TRANSMISSION OF ARBOVIRUSES WITHOUT INVOLVEMENT OF ARTHROPOD VECTORS

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Summary. – Transmission of arboviruses (arthropod-borne viruses belonging to various virus families) without involvement of arthropod vectors has been documented for years, but the reports have not been reviewed systematically. The recent report of West Nile (WN) virus isolation from a hawk in mid-winter in New York (Garmendia et al., J. Clin. Microbiol. **38**, 3110–3111, 2000) generated a considerable interest in this mode of arbovirus transmission. In this article, the data available worldwide are analyzed according to the factors involved in such a transmission under natural conditions, mode of infection, virus entry mechanism, administration and efficacy evaluation of vaccines, and significance in agricultural trade and public health. Analysis of numerous reports compiled for this review revealed that peroral and intranasal/aerosol transmissions are very common among arboviruses. The mechanism of virus infections in animals was most extensively studied for intranasal/aerosol infection, confirming two routes of virus spread to central nervous system (CNS), olfactory and hematogenous. To rule out the possibility of asymptomatic, cryptic infection the efficacy evaluation of candidates for vaccines against neurotropic arboviruses should include virus isolation from tissues of not only symptomatic but also of asymptomatic animals that survive intranasal virus challenge. Human activities, such as feeding livestock animals with food containing virus-contaminated meat and assembling a large number of livestock from many geographically-separated locations, have been identified as a cause of spread of some arboviral diseases. Despite numerous laboratory reports, the significance of this mode of transmission of arboviruses under natural conditions was rarely investigated, except for a few viruses important for veterinary medicine.

Key words: arbovirus; transmission mechanism; direct transmission; contact transmission

Introduction

The recent isolation of WN virus from a hawk in mid-winter in New York in the absence of mosquito activity (Garmendia *et al.*, 2000) offered immediately at least two possible explanations: virus persistence in vertebrate host and infection by ingestion of WN virus-infected prey. As WN virus appears to have established endemicity at least in northeastern parts of the USA, undoubtedly both the overwintering of arboviruses in temperate regions and significance of those phenomena in natural transmission will be studied in more detail.

Thus it is important to examine the accumulated data to better understand those phenomena. The persistence of

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Abbreviations: AHS = African horse sickness; ASF = African swine fever; BT = bluetongue; CCHF = Crimean-Congo hemorrhagic fever; CNS = central nervous system; EEE = eastern equine encephalomyelitis; JE = Japanese encephalitis; LI = louping ill; Nt = neutralizing; RH = relative humidity; RVF = Rift Valley fever; SFF = Semliki Forest fever; SLE = St. Louis encephalitis; TBE = tick-borne encephalitis; VEE = Venezuelan equine encephalomyelitis; VS = vesicular stomatitis; VSNJ = VS New Jersey serotype; WEE = Western equine encephalomyelitis; WN = West Nile; YF = yellow fever

arboviruses in vertebrate hosts has been recently reviewed (Kuno, 2001). The transmission of arboviruses without involvement of arthropod vectors is the focus of this article. The phenomenon of arbovirus infection without arthropod involvement has been often termed "direct" transmission. When the exact mode of infection was identifiable, it has been traditionally subclassified by the route of infection into either oral or intranasal/aerosol transmission. In this article, for the sake of clarity, these subdivisional terms are applied to the reports in which the mode of infection was known to the investigators; and all reports of so-called "contact" transmission without an identifiable route of virus entry are interpreted as examples of direct transmission without a known mode of infection. As the *in utero* transmission and congenital infection are technically another examples of infection that do not involve arthropod vectors, they are discussed separately from direct or contact transmission. Any experimental data on viral infection of vertebrate hosts through skin abrasion, naturally acquired or artificially made by means of scarification or other types of invasive techniques, including the reports of human infections through blood transfusion, are excluded. Furthermore, the references to intranasal/aerosol transmission in humans are held to minimum, since such reports have been repeatedly reviewed before in relation to biosafety and protection measures for laboratory workers.

