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# Susceptibility of various animals to the vesiculoviruses Isfahan and Chandipura

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SUMMARY

To determine the pathogenic potential of the vesiculoviruses Isfahan and Chandipura for domestic animals, two ponies, two steers, three sheep, three goats and three pigs were inoculated with each virus intradermally in the tongue or, in the case of the pigs, in the snout, heel and coronary band. The ponies were also inoculated intradermally in the right commissure of the mouth. Animals inoculated with each virus 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 ulcers at the inoculation sites, were observed in the Chandipura study in two inoculated ponies, one inoculated steer and one inoculated goat. No elevated temperature was observed. Virus was isolated from the ulcerated tongue tissue, but not from serial blood samples, oesophageal-pharyngeal mucus samples, or from the tissues which were collected at necropsy. Precipitating antibody was not detected by the immunoelectro-osmophoresis (IEOP) test in any of the pre- or post-serum samples except from two inoculated sheep at 29 days post-inoculation (D.P.I.). Low levels of neutralizing activity were detected in pre-inoculation serum from all steers, pigs, contact sheep, and one contact goat. By 15 D.P.I. all inoculated animals and contact ponies and steers exhibited increased neutralizing antibody titres.

In studies with the Isfahan virus, lesions developed only at the inoculation sites in the two ponies, and the virus was isolated. No virus was isolated from any blood, oesophageal-pharyngeal mucus samples or tisues collected at necropsy. All pre-inoculation sera were negative for neutralizing and precipitating antibodies. By 14 D.P.I. all inoculated animals exhibited neutralizing antibody, while all the contacts remained negative. The IEOP test remained negative for all animals throughout the experiment. A subpassage of a suspension of Isfahan-infected tongue tissue injected into ponies and steers also yielded only firm swellings of lesser extent than the original reaction at the inoculation sites.

With both viruses, lethal infections were produced by intracranial or intraperitoneal inoculation of day-old mice and hamsters, and by allantoic inoculation of embryonating chicken eggs. Adult mice, hamsters, guinea-pigs and rabbits produced serum antibodies but lacked clinical signs.

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## INTRODUCTION

Vesicular stomatitis (VS) is a viral disease of horses, swine, cattle and man caused by infection with certain viruses of the vesiculovirus genus in the family Rhabdoviridae (Hanson, 1981). The classical disease is caused by the serotypes New Jersey and Indiana of vesicular stomatitis virus (VSV). However, naturally occurring disease in some regions of Central and South America may be caused by subtypes of Indiana, such as Cocal, Argentina and Alagoas (Federer, Burrows & Brooksby, 1967). VS is characterized by vesicular lesions in the mucosal tissue of the mouth and in the skin of the coronary band (Hanson, 1981). In addition to being a significant disease in its own right, it can confound the diagnosis of other vesicular diseases, most notably foot-and-mouth disease.

In recent years a number of other viruses which morphologically and serologically resemble the vesiculoviruses have been isolated from a range of mammalian and arthropod hosts (Tesh, Travassos da Rosa & Travassos da Rosa, 1983).

Isfahan virus was isolated from pools of adult female *Phlebotomus papatasi* collected in Isfahan Province, Iran in 1975 (Tesh *et al.* 1977). On the basis of its bullet-shaped morphology, morphogenesis in cell culture, and serological relationship with the vesiculoviruses Piry and Chandipura, it is regarded as a member of the vesiculovirus genus (Brown *et al.* 1979; Tesh *et al.* 1983).

Chandipura virus was isolated from the blood of a human patient with a febrile illness in Central India (Bhatt & Rodrigues, 1967). Serological relationships between Chandipura and Isfahan, Piry and Cocal have been demonstrated (Tesh *et al.* 1983). Bite transmission by mosquitoes has also been shown to occur experimentally (Rao *et al.* 1967).

