Susceptibility of various animals to the vesiculovirus Piry

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SUMMARY

To determine the pathogenic potential of the vesiculovirus Piry for domestic animals, two ponies, two steers, three sheep, three goats and three pigs were inoculated intradermally in the tongue or, in the case of the pigs, in the snout, heel and coronary band. Inoculated animals were housed in one room and allowed to mingle freely with an equal number of uninoculated contact animals of each species. Clinical signs of infection, consisting of elevated temperature and ulcers at the inoculation sites, were only observed in the ponies, but all inoculated animals developed specific antibody following inoculation. In addition, one of the contact sheep had neutralizing antibody to Piry at 7 and 29 days post inoculation suggesting a contact infection. Virus was not demonstrated in tissues, other than tongue, of any animal.

The failure of Piry virus to produce lesions in steers, sheep, goats and pigs and only limited ulcerations in ponies suggests that this virus is not similar pathogenically to New Jersey and Indiana strains of vesiculoviruses which produce classical vesicular stomatitis.

Lethal infections were produced by inoculation into suckling mice and hamsters, adult hamsters and embryonating chicken eggs. Further, lethal infections followed contact of adult female hamsters with their inoculated litters.

INTRODUCTION

Vesicular stomatitis (VS) has been a recognized clinical entity for over 100 years and may be produced by infection of cattle, pigs and horses with either the New Jersey or Indiana type of vesicular stomatitis virus (VSV) (Hanson, 1981). Apart from outbreaks in France and South Africa which followed the introduction of animals from the United States, naturally occurring disease in equine, bovine and porcine animals is restricted to the western hemisphere (Hanson, 1981).

While only one type of VSV-New Jersey is recognized, VSV-Indiana has been divided into three serologically related groups: VSV-Indiana 1 (the classical strain), VSV-Indiana 2 (Cocal and Argentina strains), VSV-Indiana 3 (Alagoas) (Federer, Burrows & Brooksby, 1967). The genus, vesiculovirus (Brown *et al.*

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1979), is made up of the above four viruses which produce disease naturally in domestic animals plus the three serologically related viruses Piry, Chandipura and Isfahan, which were respectively isolated from an opossum in Brazil, man in India and sandflies in Iran (Theiler & Downs, 1973; Bhatt & Rodrigues, 1967; Tesh *et al.* 1977). The pathogenic potential of these latter three viruses for domestic animals has not been reported.

Because the viruses Piry, Chandipura and Isfahan are serologically related to one or more of the better known strains of VSV, it seemed important to determine their pathogenic potential for domestic animals and their behaviour in laboratory hosts. Such information is of value to those laboratories concerned with the diagnosis and differentiation of vesicular diseases, including foot-and-mouth disease, swine vesicular disease, vesicular exanthema of swine and San Miguel sea lion virus infection in domestic animals. This current work reports our results in domestic animals and laboratory hosts with the virus Piry.

MATERIALS AND METHODS

Virus strain and preparation of inoculum

Piry virus was obtained as infected suckling mouse brain (five passages) from Dr R. E. Shope, Yale Arbovirus Research Unit, New Haven, Connecticut. The virus was passed once in Vero cell line (American Type Culture Collection, Rockville, Maryland 20852) and then inoculated intracerebrally into day-old mice. Brains were harvested at death and a 10% (w/v) suspension made in culture medium (Eagle's basal medium supplemented with 2% fetal calf serum). Virus infectivity was titrated in Vero cells in microtitre plates (Linbro Chemicals, Hamden, Connecticut, 06517). The mouse brain suspension containing $10^{8.3}$ TCID₅₀/0.025 ml was stored at -70 °C in 5 ml aliquots.

Domestic animals

Four ponies, four steers, six sheep, six goats and six pigs were placed in one animal isolation room, allowed to mix freely and fed and watered *ad libitum* from communal troughs. Preinoculation serum samples were collected from all animals and rectal temperatures recorded daily for five days to establish baseline values. Two ponies, two steers, three sheep and three goats were given inoculations of a total of 1 ml of virus suspension intradermally (IDL) in six sites. The ponies were also inoculated intradermally in the right commissure of the mouth. Three of the pigs were inoculated intradermally in the heel and coronary band of both right feet and also in the snout. The remaining animals were left uninoculated as contacts. The animals were observed daily and rectal temperatures recorded up to slaughter at 29 days post inoculation (DPI).