Requirements for direct transmission

Requirements for direct transmission under natural conditions

For direct transmission to occur, the critical requirements that must be met include (a) shedding of infectious virus by infected animals; (b) stability of the shed virus under environmental conditions; (c) presence of susceptible, uninfected animals in the same facility or within close proximity to infected animals coincident with virus shedding; and (d) mode of virus entry that favors the infection of animals, including biologic factors, such as animal behavior and physical mechanisms, such as airflow affecting the movement of aerosols. The suspected sources of infectious viruses, analogous to the concept of fomites in etiology of human infections, are excrements, saliva, nasopharyngeal secretions, conjunctival fluid, external lesions (such as vesicles), milk, and semen. Various modes of entry, oral, intranasal, via aerosol, and by physical contact have been reported. As controlling animal behavior, which dictates the mode of entry, is difficult, experimental studies were most often conducted by arbitrarily applying a selected mode of entry and disregarding variation in animal behavior and air flow.

Experimental direct transmission in animals

Experimental data in Table 1 clearly demonstrate that direct transmission of arboviruses representing various virus families occurs in many animal species and that possible modes of infection are variable, depending on virus, host, and animal behavior. Peroral transmission has been often demonstrated by feeding virus-contaminated food to animals, by swabbing mouth (Holden and Sussman, 1955), by forced feeding virus-contaminated materials into the alimentary tract of animals through a catheter (Ilyenko, 1957), or by allowing animals to get milk from infected mothers (Shah, 1965; Woodall and Roz, 1977). Insectivorous animals, such as bats, become infected by ingesting virus-infected mosquitoes, as demonstrated for Rift Valley fever (RVF), yellow fever (YF) (Oelfsen and Van der Ryst, 1999) and Japanese encephalitis (JE) (LaMotte, 1958) infections. Intrarectal instillation of albino mice with Semliki Forest (SF) virus also led to viral infection (Reagan *et al.*, 1953).

Aerosol transmission has been demonstrated by exposing animals to sprays produced by atomizers or nebulizers in a closed chamber or animal facility (Phillpotts *et al.*, 1997). In one experiment, air from the room housing infected animals was blown with a fan to another room housing uninfected animals via an air duct (Wilkinson *et al.*, 1977). The outcome of aerosol exposure, however, may be significantly different between two methods: nose-only and whole body exposures (Stephenson *et al.*, 1988). Intranasal instillation was invariably performed by dropping a known amount of virus into a nostril of the animal, using a blunt needle, fine capillary tubes or similar utensils.

In contrast to the above modes of transmission, the exact mechanism(s) of so called "contact" transmission most likely involves one or a combination of the above mechanisms, in addition to physical contact (Zarate and Scherer, 1968; Howard, 1974; Seymour *et al.*, 1983). In VS, although contact transmission was confirmed early as one of the modes of transmission (Wagener, 1931; Patterson *et al.*, 1955), not all infected animals developed external lesions, such as vesicles, and yet they could transmit VS New Jersey serotype (VSNJ) virus to contact hosts (Patterson *et al.*, 1955). However, development of vesicles facilitated contact transmission; apparently, the bodily focus of infection influences how and where normal contact animals acquire the infection. For example, when healthy pigs were inoculated with VSNJ virus in the apex of the snout or coronary band, vesicles developed only at the sites of inoculation. When healthy pigs were then placed in contact with the inoculated ones, the contact pigs became infected, shedding virus from the tonsils of the soft palate. However, when healthy pigs were exposed to those inoculated intradermally in the coronary band or intranasally, contact transmission did not occur (Howerth *et al.*, 1997).

Evaluating the variables among the factors required for direct transmission

Virus shedding, host and virus factors

Not all infected animals shed virus. However, virus shedding into excrements, saliva, nasopharyngeal secretion, and conjunctival fluid has been documented in many species of animals infected with arboviruses. Virus shedding to the upper alimentary tract was confirmed by isolating virus from crop washings of birds and from regurgitated food used by the birds for feeding nestlings (Winn and Palmer, 1961). With pigs which began shedding VS virus as early as 12 hrs after intravenous inoculation, uninfected pigs in the same pen acquired infection by a contact 24 hrs after the donor pigs had been inoculated (Patterson *et al.*, 1955). In an intracage transmission of Venezuelan equine encephalomyelitis (VEE) virus in cotton rats, contact mates began demonstrating viremia between 4 and 10 days after an infected rat was introduced into each cage (Howard, 1974). Virus shedding does not last long in most cases, but it may last much longer in others; for example, virus was shed into urine in Modoc virus-infected hamster for as long as 153 days (Johnson, 1970) and Rio Bravo virus was shed for at least 309 days in the saliva of chronically infected bats (Baer and Woodall, 1966).