Consequently, it is important to determine whether vesiculoviruses other than New Jersey and Indiana, particularly Piry, Chandipura and Isfahan, are also capable of infecting domestic animals and producing clinical signs which would further complicate the diagnosis of vesicular diseases.

We have previously reported infection experiments with Piry virus (Wilks & House, 1984) and describe in this paper similar studies with Isfahan and Chandipura viruses.

## MATERIALS AND METHODS

### *Virus strain and preparation of inoculum*

Both Chandipura (Indian isolate) and Isfahan viruses were obtained as infected suckling mouse brain from Dr R. E. Shope, Yale Arbovirus Research Unit, New Haven, Connecticut. Each virus was passed once in the 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 bovine serum). Virus infectivity was titrated in Vero cells in microtitre plates (Linbro chemicals, Hamden, Connecticut, 06517). Suspensions were stored in 5 ml aliquots at −70 °C. The Chandipura virus suspension contained $10^{10.3}$ $TCID_{50}$/0·025 ml and the Isfahan virus suspension contained $10^{7.5}$ $TCID_{50}$/0·025 ml. These suspensions were used undiluted for all subsequent inoculations of domestic and laboratory animals and embryonating eggs.

*Domestic animals*

For the studies with Chandipura virus, four ponies, four steers, six sheep, six goats and six pigs were placed in one animal isolation room, were allowed to mix freely and were fed and watered *ad libitum* from communal troughs. Pre-inoculation serum samples were collected from all animals and rectal temperatures recorded daily for 5 days to establish baseline values. Two ponies, two steers, three sheep and three goats were given inoculations totalling 1 ml of the undiluted virus suspension intradermalingually (I.D.L.) 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 (D.P.I.). Investigators and animal handlers wore self-contained battery-operated hoods supplying HEPA filtered air during all animal work with both viruses (Vickers Limited Medical Engineering, Priestly Rd, Basingstoke, Hampshire, RG2A 9NP, England).

Blood for serological examination and for virus isolation was collected from all animals 1, 2, 3, 4, 7, 15, 22 and 29 D.P.I. Microtitre assays for neutralizing antibody, immunoelectro-osmophoresis (IEOP) for precipitating antibody and assays for infectious virus were performed as previously described (Wilks & House, 1984, Wilks, Jenney & House, 1984).

Oesophageal-pharyngeal mucus specimens were collected from all animals at 15 and 29 D.P.I. and tissues were collected from all animals at slaughter. These samples were assayed for infectious virus as previously described (Wilks & House, 1984).

To determine if a single passage of Chandipura virus in ponies affected its subsequent pathogenicity, infected tongue epithelium was collected from the two inoculated ponies, at 7 D.P.I., prepared as a 10% suspension, and inoculated I.D.L. into the contact ponies and two contact steers at 15 D.P.I. Observations continued as described to slaughter at 29 days.

The above experiment was also conducted with Isfahan virus. The only differences in protocol were that the contact ponies and steers were inoculated at 14 D.P.I. with infected tongue epithelium which had been collected at 2 D.P.I.; all sheep, goats and pigs were killed at 18 D.P.I., and the experiment concluded at 25 D.P.I.

*Studies in laboratory hosts*

The following inoculations and assays were conducted with both Chandipura and Isfahan viruses.

*Mice.* Rockefeller H/Plum Island strain mice were produced at the PIADC. Two hundred 1-day-old mice were given inoculations intracerebrally (I.C.) with 0·03 ml of each virus which had been passed once in Vero cells. A 10% brain suspension prepared from these mice at death was used for all subsequent inoculations.

Each virus was inoculated intraperitoneally (I.P.), into 20 1-day-old and into six adult (6- to 8-week old) mice, and six adults were given inoculations subcutaneously (S.C.) with 0·1 ml of virus suspension. The mice were observed daily for up to 14 days.

