidemiology of fowlpox has been recognized since the 1930s (Brody, 1936), and new vector species continue to be identified (Fukuda *et al.*, 1979). It was noted by Brody (1936), that to transmit effectively the mosquito must feed on lesions, and not unaffected areas of skin. The virus was shown to persist for long periods of time on infected mosquito mouthparts. Transmission was successful in four out of six mosquitoes after 39–41 days, and in the same experiment three out of 15 pins used to probe the lesions were still able to transmit disease after 40–45 days.

Fenner *et al.* (1952), concluded that, with respect to its ability to transmit myxomatosis, the mosquito *Aedes aegypti* was essentially a flying pin. Mosquitoes were marginally more effective than pins at transmission of myxoma virus, perhaps reflecting a relatively protected environment for the virus on the insect's mouthparts. Mosquitoes were not infective unless they contaminated their probosces by penetrating infected epithelial cells and, due to the irregular distribution of virus within the lesion, it was chance whether a mosquito or pin became infective. Similarly, when either insect or pin were probing following infection, it was chance if an infective dose of virus was dislodged. The probability of infecting a mosquito increased with the age of the lesion: although virus was present before the clinical manifestations of the disease, maximum titres were reached after the signs were well developed, and so it was concluded that those rabbits with numerous and extensive skin lesions were the most important in the transmission of the disease.

Viruses which are predominantly mechanically transmitted by vectors undergo rigorous selection of available generic variants, based on titre of virus accessible to the mouthparts of the insects and the longevity of the source of virus in the vertebrate host (Fenner & Ratcliffe, 1965). The evolution of Myxoma virus is the classical example of this. In Australia in the 1950s, the KM13 strain of Myxoma virus, although not the most virulent field strain, rapidly became predominant. Some other strains killed the vertebrate host within 12 days, allowing only 4–5 days when the virus was at high enough titres in the skin overlying the tumours for effective transmission. Strains less virulent than KM13 produced high titres, but the lesions quickly broke down and formed scabs so the infective tissue was not accessible to the biting insect despite the animal surviving with lesions for many weeks, hence decreasing the time available for transmission to about 7 days. Following infection with the KM13 strain, the host survived with virus at a high enough titre for effective transmission for 17 days before death, and consequently this strain was heavily selected for in the field (Fenner & Ratcliffe, 1965).

Poxviruses may also be transmitted by insects that do not belong to the order Diptera. Swinepox, which may be spread through direct contact and skin abrasions, is mechanically transmitted by the louse *Haematopinus suis*. The louse greatly influences the severity and course of the disease, infestations resulting in generalized skin lesions. The louse carries infectious virus for weeks or months and biting insects are suspected of being responsible for spread between premises (Tripathy *et al.*, 1981).

Obligate association between virus and vector

Most viruses that are mechanically transmitted may also be transmitted by other means. It is difficult to prove conclusively by experiment that no modes of transmission other than by vectors can occur, and it is subsequently hard to ensure accuracy when attributing viruses to this group. Equine infectious anaemia virus has not been shown to be naturally transmitted horizontally by any means other than vectors. LSDV appears to be similarly constrained (Carn & Kitching, 1995).

In contrast to the capripoxviruses causing capripox of small ruminants (sheep pox and goat pox), LSDV, which causes the capripox disease of cattle, is highly dependent on vector transmission. In common with the other pox diseases, LSD is characterized by skin lesions. Infected animals are pyrexic, internal organs may be affected, and losses are increased due to myiasis and secondary infection, particularly of the udder (Davies, 1991). It is a highly seasonal disease, and has long been associated with biting insects. The first reports of LSD, in 1929, were from Northern Rhodesia (Zambia) and the disease was then assumed to be a hypersensitivity type reaction to the bites of insects, and was therefore called 'pseudo-urticaria' (MacDonald, 1931). It was noted at that time to be most prevalent along low-lying river banks where there were large numbers of flies.

Recent experimental data (Carn & Kitching, 1995) support the hypothesis that transmission of LSDV in the absence of arthropods does not occur. Naïve animals in contact with severely diseased animals did not become infected, sero-convert or become immune to challenge. In contrast, it has been shown that less than five probings from a pin that has contacted a high titre of virus are sufficient to infect an animal (Carn & Kitching, unpublished results). Carn and Kitching (1995) suggest that intravenously feeding vectors, such as mosquitoes, are required for an epidemic to become established, and that intradermally feeding vectors are responsible for the endemic status.