Virus is often shed at irregular intervals, and the shedding lasts for various lengths of time, depending on virus and host factors. Virus factors usually include virus species, virus strain, passage history, and dosage; host factors include animal species, breed, age, and route of viral infection. For example, in chickens, WN virus was shed in excrements for only 4–5 days (Senne *et al.*, 2000), although infectious virus persisted in many organs for 7–10 days (Kundin, 1963; Senne *et al.*, 2000); but in a duck species (*Anas*), the viral shedding into excrement lasted as long as 22 days (Fedrova and Stavskiy, 1972). In eastern equine encephalomyelitis (EEE) virus-infected pheasants, virus could be isolated from quill 1–2 days longer than from blood; this was an interesting finding since this bird transmits the virus by feather picking and canniablism among pen mates (Satriano *et al.*, 1959). For experimental demonstration of direct transmission, usually animals at young ages have been preferred. Thus, for avian hosts, newly hatched "wet" chicks have been most frequently used (Winn *et al.*, 1957; Reeves *et al.*, 1958; Bourke, 1964). When virus strain, passage history, route of inoculation, vertebrate host, and period of observation in experimental infection are all different, naturally quite different results are obtained regarding viral shedding, as demonstrated in two studies of Modoc virus infection (Davis and Hardy, 1974; Fairbrother and Yuill, 1987).

Virus stability

The rate of infectivity decay under particular environmental conditions varies considerably among viruses. For example, at 21°C and relative humidity (RH) ranging from 23% to 80%, no significant decay was observed for at least the first 6 hrs for St. Louis encephalitis (SLE) virus (Rabey *et al.*, 1969). On the other hand, the survival of JE virus was found to be inversely related to RH; its half lives at RH of 80%, 55%, and 30% were 28, 38, and 62 mins, respectively (Larson *et al.*, 1980). High RH was favorable for retaining the infectivity of YF and RVF viruses (Miller *et al.*, 1963). In one of the most puzzling reports regarding arbovirus stability, RVF virus was isolated from a pharyngeal washing of a laboratory worker who had never worked with the virus or infected animals, but who became sick after he assisted in washing and repainting walls and cleaning the floor of a room where RVF virus had been last used more than 3 months before the cleaning assignment (Francis and Magill, 1935). Regarding the stability of other viruses at 23°C , the survival of aerosolized VS virus was best at 10% RH (Songer, 1967). VEE virus survived better, for 23 hrs after aerosolization at lower temperature (9–9.5°C) and at RH of 19% (Harper, 1961). African swine fever (ASF) virus is notable for its survival at 56°C for 30 mins (Bengis, 1997).

Under artificial conditions, infectivity of arboviruses can be maintained longer. Lyophilized, attenuated YF (17D) virus vaccines manufactured by many institutions, in the presence of stabilizers, lost less than 0.5 log from log LD_{50} (titrated on mice) at 37°C in 14 days (Monath, 1996). A similar observation was reported elsewhere (Perraut *et al.*, 2000).

Generally, proteins increases the survival of arboviruses (Benbough, 1971; Gresikova *et al.*, 1975). Tick-borne viruses are discharged in feces together with crystallized hemoglobin which is derived from the blood of the vertebrate host. In an unusual experiment, in which ticks were infected with EEE virus, the virus survived in crystallized hemoglobin for as long as 11 and 8 days at 18–23°C and 23–30°C, respectively (Rehacek, 1958). ASF virus remains infectious in serum at room temperature for 18 months and for 3–6 months in processed hams, salamis, and smoked sausages (Bengis, 1997).

Animal behavior

Although the mode of entry of virus into susceptible host in direct transmission is largely determined by the behavior of animal, the difficulty of standardizing this variable contributed sometimes to discrepant results and skepticism about direct transmission. Any body parts (beak, snout, mouth, leg, tail, hair, feathers, etc.) may be contaminated with shed virus through a variety of behaviors, such as

sniffing, licking, pecking, grooming, nuzzling, mutual preening, eating (including cannibalism), drinking, and all forms of physical contacts among animals, which are facilitated by crowded conditions. Unfortunately, too often the information necessary for evaluation of the probability of contact, such as dimensions of animal facility, body size of animal, animal density, spatial distribution of food and water sources, and availability of bedding materials or nests, are not described in publications. If flying birds are studied, tridimensional spacial distribution of resting sites and flying activity become another factors modulating crowding and mobility of aerosols.