*Hamsters.* Hamsters were purchased as young adults (6–8 weeks) and as

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pregnant females (Charles River laboratories, Wilmington, Massachusetts, 01887). Two litters (20 animals) of day-old hamsters were given inoculations I.C. (0·03 ml) and two litters (20 animals) were given inoculations I.P. (0·1 ml). At death, tissues were collected and the brains and pools of liver and spleen were titrated in Vero cells. Five adults hamsters were inoculated I.P. (0·1 ml) and five S.C. (0·1 ml) and observed daily for up to 14 days, when blood was collected for serological tests.

*Guinea-pigs.* Guinea-pigs (400–450 g) were obtained from Dutchland Farms, Denver, Pennyslvania 17517. Blood for serological examination was collected prior to inoculation with virus. Four guinea-pigs were given inoculations in the left hind footpad (0·1 ml) and four were inoculated I.P. (0·1 ml) with each virus. They were observed daily for 28 days and blood for serological tests collected at 14 and 28 D.P.I.

*Rabbits.* Pre-inoculation serum samples were collected from three rabbits (Dutchland Farms, which were then given inoculations intravenously (I.V.) with 0·5 ml of each virus suspension. Serum was collected at 14 D.P.I. when the inoculations were repeated and the animals exsanguinated under anaesthesia at 28 D.P.I.

*Eggs.* Groups of 5-day-old embryonating chicken eggs (Westbrook Farms, Bohemia, New York 11716) were given inoculations of 0·1 ml of either virus suspension into the allantoic cavity. Control eggs were inoculated with 0·1 ml of uninfected culture medium into the allantoic cavity. Viability was assessed by trans-illumination daily and, when embryos were considered dead, the eggs were chilled at 4 °C and the allantoic fluids harvested and titrated for infectious virus in Vero cells.

## RESULTS

### *Domestic animals*

*Chandipura virus*

Clinical signs of infection were only observed in the two inoculated ponies, one of the two inoculated steers and one of the three inoculated goats. Temperatures were not elevated in any of the inoculated or contact animals. At 1 D.P.I., raised blanched areas resembling an early vesicle and approximately 2 cm in diameter were observed at the six inoculation sites on the tongues of the ponies. At 2 D.P.I. they had ruptured, forming deep ulcers, but did not progress peripherally. Ingrowth of healthy tissue was apparent by 7 D.P.I. and only small scars marked the sites of the lesions by 15 D.P.I. Tongue tissue collected from the two ponies at 1 D.P.I. had more than $10^{8.1}$ $TCID_{50}$ of infectious virus per gram of tissue and at 7 D.P.I. had $10^{5.9}$ $TCID_{50}$/g. One steer and one goat developed tongue lesions, which were first observed at 3 D.P.I. as pale areas corresponding to the inoculation sites. These areas had sloughed at 4 D.P.I. to reveal shallow ulcers which had healed by 7 D.P.I. Tongue tissue collected from the steer and goat at 3 D.P.I. contained $10^{5.4}$ and $10^{3.9}$ $TCID_{50}$/g of infectious virus respectively.

Infectious virus was not detected in blood samples, oesophageal-pharyngeal mucus or from the tissues which were collected at necropsy.

Precipitating antibody was not detected by the IEOP test in any of the pre- or post-inoculation serum samples except in those collected from two of the three