Blood for serological examination and for virus isolation was collected from all animals at 1, 2, 3, 4, 7, 15, 22 and 29 DPI. Neutralizing antibody was assayed by making twofold dilutions (final dilution 1 in 2 to 1 in 256) of each serum in microtitre plates and reacting with 100–200 TCID₅₀ of Piry virus for 45 min at 37 °C in an atmosphere of 5% CO₂. After the addition of Vero cells, the plates were incubated for 3 days at 37 °C in a humidified atmosphere with 5% CO₂. The final concentration of fetal bovine serum was 5%. The plates were stained with crystal

violet in 2.5 % (v/v) glutaraldehyde and 50 % endpoints calculated for each serum: (Finney, 1981).

Sera were also tested for precipitating antibody by the immunoelectroosmophoresis (IEOP) test using infected cell extract antigen as previously described (Wilks, Jenney & House, 1984).

Blood for virus isolation was mixed with heparin (final concentration 15 i.u. per ml) and stored at -70°C for up to 21 days before inoculation at 10^0 and 10^{-1} dilutions onto Vero cells in microtitre plates. Plates were observed for 5 days for the appearance of viral-induced cytopathic effect.

Samples of oesophageal-pharyngeal mucus were collected by probang from selected animals at 15 DPI and from all animals at 29 DPI. The samples were mixed immediately with an approximately equal volume of culture medium and stored at -70°C for up to 21 days before inoculation onto established monolayers of Vero cells in 25 cm² plastic flasks. The flasks were observed for 5 days for the appearance of viral-induced cytopathic effect.

In those animals in which lesions appeared at the inoculation sites, affected tissue was collected and a 10 % w/v suspension made in culture medium. Tissue suspensions were stored for up to 14 days at -70°C before being titrated by tenfold dilutions in Vero cells in microtitre plates.

At slaughter, a retropharyngeal lymph node and approximately 2 g each of liver and spleen was collected from each animal. Tissues were pooled for each animal and suspended by grinding to 10 % w/v in culture medium. Suspensions were stored at -70°C for up to 14 days before inoculation into established monolayers of Vero cells in 25 cm² plastic flasks and observed for cytopathic effect for 5 days.

Subpassage of virus in domestic animals

In a separate experiment to determine whether a single passage of virus in ponies and steers affected subsequent pathogenicity of the virus, four ponies and four steers were housed together. Two of the ponies and two of the steers were inoculated with Piry virus as described. Samples of tongue epithelium were collected from the two ponies at two DPI and a 10 % w/v suspension prepared and titrated in Vero cells.

At seven DPI, blood for serological examination was collected from all animals and the two contact ponies and two contact steers were given inoculations with the equine tongue epithelium suspension. All animals were observed for a further 4 days when blood was again collected and the experiment terminated.

Studies in laboratory hosts

Mice. Rockefeller H/Plum Island strain mice were produced at the PIADC. Two hundred one-day-old mice were given inoculations intracerebrally (IC) with 0.03 ml of Piry virus propagated in Vero cells. A 10 % brain suspension prepared from these mice at death was used for all subsequent inoculations.

Twenty one-day-old and six adult (6–8 weeks old) mice were given inoculations intraperitoneally (IP) and six adults were given inoculations subcutaneously (SC) with 0.1 ml of Piry virus. Mice were observed daily for up to 14 days.

Hamsters. Hamsters were purchased as young adults (6–8 weeks) and as pregnant females (Charles River Laboratories, Wilmington, Massachusetts,

01887). Two litters (20 animals) of day-old hamsters were given inoculations IC (0.03 ml) and two litters (19 animals) were given inoculations IP (0.1 ml). At death, the brains and a pool of liver and spleen were harvested and titrated in Vero cells. Five adult hamsters were inoculated IP (0.1 ml) and five SC (0.1 ml) and observed daily for up to 14 days. A pool of liver and spleen was prepared from those which died and titrated in Vero cells. Blood for serological tests were collected from survivors at 14 DPI.

Guinea pigs. Guinea pigs (400–450 g) were obtained from Dutchland Farms, Denver, Pennsylvania, 17517 and blood for serological examination was collected prior to inoculation. Four guinea pigs were given inoculations in the left hind footpad (0.1 ml) and four were inoculated IP (0.1 ml). They were observed daily for 28 days and blood for serological tests was obtained at 14 and 28 DPI.