In one of a few well designed studies, the modes of transmission of Highlands J virus infection in chickens were investigated by restricting the movement of infected birds (Bourke, 1964). The study revealed that the oral-oral route of infection through contact between beaks rather than fecal-oral route was responsible for direct transmission of this virus. Regarding the importance of the beak, the aforementioned direct transmission of EEE virus in pheasants (Satriano *et al.*, 1959) was confirmed by rendering the birds unable to engage in feather picking and cannibalism In another case, when a bushpig was infected with a particular strain of ASF virus, placed in the same pen with healthy domestic pigs immediately after it was infected and held for only 3 hrs before immediate transfer to another pen to prevent further physical contact, the transmission to the domestic pigs still occurred. On the other hand, when the roles of the two species were reversed, direct transmission did not occur (Anderson *et al.*, 1998). In VSNJ virus infection, the contact transmission in domestic pigs occurred when normal pigs were put in contact with the pigs inoculated in the apex of the snout but not in contact animals exposed to the pigs infected intradermally or intranasally (Howerth *et al.*, 1997).

Virus entry mechanisms in direct transmission

Howitt (1932) has demonstrated a direct western equine encephalomyelitis (WEE) virus dissemination in blood before viral invasion of CNS in the animals from which olfactory bulbs had been surgically removed; this suggested hematogenous route of CNS infection. Although a similar result was obtained in EEE virus infection of guinea pigs and monkeys, it could not necessarily be interpreted that the intranasally introduced virus would bypass olfactory bulbs to infect CNS (Hurst, 1936). Later experiments demonstrated that the process of CNS invasion largely depended on the route of inoculation and properties of the virus. For example, intranasal and aerosol applications of VEE virus resulted in virus spread to CNS by both vascular (hematogenous) and olfactory routes in healthy mice, but primarily by the olfactory route in mice passively immunized by the injection of a rabbit VEE virus antiserum (Ryzhikov *et al.*, 1995). In aerosol infection, the virus appeared first in the olfactory area including the olfactory bulbs and then in the brain before the virus appeared in low titers in blood (Ryzhikov *et al.*, 1995).

Other experiments demonstrated that subcutaneous inoculation of mice with VEE virus led to either direct invasion of the CNS through the olfactory neuroepithelium, the olfactory bulb from blood or the trigeminal nerve. This revealed that the presence of the olfactory bulb was not an absolute requirement (Ryzhikov *et al.*, 1995; Charles *et al.*, 1995).

In mice infected with aerosolized JE virus, lesions developed first in the olfactory bulb, frontal lobe and olfactory portions of the cerebrum, followed a few days later by the development of necrotic lesions in the olfactory bulb, cerebrum, brain stem, and spinal cord (Larson *et al.*, 1980). The lesions were not observed in the olfactory epithelium of the nasal cavity for a few more days, indicating very late infection of the nasal cavity. Thus, in the absence of viremia, these results strongly suggest virus transport from the nasopharynx directly across the foramina of the cribriform plate to the olfactory bulb, rather than by the hematogenous route (Larson *et al.*, 1980). Similarly, in Syrian hamsters infected peripherally with SLE virus, after a brief period of viremia, cells in the olfactory neuroepithelium were infected; this was followed by axonal transport of the virus to the olfactory bulb and finally by viral invasion of the CNS; the virus was recovered from nasal washings on day 4 post inoculation (Monath *et al.*, 1983).

The process of viral invasion by oral transmission is much less known. When young pigs were infected with ASF virus, whether the animal was inoculated orally or intranasally, the primary infection usually occurred in the upper respiratory tract; the virus spread to lymph nodes in the cephalic region as early as 1 day post inoculation. Exposure of other parts of animal digestive system also led to infection, as demonstrated in SF virus infection of mice by intrarectal instillation (Reagan *et al.*, 1956b) and intake of JE virus vaccine-loaded biodegradable microspheres into Peyer's patch (Khang *et al.*, 1999).