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

| Species | Identification no. | Days post-inoculation −5 | 7 | 15 | 29 |
|---|---|---|---|---|---|
| Equine | | | | | |
| Inoculation * | 61 | < 2(−) † | 11(−) | 220(−) | 89(−) |
| | 62 | < 2(−) | 18(−) | 280(−) | 180(−) |
| Contact † | 63 | 2(−) | < 2(−) | 280(−) | 45(−) |
| | 64 | < 2(−) | < 2(−) | 45(−) | 11(−) |
| Bovine | | | | | |
| Inoculation | 35 | 6(−) | 110(−) | > 360(−) | > 360(−) |
| | 36 | 3(−) | < 360(−) | 280(−) | 89(−) |
| Contact | 37 | 3(−) | < 2(−) | 280(−) | NT |
| | 40 | 3(−) | < 2(−) | 11(−) | 280(−) |
| Ovine | | | | | |
| Inoculation | 84 | < 2(−) | 140(−) | < 360(−) | 220(+) |
| | 85 | < 2(−) | 180(−) | < 360(−) | > 360(−) |
| | 86 | < 2(−) | 140(−) | < 360(−) | 280(+) |
| Contact | 513 | 2(−) | < 2(−) | < 2(−) | 2(−) |
| | 905 | < 2(−) | < 2(−) | < 2(−) | 2(−) |
| | 909 | < 2(−) | < 2(−) | < 2(−) | 2(−) |
| Caprine | | | | | |
| Inoculation | 6 | < 2(−) | 28(−) | 280(−) | > 360(−) |
| | 65 | < 2(−) | 71(−) | 110(−) | 140(−) |
| | 67 | < 2(−) | 89(−) | 220(−) | > 360(−) |
| Contact | 68 | < 2(−) | < 2(−) | < 2(−) | 4(−) |
| | 69 | < 2(−) | < 2(−) | < 2(−) | 2(−) |
| | 71 | 3(−) | 6(−) | 2(−) | 4(−) |
| Porcine | | | | | |
| Inoculation | 91 | 5(−) | 280(−) | 45(−) | 89(−) |
| | 92 | 3(−) | > 360(−) | 280(−) | 280(−) |
| | 93 | 2(−) | > 360(−) | > 360(−) | 45(−) |
| Contact | 94 | 2(−) | 2(−) | 3(−) | 4(−) |
| | 95 | 2(−) | < 2(−) | 2(−) | NT |
| | 96 | 3(−) | 9(−) | 3(−) | 2(−) |

NT Indicates that serum was not tested.
* Animals inoculated as described in 'Materials and Methods' section.
† Uninoculated animals present in the room as contact controls.
‡ Reciprocal of neutralizing antibody titre calculated by Spearman–Kärber method of estimating 50% end-points (Finney, 1978); IEOP results in parentheses.

inoculated sheep at 29 D.P.I. (Table 1). Low levels of neutralizing activity were detected in the pre-inoculation sera from all the steers, one contact sheep, one contact goat and all the pigs. By 7 D.P.I. increased levels of neutralizing antibody were detected in serum from all the inoculated animals, and by 15 D.P.I. the contact ponies and steers also had increased levels of neutralizing antibody. The low levels of neutralizing activity in the contact sheep, goats and pigs did not rise substantially up to 29 D.P.I.

*Isfahan virus*

None of the animals had a pyrexia following inoculation, and lesions only developed at the inoculation sites in the two ponies. The lesions appeared as firm

Table 2. *Serological responses of animals following inoculation with Isfahan virus measured by the virus neutralization and immunoelectro-osmophoresis tests**