Rabbits. Preinoculation serum samples were collected from three rabbits (Dutchland Farms, Denver, Pennsylvania, 17517) who were then given inoculations intravenously (IV) with 0.5 ml of Piry virus. Serum was collected at 14 DPI when the inoculations were repeated and the animals were exsanguinated under anaesthesia at 28 DPI.

Eggs. Five-day-old embryonated chicken eggs (Westbrook Farms, Bohemia, New York, 11716) were given inoculations into the allantoic cavity with 0.1 ml of Piry virus. As controls, five eggs were given inoculations with 0.1 ml of VSV-New Jersey (propagated in Vero cells) and five with 0.1 ml of culture medium. Eggs were examined daily by transillumination for viability. When embryos were considered dead, the eggs were chilled at 4 °C and allantoic fluids harvested and titrated for infectious virus in Vero cells.

RESULTS

Domestic animals

Clinical signs of infection were not observed in any of the domestic animals except the two inoculated ponies. At one DPI, both inoculated ponies had elevated temperatures (a rise of 2.0 and 2.2 °C) but these had returned to baseline values by two DPI and remained so throughout the experiments. At one DPI, the surface epithelium had been lost over the inoculation sites on the tongue and in the lip commissure of both ponies leaving shallow ulcers. Vesicular lesions were not observed. Over the next 3 days, the tongue lesions extended peripherally to a limited degree by underrunning of the epithelium to produce ulcers up to 3 cm in diameter. By seven DPI, healing had commenced and resolution was complete by 14 DPI.

Affected tongue epithelium collected from both ponies at three DPI had titres of $10^{3.6}$ and $10^{3.1}$ TCID₅₀/g. Tongue epithelium collected from one pony at seven DPI had a titre of $10^{6.9}$ TCID₅₀/g.

Infectious virus was not detected ($< 10^{0.25}$ TCID₅₀/0.025 ml) in any of the blood samples, oesophageal-pharyngeal samples or pools of tissue.

Precipitating antibody was not detected with the IEOP test in any of the preinoculation blood samples. Low levels of virus neutralizing activity were present in serum from five of the six pigs (Table 1). At seven DPI, all of the inoculated animals had neutralizing antibody, and this was present at higher titre

Table 1. Serological responses of animals following inoculation with Piry virus measured by the virus neutralization and immunoelectro-osmophoresis tests

Species	(Identification)	Days from inoculation				
		-5	7	15	22	29
Equine	Inoc†	(67)	< 2 (-)*	45 (+)	≥ 360 (+)	≥ 360 (+)
		(58)	< 2 (-)	9 (+)	45 (+)	≥ 360 (+)
	Contact‡	(59)	< 2 (-)	< 2 (-)	< 2 (-)	< 2 (-)
		(60)	< 2 (-)	< 2 (-)	< 2 (-)	< 2 (-)
Bovine	Inoc	(7)	< 2 (-)	18 (-)	36 (-)	71 (+)
		(8)	< 2 (-)	36 (-)	89 (-)	45 (-)
	Contact	(9)	< 2 (-)	< 2 (-)	< 2 (-)	< 2 (-)
		(10)	< 2 (-)	< 2 (-)	< 2 (-)	< 2 (-)
Ovine	Inoc	(81)	< 2 (-)	28 (-)	§NT (-)	NT (-)
		(82)	< 2 (-)	9 (-)	NT (-)	NT (-)
		(83)	< 2 (-)	45 (-)	NT (-)	NT (-)
	Contact	(894)	< 2 (-)	< 2 (-)	NT (-)	NT (-)
		(913)	< 2 (-)	89 (-)	NT (-)	NT (-)
		(946)	< 2 (-)	< 2 (-)	NT (-)	NT (-)
Caprine	Inoc	(12)	< 2 (-)	7 (-)	NT (-)	NT (-)
		(76)	< 2 (-)	7 (-)	NT (-)	NT (+)
		(77)	< 2 (-)	45 (+)	NT (+)	NT (+)
	Contact	(78)	< 2 (-)	< 2 (-)	NT (-)	NT (-)
		(79)	< 2 (-)	< 2 (-)	NT (-)	NT (-)
		(80)	< 2 (-)	< 2 (-)	NT (-)	NT (-)
Porcine	Inoc	(65)	< 7 (-)	28 (-)	NT (-)	NT (+)
		(66)	< 2 (-)	36 (-)	NT (-)	NT (-)
		(67)	3 (-)	22 (-)	NT (-)	NT (+)
	Contact	(68)	5 (-)	< 2 (-)	NT (-)	NT (-)
		(69)	4 (-)	7 (-)	NT (-)	NT (-)
		(70)	6 (-)	2 (-)	NT (-)	NT (-)

* Neutralizing antibody titre (IEOP results).