Although intranasal/aerosol transmission of VSNJ virus in pigs has been reported (Howerth *et al.*, 1997), it was found to be ineffective (Stallknecht *et al.*, 1999). Instead, although viremia has been documented, contact transmission was considered the most likely major mechanism, as virus titers in vesicles were reasonably high (Howerth *et al.*, 1997). Accordingly, this virus infection may occur through contact with skin, tonsils or nasopharyngeal excretion or mucous tissues (Stallknecht *et al.*, 1999). Similarly, many reports of direct arboviral transmission are classified as examples of contact transmission because of the difficulty of identifying the exact route of virus entry (Zarate and Scherer, 1968;

Howard, 1974; Sonenshine, 1993; Anderson *et al.*, 1998). For elucidating the exact mechanism for each of those cases of contact transmission by arboviruses sensitive molecular biological techniques in time-course studies, as those which were found useful for unraveling the similar mode of transmission of foot-and-mouth disease virus (Alexandersen *et al.*, 2001), should be used.

Role of direct transmission in nature

The role of direct transmission of arboviruses in natural transmission has been the focus of interest. In theory, the longer virus is shed and remains stable in natural environment and the more often and longer uninfected control animals are exposed to the source of infectious virus through any of the aforementioned routes, the higher the probability of direct transmission in natural conditions. Thus, the role of vertebrate hosts demonstrating asymptomatic, prolonged infection and virus shedding (Kuno, 2001) holds a particular importance in this regard. For example, the congested living condition of deer mice (up to 20 mice/nest in winter) clearly favors a perpetual direct transmission of Modoc virus, a virus without a known vector (Johnson, 1970).

Transmission of VS under natural conditions is one of a few intensely investigated disease problems. The 1982 outbreak of VS in western parts of the USA that continued into winter and other earlier outbreaks that occurred when snow was on the ground (Jenny *et al.*, 1984) strongly suggest maintenance of the virus through contact transmission. It has been noted that the spread of VS in widely-separated geographic locations in the western USA often coincides with seasonal increase in such activities as stock trade/show, state fair, rodeo, and others that favor a congregation of a large number of animals (Bridges *et al.*, 1997). In fact, historically, the assembly of a huge number of horses and mules from many parts of the country for shipment to Europe to assist during the World War I has been strongly speculated to have contributed to the dissemination of VS in the USA (Cotton, 1927). Other human activities also contribute to artificial dissemination of arbovirus diseases. For example, the spread of African swine fever (ASF) outside Africa in the past was caused by the common agricultural practice of feeding foods consisting of underprocessed meat of infected animals to livestock animals (Bengis, 1997). An outbreak of African horse sickness (AHS) occurred in dogs that were fed meat of horse carcasses (Van Rensburg *et al.*, 1981).

Among the known modes of direct transmission (Table 1), the oral route of infection is especially important for birds that feed regurgitated foods to their nestlings and for omnivorous or predatory birds. Thus, it is highly probable that the hawk from which WN virus was isolated in New York in winter had acquired the infection by ingesting infected prey, since the pathological examination revealed that the hawk had been recently infected (Garmendia *et al.*, 2000). It was also demonstrated that EEE among captive birds, such as pheasants, occurred by feather picking or eating diseased pen mates (Holden *et al.*, 1955; Satriano and Sussman, 1959). Also, crows became infected by eating EEE virus-infected chick embryos (Karstad *et al.*, 1959). However, whether this mode of transmission occurs in free-ranging birds under natural conditions still needs to be determined.

Application of the direct transmission mechanism to administration and efficacy evaluation of vaccines