| Species | Identification no. | Days from inoculation −5 | 7 | 14 | 21 | 25 |
|---|---|---|---|---|---|---|
| Equine | | | | | | |
| Inoculation † | 65 | < 2 | 6 | 18 | 6 | 14 |
| | 66 | < 2 | 6 | 18 | 11 | 9 |
| Contact‡ | 67 | < 2 | < 2 | < 2 | < 21 | 5 |
| | 68 | < 2 | < 2 | < 2 | < 2 | < 2 |
| Bovine | | | | | | |
| Inoculation | 75 | < 2 | 6 | 180 | 45 | 6 |
| | 76 | < 2 | < 2 | 200 | 28 | 14 |
| Contact | 77 | < 2 | < 2 | < 2 | < 2 | < 2 |
| | 78 | < 2 | < 2 | < 2 | < 2 | < 2 |
| Ovine | | | | | | |
| Inoculation | 113 | < 2 | 22 | 14 | —§ | — |
| | 125 | < 2 | 14 | 9 | — | — |
| | 127 | < 2 | 11 | 14 | — | — |
| Contact | 128 | < 2 | < 2 | < 2 | — | — |
| | 129 | < 2 | < 2 | < 2 | — | — |
| | 130 | < 2 | < 2 | < 2 | — | — |
| Caprine | | | | | | |
| Inoculation | 116 | < 2 | 11 | 110 | — | — |
| | 117 | < 2 | 11 | 71 | — | — |
| | 118 | < 2 | 18 | 11 | — | — |
| Contact | 119 | < 2 | < 2 | < 2 | — | — |
| | 120 | < 2 | < 2 | < 2 | — | — |
| | 121 | < 2 | < 2 | < 2 | — | — |
| Porcine | | | | | | |
| Inoculation | 145 | < 2 | 110 | 45 | — | — |
| | 146 | < 2 | 89 | 36 | — | — |
| | 147 | < 2 | 89 | 280 | — | — |
| Contact | 148 | < 2 | < 2 | < 2 | — | — |
| | 149 | < 2 | < 2 | < 2 | — | — |
| | 150 | < 2 | < 2 | < 2 | — | — |

* IEOP test was negative with all sera, so only the reciprocal of the neutralizing antibody titre calculated by the Spearman–Kärber method of estimating 50% end-points is reported.

† Animals inoculated as described in 'Materials and Methods' section.

‡ Uninoculated animals present in the room as contact controls.

§ Sheep, goats and pigs were removed from the experiment at 18 D.P.I.

swellings 1 cm in diameter at 1 D.P.I. and these had ulcerated by 2 D.P.I. The lesions did not extend peripherally and healing was complete by 8 D.P.I. with minimal scarring. Affected tongue tissue collected at 3 D.P.I. contained $10^{4.6}$ $TCID_{50}/g$.

Infectious virus was not detected in blood samples, oesophageal-pharyngeal mucus or from tissues collected at necropsy.

Antibody to Isfahan virus was not detected in any of the pre-inoculation blood samples by either the IEOP or virus neutralization tests (Table 2). Although the IEOP test remained negative with serum from all animals throughout the experiment, neutralizing antibody was detected in 12 of the 13 inoculated animals by 7 D.P.I. and in all 13 by 14 D.P.I. However, with the exception of the two

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inoculated steers tested at 14 D.P.I., the antibody titres were low. Neutralizing antibody was not detected in any of the contact animals except for pony no. 67 at 25 D.P.I., which was 10 days after that pony had been inoculated with infected equine tongue tissue.

*Subpassage in domestic animals*

Apart from small (0·5 cm) firm swellings at the inoculation sites of the two ponies injected with Chandipura-infected tongue tissue, no clinical sign of infection was detected in ponies or steers receiving injections of Chandipura- or Isfahan-infected tongue tissue. These swellings were only detected on the first day after inoculation. Moderate levels of virus neutralizing antibody were detected in the serum collected from ponies and steers prior to inoculation with Chandipura-infected tongue tissue (Table 1). By 29 D.P.I. the titre had fallen in the two ponies but had risen in the one surviving steer. (One steer had to be killed following an accidental injury.)

Antibody was not detected by either test in the serum of ponies and steers prior to their inoculation with Isfahan-infected tongue tissue, and was only detected by the virus neutralization test at 25 D.P.I. in one (pony no. 67) of the two inoculated ponies but not in either steer (Table 2).

*Laboratory hosts*

*Chandipura virus*

Death followed within 24 h of I.C. or I.P. inoculation in day-old mice with the virus growing to a titre of $10^{12.9}$ $TCID_{50}$/g in brain. Adult mice were resistant to both I.P. and S.C. inoculation.

One-day-old hamsters died within 24 h of I.P. or I.C. inoculation, while adults were resistant to both routes of challenge. Pooled serum collected from the adults at 14 D.P.I. contained virus neutralizing antibody (with titres > 360) but precipitating antibody was not detected.