† Animal inoculated as described in Materials and Methods Section.

‡ Uninoculated animals present in the room as contacts.

§ NT indicates that neutralizing antibody titre was not measured for this serum.

at 29 days. Neutralizing activity remained at a low level in the uninoculated pigs in contrast to those inoculated. Precipitating antibody was detected only in inoculated animals, first appearing at seven DPI. Neutralizing antibody was present in one contact sheep when tested at seven and 29 DPI.

Subpassage in domestic animals

Lesions resembling early vesicle formation were present at the inoculation sites on the tongues of the two ponies given inoculations of mouse brain suspension at one DPI. Limited progression followed by resolution of lesions was similar to that already described. No clinical signs of infection were detected in the two inoculated steers.

Affected tongue epithelium was collected from the two ponies at two DPI, pooled and titrated in Vero cells ($10^{6.2}$ TCID₅₀/g). No clinical signs of infection followed inoculation of this equine tongue tissue suspension into the two contact ponies and two calves.

Virus neutralizing antibody was not detected (titre less than 2) in any of the animals prior to inoculation or at seven DPI. At the termination of the experiment (11 DPI) low levels of neutralizing antibody was detected in both of the originally inoculated ponies (titres of 4 and 3) and both calves (titres of 5 and 5) but not in any of the ponies or calves which were inoculated with equine tongue tissue.

Laboratory hosts

Mice. Piry virus was lethal for day-old mice within 30 h of injection by either the IC or IP route but not for adult mice inoculated either IP or SC.

Hamsters. Lethal infection occurred by 24 h in day-old hamsters inoculated IC or IP and high titres of virus were found in the brain and liver/spleen suspensions ($10^{9.8}$ and $10^{10.6}$ TCID₅₀/g, respectively). Also, both of the mothers of the IC inoculated hamsters died (three and four DPI) as did one of the two mothers of the IP inoculated hamsters. Only one mother (IC inoculated hamsters) had cannibalized her young. Virus was recovered from suspensions of liver and spleen from each of these mothers ($10^{8.7}$ TCID₅₀/g).

Two of five adult hamsters inoculated IP died (4 and 10 DPI, respectively) but no deaths occurred in the group of five inoculated SC. Both precipitating and virus neutralizing antibodies (titre ≥ 360) were detected in all surviving adults at 14 DPI.

Guinea pigs and rabbits. Both neutralizing and precipitating antibodies were detected at 14 and 28 DPI, but no signs of disease were observed.

Embryonated chicken eggs. All of the embryonated eggs given inoculations with Piry or VSV-New Jersey virus were dead by three DPI. Eggs inoculated with culture medium were alive at four DPI. Allantoic fluid was harvested from the eggs inoculated with Piry and VSV-New Jersey viruses and titrated in Vero cells. Piry virus allantoic fluid had a titre of $10^{7.8}$ TCID₅₀/0.025 ml and VSV-New Jersey $10^{8.0}$ TCID₅₀/0.025 ml.

DISCUSSION

There have been no subsequent field isolations of Piry virus reported since the prototype was recovered from organs of a marsupial *Philander opossum* in 1960 (Theiler & Downs, 1973). Piry virus is pathogenic for man as demonstrated by several confirmed infections in laboratory workers, and there is serological evidence for natural infection in both man and wild mammals in Brazil (Theiler & Downs, 1973). Although there is no clinical or serological evidence suggesting infection of horses or cattle with Piry virus in Brazil, the presence of other vesicular diseases including classical VSV and foot-and-mouth disease in this area makes it important to know if Piry virus is capable of producing lesions which could confuse diagnosis.