The non-invasive routes of transmission of arboviruses for a vaccination and challenge test have been found to be potentially useful alternatives to traditional parenteral administration for efficacy evaluation of arbovirus vaccines (Walker, 1994; Raengsakulrach *et al.*, 1999a). For example, YF (17D) virus vaccine was administered intranasally to monkeys (Niedrig *et al.*, 1999) and VEE and JE virus vaccines were administered orally to mice (Hart *et al.*, 1997; Ramakrishna *et al.*, 1999). Moreover, three human volunteers orally immunized with an avirulent Langat virus strain for protection against TBE viral infection all seroconverted (Mayer *et al.*, 1976). The efficacy of vaccine candidates is most often evaluated in animal models on the basis of the following factors: absence of clinical symptoms, a favorable immune response indicated by the rise in neutralizing antibody titer, and lack of viremia. However, it has been recognized that the routes of inoculation for vaccination and challenge as well as the qualitiy of vaccine determine the outcome. For example, although peripheral inoculation of an attenuated VEE virus vaccine (TC-83) in mice conferred full protection against an intranasal challenge, immunization with formalin-inactivated recombinant vaccine did not (Jahrling *et al.*, 1984; Charles *et al.*, 1995; Phillpotts *et al.*, 2000). Similarly, in mice passively immunized by subcutaneous inoculation of a rabbit VEE virus antiserum, intranasal virus challenge elicited virus replication in the olfactory bulbs and brain (Ryzhikov *et al.*, 1995). Thus, for full protection against intranasal challenge, three subcutaneous (Hart *et al.*, 1997) or two (one subcutaneous followed by one intratracheal) immunizations (Greenway *et al.*, 1997) were necessary. In contrast to mice, subhuman primates (Monath *et al.*, 1992) and rabbits (Sviatchenko *et al.*, 2000) vaccinated with recombinant VEE virus vaccines were protected against intranasal challenge.

The importance of nasal mucosal immunity was further illustrated by an evaluation of an RVF virus vaccine (Anderson *et al.*, 1991). In that study, rats were intranasally challenged after vaccination. Surprisingly, infectious virus could be isolated from 7% of apparently healthy rats

Table 1. Selected records of experimental infections by direct transmission of arboviruses in animals

Disease/Virus	Animal	Mode of infection[a] (reference)
African horse sickness	Dog	OR (Van Rensburg, *et al.*, 1981)
African swine fever	Pig	NA (Wilkinson, *et al.*, 1977); OR(Hess, 1988)
Bluetongue	Bull	IU (Luedke, *et al.*, 1977)
Eastern equine encephalitis	Mouse,	NA (Sabin, 1938)
	Pheasant,	OR (Holden and Sussman, 1955; Satriano, *et al.*, 1959)
	White Pekin duckling	NA and OR (Dougherty and Price, 1960)
	Crow	OR (Karstad, *et al.*, 1959)
	Emu	CT (Tengelsen, *et al.*, 2001)
Germiston	Monkey	NA (Schwartz and Allen, 1970)
Getah	Horse	NA(Kumada, *et al.*, 1991)
Japanese encephalitis	Monkey	NA(Hsieh, *et al.*, 1961;Lee, *et al.*, 1969; Harrington, *et al.*, 1977; Monath, *et al.*, 1999)
	Bat	OR (LaMotte, 1958)
	Pig	VT (Habu, *et al.*, 1977)
Kyasanur Forest disease	Monkey	OR (Shah, 1965)
La Crosse encephalitis	Cat	OR (Godsey, *et al.*, 1988)
Louping ill	Pig	OR (Bannatyne, *et al.*, 1980)
	Rat	NA (Burnet, 1936)
	Goat	OR (Reid, *et al.*, 1984)
Modoc	Hamster	NA and IU (Davis and Hardy, 1974)
Powassan	Goat	OR (Woodall and Roz, 1977)
Rift Valley fever	Sheep	NA, OR, and CJ (Easterday, *et al.*, 1962)
	Rat/mouse	NA (Easterday and Murphy, 1963)
Rift Valley fever		OR (Mims, *et al.*, 1956)
	Ferret	NA (Francis and Magill, 1935)
	Bat	OR (Oelofsen and Van der Ryst, 1999)
Russian spring-summer encephalitis	Sheep	NA (Zilber, 1982)
Semliki Forest	Mouse	NA (Bradish and Allner, 1972; Kaluza, *et al.*, 1987)
		IR (Reagan, *et al.*, 1953)
Sindbis	Cave bat	NA and IR(Reagan, *et al.*, 1956a)
	Hamster	NA and IR(Reagan, *et al.*, 1956b)
St. Louis encephalitis	Mouse	NA (Webster and Clow, 1936; Slavin, 1943)
	Sloth	CT (Seymour, *et al.*, 1983)
Tick-borne encephalitis	Mouse	OR (Ilyenko, 1957; Pogodina, 1962; Elečková and Grešíková, 1992)
		NA (Daneš, *et al.*, 1962)
	Monkey	NA (Hambleton, *et al.*, 1983)
Venezuelan equine encephalitis	Guinea-pig	NA (Daneš, *et al.*, 1973a; Hrušková, *et al.*, 1969)
	Horse	NA and CT (Kissling, *et al.*, 1956)
	Bat	NA (Corristan, *et al.*, 1956)
	Cotton rat	CT (Zarate and Scherer, 1968; Howard, 1974)
	Monkey	NA (Daneš, et al, 1973b)
	Rabbit	NA (Daneš, *et al.*, 1973a)
	Hamster	NA (Jahrling, *et al.*, 1984)
	Mouse	NA (Kinney, *et al.*, 1988; Stephenson, *et al.*, 1988; Charles, *et al.*, 1995; Ryzhikov, *et al.*, 1995)
Vesicular stomatitis-NJ	Mouse	NA (Sabin, 1938)
	Pig	CT (Patterson, *et al.*, 1955; Howerth, *et al.*, 1997)
Western equine encephalomyelitis	Duck	OR (Burton, *et al.*, 1961)
	Guinea-pig	NA (Howitt, 1932 and 1935)
	Monkey	NA (Howitt, 1932)
	Mouse	NA (Froeschle, 1964)
West Nile	Monkey	NA (Nir, 1959)
	Mouse	NA (Nir, *et al.*, 1965)
	Hamster	NA(Nir, 1959)
	Mouse	OR (Odcola and Oduye, 1977)
	Goose	CT (Swayne, *et al.*, 2001)
Yellow fever	Bat	OR (Oelofsen, *et al.*, 1999)
	Monkey	NA and OR (Niedrig, *et al.*, 1999)