One of four guinea-pigs which were given an I.P. injection of virus died, and virus was recovered from a pool of liver and spleen. One of the five guinea-pigs which received an injection into the footpad developed a vesicle-like lesion at 4 D.P.I., but virus was not detected in fluid or tissue collected from the lesion. Pooled serum collected at 14 D.P.I. contained virus neutralizing antibody with a titre > 360, but not precipitating antibody.

No clinical sign of infection was recorded in rabbits receiving I.V. injections of virus, but both virus neutralizing antibody (titre > 360) and precipitating antibodies were detected in serum pools collected at 14 and 28 D.P.I.

Embryonating chicken eggs were dead at 2 D.P.I. and harvested allantoic fluid had a titre of $10^{7.8}$ $TCID_{5.0}$/0·025 ml.

*Isfahan virus.*

One-day-old mice died within 48 h of I.C. injection of virus and within 72 h of receiving an I.P. injection. Harvested brain tissue contained $10^{10.1}$ $TCID_{50}$ of infectious virus/g. Death did not follow either I.P. or S.C. injection of adult mice.

One-day-old hamsters died within 48 h of receiving an I.P. or I.C. injection of virus, while adult hamsters survived I.P. or S.C. inoculation. Virus neutralizing

antibody (with a titre > 360) as well as precipitating antibodies, were detected in serum collected from the adults at 14 D.P.I.

No lesion or deaths was observed in the guinea-pigs which received injections into the footpad or in those given I.P. injections. Both neutralizing (titre 89) and precipitating antibodies were detected at 14 D.P.I.

No clinical sign of infection was observed in the rabbits receiving I.V. injections, but neutralizing antibody with a titre > 360 was found at 28 D.P.I.

Two of the five embryonating eggs which were inoculated were dead at 2 D.P.I., and the other three died at 3 D.P.I. Harvested allantoic fluid had a titre of $10^{5.8}$ $TCID_{50}$/0·025 ml.

## DISCUSSION

Natural outbreaks of VS caused by the New Jersey or Indiana serotypes of VSV are characterized by the formation of vesicles on tongues and coronary bands of horses and cattle, and on the snouts and coronary bands of pigs. (Hanson, 1981). When these vesicles extend they usually rupture, forming an ulcer leading to the loss of lingual epithelium and occasionally extending into the oesophagus. Using a protocol similar to the one reported here we have previously recorded lesions of this severity, accompanied by a febrile response, in ponies, steers and pigs inoculated with a field isolate of New Jersey VSV (House & Wilks, 1983).

The lesions produced by Chandipura and Isfahan viruses were much milder than those associated with either natural or experimental infection with New Jersey or Indiana viruses. Febrile reactions were not recorded in any of the animals. Vesicular lesions were seen on the tongues of the ponies receiving injections of either Chandipura or Isfahan virus but, following rupture of the vesicles, there was no extension of the lesions and healing was rapid. Only Chandipura virus caused lesions in other species of domestic animals, in one of two steers and one of two goats, but these lesions also were mild and resolved rapidly.

It should be noted that there was a considerable difference in the titres of the virus suspensions used ($10^{10.3}$ $TCID_{50}$/0·025 ml and $10^{7.5}$ $TCID_{50}$/0·025 ml of Chandipura and Isfahan respectively). This may have accounted for the milder lesions observed in the ponies after Isfahan inoculations and for the more restricted host range found with this virus. In our previously reported study with Piry virus (Wilks & House, 1984), where the viral titre of the inoculum was $10^{8.3}$ $TCID_{50}$/0·025 ml, ponies were the only domestic animals to develop lesions and these were restricted to ulcerations at the site of inoculation.

There was serological evidence that Chandipura virus spread from the inoculated animals to the contact ponies and steers, but no clinical sign of infection was detected. Subpassage of these viruses using infected tongue tissue did not enhance their pathogenicity for ponies or steers; on the contrary, the animal responses were even less apparent, possibly due to lower titres of virus in the inoculum.