Equine infectious anaemia (EIA), or 'swamp fever', so called because of the association of the disease with damp, low-lying areas, is a persistent virus infection of horses which presents as occasional febrile episodes between periods of clinical quiescence (Powell, 1976). During recrudescence, or acute disease, signs include pyrexia, depression, anorexia, anaemia, oedema of the limbs and a raised, arrythmic heartbeat. Mortality in young horses may be high. The epidemiology of the disease is closely associated with the prevalence of biting flies, especially tabanids (Kemen *et al.*, 1978). *Stomoxys calcitrans* has also been implicated. Horses that are pastured together may all become infected, although horses in adjacent fields do not. This is thought to be due to the very persistent biting behaviour of the *Tabanus* spp. involved, their low potential for moving between feeding periods, and the requirement for transmission of the virus to take place within a few hours of feeding on the infected animal (Powell, 1976). EIA virus does not appear to be naturally transmitted horizontally in the absence of arthropods and vampire bats, although man's intervention with needles, tooth rasps and twitches, may result in transmission. It has been shown that EIAV can be transmitted by a single fly (Hawkins *et al.*, 1976), and that multiple interrupted feedings of an afebrile carrier followed by immediate feeding on a susceptible horse may lead to transmission (Kemen *et al.*, 1978). This is of particular significance as most horses with EIA virus are without clinical signs. Once infected, horses have a persistent viraemia, the virus being free in the plasma. Virus titres are high during clinical signs, and drop when the horse recovers, although during the sub-clinical period following acute infection virus titres fluctuate, and the likelihood of the animal acting as an infectious focus therefore changes with time. The significance of the threat caused by a potentially infective animal will depend on vector burdens, vector species and the physical distance between infected and susceptible horses (Foil *et al.*, 1988b).

CONTROL OF VIRUSES SPREAD BY INVERTEBRATES TO ANIMALS

Transmission of disease is governed by certain general principles, the complexity of which is increased by the addition of an arthropod into the disease cycle. Transmission rates depend on the extent of effective contact between infected and susceptible hosts. For a mechanically transmitted virus, there must be sufficient virus to contaminate the arthropod during its feeding to the extent that it can then transmit the virus to another susceptible vertebrate. In both cases, the probability of this contact is dependent on both the duration of a sufficiently high virus titre, and the number of arthropods feeding. Diptera typically have a short lifespan: 90% survive for 24 h, thus only one in three of a feeding population will still be alive 10 days later in favourable conditions. Longevity of arthropods is greatly affected by ambient temperature and relative humidity, and humidity of the insects' resting place is especially important. For some viruses, for example EIA virus, transmission has to take place within a few hours of the vector becoming contaminated, or the virus will be inactivated. Other viruses, for example pox viruses, can persist on mouthparts for at least 30 days (Kilham & Dalmat, 1955), and so longevity of the vector is more important.

Successful control of vector transmitted diseases relies on knowledge of the host–vector–virus relationship. In the endemic situation, control may be based on decreasing the effective contact rate or reducing the numbers of arthropods. Decreasing effective contact may be achieved by clearing belts of scrub or forest (the endemic environment) so reducing opportunities for arthropod vectors to feed on both wild vertebrate hosts and domestic animals or man (Jordan, 1986). The use of solid housing construction and mosquito netting also reduces feeding opportunities. Population control can be directed at either the vertebrate or invertebrate. The susceptible vertebrate host population may be reduced by control of wild hosts or effectively reduced by immunization.

Arthropod-borne diseases have distinct patterns, both temporal and spatial, that are determined by the distribution and population density of the vector (Bailey &

Linthicum, 1989). Control may therefore be achieved by vector habitat alterations, such as the clearing of undergrowth or the altering of irrigation or draining practices. Other methods of insect control include the use of traps, the introduction of insect diseases, predators and population manipulation. The control of Rift Valley fever has been aided by satellite observation of vegetational changes (Linthicum *et al.*, 1987), which allow rapid focusing of vector control efforts, and if appropriate vaccination (Hayes *et al.*, 1985).

The risk of disease is not limited to the importation of infected animals or their products. The widespread and frequent travel by aircraft has increased the possibility of transfer of infected vectors between distant countries. The possibility of migration of an infected vector should also be considered. If global warming occurs the resulting extension of the endemic range of tropical and sub-tropical dipterous species will increase the threat of windborne vectors importing exotic viral diseases into Europe.

CONCLUSION

Mechanical transmission by arthropod vectors is important in the epidemiology of many virus diseases of animals and frequently occurs in situations where other modes of transmission dominate. Although many viruses produce high titres in the skin of the infected vertebrate host, which facilitates transmission, other viruses are transmitted even during relatively low levels of viraemia. This latter observation may be of consequence when considering the potential for sub-clinical carriers to infect naïve hosts, and given the morphological similarities between EIA virus and HIV, raises the possibility of insect transmission of this important human pathogen. It has been shown that mechanical transmission produces strong selection pressures on the virus, with subsequent genetic drift, and that even very closely related viruses may differ in their requirements or otherwise for vectors. Control of diseases for which vectors have been implicated may be approached by systematic vaccination and vector eradication, or by targeted control methods. The possibility of extension of diseases due to vector spread following changing meteorological conditions or man's intervention should not be ignored.

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