[a]CJ = conjunctival transmission; CT = contact transmission; IR = intrarectal transmission; IU = *in utero* transmission; NA = intranasal or aerosol transmission; OR = oral transmission; VT = venereal transmission

sacrificed at 28 days after challenge despite high levels of neutralizing (Nt) antibodies in their blood.

This report raised a serious question about the relevance of serum Nt antibody titer as an indicator of protection. The aforementioned observations suggested that (i) the nasal mucosal protection was an absolute requirement of a neurotropic arbovirus vaccine, "if it was ever used to vaccinate laboratory workers who might be exposed to a high dose of virus aerosol" (Kinney *et al.*, 1988), and (ii) the tissue examination of the animals surviving intranasal challenge was necessary to rule out the possibility of cryptic virus replication in CNS. Thus, it is puzzling that two poxvirus recombinant vaccines of JE virus were considered safe despite virus isolation and development of encephalitis in some of the vaccinated monkeys after intranasal challenge (Raengsukulrach *et al.*, 1999b).

In mucosal immunity the involvement of IgA has been recognized. As expected, small amounts of IgA have been detected in fecal, vaginal, intestinal, and bronchial-alveolar wash fluids of mice immunized with VEE vaccines (Hart *et al.*, 1997, Greenway *et al.*, 1997). However, in another study, the immunoglobulins responding to vaccination were IgM and IgG rather than IgA (Phillpotts, 1999). Furthermore, the IgA response of mice differed considerably among various breeds of the animal (Hart *et al.*, 1997).

In utero and congenital transmissions

Congenital infection as a result of infection of pregnant vertebrate hosts has been documented for arboviruses representing many virus families (Burns, 1950, Luedke *et al.*, 1970; Spertzel *et al.*, 1972; Barnard and Voges, 1986; Goto *et al.*, 1988; Chung *et al.*, 1990). Typically, the outcome is either abortion, stillbirth or very short life-span of neonates due to multiple organ dysfunctions. Thus, the examples of congenital infection leading to long-term persistence of JE virus in asymptomatic infant mice (Mathur *et al.*, 1982, 1986) are atypical.

On the other hand, a report of congenital bluetongue (BT) virus infection in cattle and long-term seminal shedding of the virus by a bull (Luedke *et al.*, 1977), and a speculation that transplacental BT infection in sheep is a possible mechanism of virus persistence in nature (Gibbs *et al.*, 1979) have been the source of serious controversy with negative impact on international agricultural trade (Roberts *et al*, 1993). However, in later investigations, it was concluded that some of the original reports of congenital infection of cattle by BT virus could not be reproduced (MacLachlan *et al*, 1992; Melville and Gard, 1992; Roeder *et al.*, 1992).