Although Chandipura virus is considered exotic to the United States of America, low levels of virus neutralizing antibody were found in the pre-inoculation sera of some of the domestic animals, particularly the steers and pigs. We have reported similar findings previously in native American cattle and pigs with Piry virus (Wilks & House, 1984), and with Indiana 2 (Cocal) and Indiana 3 (Alagoas) viruses

(House & Wilks, 1983), also believed to be exotic to the United States of America. All these titres were low and are considered to be non-specific reactions, possibly similar to that described with some human sera against Indiana virus (Mills, Beebe & Cooper, 1979). No such activity was found against Isfahan virus.

The IEOP test, employing viral glycoprotein as antigen, is useful for the detection of antibody in domestic species following infection with New Jersey or Indiana Serotypes of VSV (Wilks, Jenney & House, 1984) and Piry virus (Wilks & House, 1984). It appears, however, to be of little value in Chandipura and Isfahan infection. Differences in the nature of the glycoproteins (Wilks & House, 1985) or in the antibody response elicited by these viruses may account for this phenomenon.

Death in day-old mice following I.P. or I.C. injections of Chandipura virus agrees with previous findings (Anon, 1967; Berge, 1975), which also recorded deaths in 3- to 4-week-old mice given I.C. or I.P. injections. Our findings suggest that day-old hamsters are also a very sensitive host, but adult hamsters, guinea-pigs and rabbits may only be valuable as a source of serum antibodies. The one guinea-pig which died following an I.P. injection may indicate that, if younger animals had been used, they would also have been susceptible to infection. Virus was not isolated from the single footpad lesion that occurred, and it may have been simply a local reaction to the trauma of the injection.

Day-old mice have previously been shown to be susceptible to I.C. injection of Isfahan virus (Tesh *et al.* 1977); we have confirmed this and shown that the I.P. route is also an acceptable alternative. Day-old hamsters also appear to be a highly susceptible host for *in vivo* work with this virus while adult hamsters, guinea-pigs and rabbits are, again, good sources of serum antibodies due to their resistance.

Studies of laboratory animal susceptibility to Piry virus have yielded similar results (Wilks & House, 1984), although Piry virus also produced lethal infections in two of five adult hamsters inoculated I.P. and, presumably, by contact with adult female hamsters who had young inoculated I.C.

Embryonating eggs are susceptible to both Chandipura and Isfahan viruses when inoculated by the allantoic route, indicating that they also may be useful for virus isolation from field material. However, in view of the low titre of virus found in allantoic fluid ($10^{7.8}$ $TCID_{50}$/0·0215 ml and $10^{5.8}$ $TCID_{50}$/0·25 ml for Chandipura and Isfahan viruses respectively) these appear to be a far less sensitive host than day-old mice or hamsters.

Serological surveys in Iran following the isolation of Isfahan virus detected neutralizing antibodies in humans and in rodents but not in sheep, goats, cattle, chickens or pigeons (Tesh *et al.* 1977). While this suggests that Isfahan virus is not readily transmitted to the latter species, at least under the prevailing ecological conditions, it does not rule out the possibility that transmission could occur elsewhere.

Since the original isolation of Chandipura virus from man in India, it has also been recovered from *Phlebotomus* sp. in India, and the hedgehog (*Atelerix albiventris*) in Nigeria (Anon, 1967). Serological surveys in India have revealed neutralizing antibody in the sera of man, horses, donkeys, cattle, sheep, goats and rhesus monkeys.

There is no evidence that natural infection with either of these viruses produces

a clinically obvious vesicular disease in domestic animals. Even following experimental infection of domestic animals, as recorded here, these viruses induce a much milder disease than classical VS and have a more limited host range. It is, therefore, concluded that infection with either Chandipura or Isfahan virus would not likely be a source of confusion with foot-and-mouth disease or classical vesicular stomatitis.

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