Clinical disease occurred in the ponies inoculated with Piry virus. The pathogenicity of this virus for domestic animals differed from that described for VSV-New Jersey and VSV-Indiana 1 in both the severity of the lesions produced and the

range of animal species which were susceptible (Hanson, 1981). Both VSV-New Jersey and VSV-Indiana 1 produce vesicular disease in the horse, cow and pig whereas the Piry infection was only manifested clinically in the ponies and only progressed to a limited degree before healing commenced. No discomfort or salivation as described in infection with the classical strains was observed and lesions at other than the inoculation sites were not detected.

Temperatures were elevated following inoculation of Piry virus into the ponies, but direct evidence for viraemia was not obtained. Virus could not be detected by the methods employed in either oesophageal-pharyngeal samples or the tissues collected at autopsy. The recovery of Piry virus from the affected pony tongue tissue at three DPI and at higher titre from one pony at seven DPI indicated that local replication of virus at and around the inoculation sites had occurred.

The virus neutralization test was sensitive for the detection of antibody following exposure to Piry, as activity was demonstrated in the serum of all inoculated animals by seven DPI. The demonstration of neutralizing activity in one contact sheep serum at seven and 29 DPI shows that this animal was exposed to Piry virus by contact with the inoculated animals. Evidence of spread to any other contact animals was not detected. The demonstration of low levels of neutralizing activity in the pre-inoculation sera of five of the six pigs is probably indicative of nonspecific neutralization similar to that described for certain human sera with VSV-Indiana 1 (Mills, Beebe & Cooper, 1979).

The IEOP test appeared to be more efficient at detecting antibody in the ponies than in other species but by 29 DPI, nine of the 13 inoculated animals gave positive reactions with this test. Although both the virus neutralization and the IEOP tests depend on interaction of antibody with viral glycoprotein (Kelley, Emerson & Wagner, 1972; Wilks, Jenney & House, 1984), they do not appear to precisely parallel each other. For example, a pony serum with a neutralizing titre of 9 at seven DPI gave a positive IEOP reaction whereas a sheep serum with a titre of 220 at 29 DPI gave a negative reaction. The IEOP test for Piry virus may be more suitable for surveys than for individual animal serology and appears to be more sensitive with equine serum than with that of other domestic species.

Since the mouse brain suspension of Piry virus used for animal inoculation had a history of three passages in suckling mice and one in Vero cells prior to preparation of the stock inoculum in suckling mice, there was the possibility of attenuation for domestic animals. It was decided to pass this virus in ponies to determine if the virulence for domestic animals was increased. Inoculation of ponies and steers with the infected equine tongue tissue (total of $10^{5.2}$ TCID₅₀ in 1 ml inoculated into each animal) failed to produce lesions. Subpassage of this material was, therefore, not continued. We have also demonstrated subsequently (Wilks & House, unpublished data) that a field isolate of VSV-New Jersey after a similar passage history in mice and Vero cells retained virulence for ponies, steers and pigs. Therefore, it seems unlikely that Piry virus was greatly attenuated for domestic animals by its passage in mice and Vero cells.

In agreement with previous reports (Anon, 1967), Piry virus produced lethal infections in day-old mice, adult hamsters by the IP route and chicken embryos but not in adult guinea pigs. We have extended this to show that adult mice and hamsters are resistant to SC inoculation as are rabbits to IV inoculation. Of

particular interest are the deaths of mothers of the day-old hamsters which were inoculated IC or IP. These mothers apparently acquired their lethal infections by contact, possibly by ingestion. Similar contact transmission of VSV-New Jersey and VSV-Indiana 1 has been previously described in mice (Olitsky, Cox & Syverton, 1934; Skinner, 1957). The failure to produce lesions in guinea pigs by footpad inoculation contrasts with similar inoculation schedules with VSV-New Jersey or VSV-Indiana 1 which produce local vesiculation and occasional secondary spread (Skinner, 1957), however, the strains used in that study had had many passages in guinea pigs and were probably adapted strains.

The rapid growth of Piry virus to a high titre in embryonated chicken eggs indicated that this host, along with suckling mice or hamsters may be suitable for virus isolation from field material.

Although Piry virus differs from VSV-New Jersey and VSV-Indiana 1 in the range of species clinically susceptible to infection, our results show that it does have the potential to produce lesions in ponies which could be confused with classical VS. Unlike classical VS, however, dramatic extension from the sites of inoculation did not occur, and disease was not produced by backpassage of virus from infected epithelium in ponies. The absence of clinical signs in cattle and pigs suggests that it would not be a source of confusion with foot-and-mouth disease or classical VS diagnosis in these species.

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