Regarding venereal transmission of arboviruses, in a simulated experiment, sows artificially inseminated with the semen from a boar experimentally infected with JE virus became infected, demonstrating viremia (Habu *et al.*, 1977). Similarly, artificial insemination of heifers with the semen from an experimentally BT virus-infected bull resulted in development of viremia in 3 of 9 heifers (Bowen and Howard, 1984).

In the cases of congenital infection of humans, two pregnant women infected with JE virus aborted and the others delivered apparently healthy children. JE virus was isolated from the brain, liver, and placental tissues of one of the aborted fetuses (Chaturvedi *et al.*, 1980). A pregnant woman who was immunized inadvertently during the first trimester delivered a baby more than 5 months later, who had specific IgM in cord blood (Tsai *et al.*, 1993). In a study of a group of women vaccinated with a YF vaccine, an increased rate (statistically significant) of spontaneous abortion was observed (Nishioka *et al.*, 1998). In yet another study, a YF virus strain recovered from a fatal case of vaccination-associated human encephalitis was intranasally inoculated into mice to determine its neurovirulence (Jennings *et al.*, 1994). Persistent infection of Ross River virus in fetus was strongly speculated in several babies with virus-specific serum IgM whose mothers had been infected between the 11th and 14th week of pregnancy (Aaskov *et al.*, 1981).

Biosafety and public health

The significance of direct transmission was recognized early with respect to laboratory-acquired human infections. In one survey, accidents accounted for only 10% of human infections, whereas handling of infected animals, aerosols, and other cases without readily identifiable causes accounted for 86% of the reported infections (Hanson and Sulkin, 1967). This and many other reports have served the basis for assigning a biosafety level to each arbovirus for the protection of laboratory workers (U.S. Department of Health and Human Services, 1999). Although the frequency of laboratory infections has been reduced considerably as the result of the enforcement of strict safety guidelines, the risk of arbovirus infection among certain professions and consumers has not abated. In a recent RVF outbreak in Yemen, 815 laboratory-confirmed patients exposed to sick sheep or meat were animal handlers, butchers, meat traders, and meat consumers (Nasher *et al.*, 2000). Similarly, persons infected with VS, louping ill (LI) and Wesselsbron viruses have been primarily animal breeders, shepherds, butchers, and veterinarians (Hanson *et al.*, 1950; Davidson *et al.*, 1991; Tomori *et al.*, 1981); Omsk hemorrhagic fever virus infection has been common among muskrat trappers (Sonenshine, 1993). TBE among persons who drank unpasteurized goat milk has been repeatedly documented in Europe (World Health Organization, 1994). During an outbreak of Kyasanur Forest disease in India, six lactating

women were infected, but no evidence of virus secretion was observed (Shah and Murthy, 1960), which contrasted to the virus secretion in milk and transmission to offspring among monkeys (Shah, 1965).

In the WN virus-endemic northeastern parts of the USA, the virus has been isolated, among many species of animals, from racoons and bats (Anonymous, 2000). Specimens from these species are usually tested first for rabies virus by rabies-vaccinated personnel before the rabies-negative specimens are processed for arboviruses. During field investigation of WN virus, a closely-related virus, SLE virus, may also be isolated from the same group of mosquitoes known to be involved in WN virus transmission. Currently, no vaccine exists against these two viruses. Special precautions are required for handling acetone-fixed cells or tissue smears of those specimens for disposing fixed specimens by unvaccinated personnel (such as custodians), since cold acetone alone may not completely inactivate both rabies and SLE viruses (Yabrov *et al.*, 1978; White and Chappell, 1982). Also, nosocomial infections among hospital staff must be prevented, as occurred when hospital staff assisted patients with Crimean-Congo hemorrhagic fever (CCHF) virus infection (Joubert *et al.*, 1985). A case of YF in a hospital technician assigned to work on blood analysis of an YF patient in London that occurred in 1931 is now strongly suspected to represent an infection without involvement of vector, because meticulously recorded documents of the incident clearly exclude any laboratory accident, contact with animals, skin abrasion, and presence of mosquito vectors, even though laboratory evidence is lacking due to the fact that a reliable diagnostic test was unavailable at that time (Cook *et al.*, 1994).

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