3. Rangaswami, G., *Bull. Indian Phytopath. Soc.*, 1968, 4, 120.
4. Balasubramanian, A. and Rangaswami, G., *Mysore J. agric. Sci.*, 1967, 1, 233.
5. Husain, S. S. and McKeen, W. E., *Phytopathology*, 1963, 52, 541.
6. Demetriades, S. P., *Nature* (Lond.), 1956, 177, 95.
7. Wallace, R. H. and Lochhead, A. G., *Soil Sci.*, 1950, 28 C, 1.

AFFINITY OF SHEEP POX VIRUS (SP_s STRAIN) FOR HETEROLOGOUS SYSTEMS

ON reviewing the literature on sheep pox it has been observed that workers have mostly attempted to cultivate sheep pox virus in homologous system in the hope to produce vaccine virus for large scale immunization of sheep. Evidently, heterologous system need to be investigated in greater detail to that virulence is lowered; therefore, this study was undertaken to passage sheep pox virus in different heterologous systems.

Sheep pox virus (SPV) was received as infected skin of lambs suspended in buffered glycerine saline from the department of bacteriology of U.P., Vety. College, Mathura. The virus was purified by Arcton according to the method of Epstein (1958).

Tissue Culture

Mouse embryo fibroblast monolayers were prepared, according to the method of Evans and Salaman (1965) except we used inactivated lamb serum.

Chick embryo fibroblast were prepared from 10-day-old white leghorn embryonated eggs. Cell preparation was same as in mouse embryo except tissue was trypsinised for 40 min. and number of cells was adjusted to 300,000/ml.

Inoculation in Unirradiated Animals

The SPV (10,000 SID 50) was inoculated intracranially in 4-day-old mice and rats and intratesticularly in 20, 40, 60-day-old male mice and rats at the rate of 0·03 ml/animal. After 6 days of observation the animals were sacrified and such 6 and 4 blind passages were given respectively.

Rabbits and guinea pigs (6 months old) were given, in each testis, 0·5 ml of SPV (10,000 SID 50). The testis and animals were observed for 6 days. Such four successive passages were given.

The sheep pox virus (10,000 and 100 SID 50) was inoculated in 12-day-old embryonated eggs by chorio-allantoic (CAM) route at the rate of 0·2 ml/egg. After 96 hours at 37° C virus was harvested and such 4 blind passages were given each time the CAM was examined visually.

In Irradiated Mice

X-Ray irradiation, A200 KV-X-Ray apparatus (Siemens) with an irradiation rate of 200 *r*/3 mins. 10 sec., of 252·5 and 505 *r*, was given to mice in two groups of 20 each according to their body weight. Ten mice, in each group (5 gm and 10 gm), were not exposed to serve as control. Mice from each high and low dose irradiated groups and the unirradiated control were then challenged with SPV (10,000 SID 50) intracranially at the rate of 0·03 ml per mouse. Encephalitic symptoms and brains were observed at the end of 10 days for any gross lesions.

In Tissue Culture

The monolayers of chick and mouse embryo fibroblast in 4 oz. bottle were infected with SPV (10,000 SID 50) by inoculating 1 ml per bottle. After virus adsorption at 37° C for 1 hr 2 ml of maintenance medium was added and cultures were reincubated at 37° C for 140 hours. The virus was liberated by freezing and thawing and centrifugated at 3000 rpm. for 10 min. to remove coarse particles. Such four successive passages were given.

The virus from passaged material from animals and tissue cultures were inoculated i/d in lambs to observe any loss in virulence.

In Intact Animals

Macroscopic examination of brains of 4-day-old mice and rats inoculated intracranially in each passage, revealed no lesions.

Hyaluronidase is known to facilitate the spreading of virus, and testis are known to be rich in this enzyme (Monroe *et al.*, 1949; Sen, 1968). Male mice and rats of varying age groups, *i.e.*, 20, 40 and 60 days, were, therefore, inoculated intratesticularly. Macroscopic examination revealed no change.

Passage of SPV in rabbits and guinea pig testis did not bring about any gross change.

The visual examination of the SPV inoculated CAM revealed thicknening of the membrane which increased progressively on successive passages, such thickening was not found in the control membranes.

The passaged materials obtained from mice, rats, rabbits, guinea pigs and embryonated eggs were inoculated in lambs skin intradermally, neither they produce any gross lesion nor any rise of body temperature upto 10th day. On challenge with virulent SPV, no protection of the inoculated lambs was observed, showing that SPV apparently did not multiply in mice and rats brain, mice, rats, rabbits, guinea pig testis as well as on CAM of eggs.

Groups of mice were irradiated with 252·5 *r* and 505 *r* prior to inoculation of the SPV intracranially, observation revealed that irradiation of mice apparently did not have any effect on virus adapta-

tion. No symptoms of encephalitis or gross lesions in brain were found in irradiated mice.

Cultured Tissue Cells

Mouse and chick embryo fibroblast passaged SPV when inoculated in lamb skin, only mouse embryo passage was able to cause a certain visible reaction in lambs and infected animals died after 14 days with specific symptoms of sheep pox infection. While no reaction was observed with chick embryo passaged material..

On challenge with virulent SPV the lambs died after 10 days of post-infection.

The fact that attempts by workers to adapt and attenuate sheep pox virus, in heterologous intact hosts and their cultured tissue cells, have failed suggests that we may have inadequate information about the physico-biological properties of the virus itself. Our attempt to adapt SPV in suckling mice and rats and adult male mice, rats, rabbits, guinea pig and chick embryo, intracranially and intratesticularly, met with failure. Similar negative results have also been reported by Angeloff (1940), Sen (1968), Ortenzi (1954), and Ozcebe *et al.* (1958), while, Trotsenko (1961) had reported success in the adaptation of a chinese strain of sheep pox virus in chorio-allantoic membrane.

In the present studies, cultured tissue cells of mouse embryo could be infected by SPV. Passaged virus produce specific symptom of sheep pox disease in lambs preceded by death.

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1. Angeloff, S., *Dtsch. Tierarztl. wschr.*, 1940, 48, 385 ; *Vet. Bull.*, 12, 584.
2. Epstein, M. A., *Brit. J. exp. Path.*, 1958, 39, 436.
3. Evans, R. and Salaman, M. H., *J. exp. Med.*, 1965, 122, 993.
4. Monroe, E., Freeman, P. A., Marian, O. and Albert, D., *J. biol. Chem.*, 1949, 180, 655.
5. Ortenzi. R. and Tiecco, G., *Zooprofilassi*, 1954, 9, 79 ; *Vet. Bull.*, 25, 669.
6. Ozcebe, I., Guley, M. and Ozaksit, A., *Turk. vet. Hekim. dern. Derg.*, 1958, 28, 4 ; *Vet. Bull.*, 29, 705.
7. Sen. K. C., *Indian J. med. Res.*, 1968, 56, 1157.
8. Trotsenko, N. I., *Trudy nauchno-Kontrol. Inst. vet. preparatov*, 1961, 9, 24 ; *Vet. Bull.*, 32, 451.

ON THE FREQUENCY OF OCCURRENCE OF *PINNOTHERES* SP. IN THE WINDOW-PANE OYSTER, *PLACENTA PLACENTA* (LINNE)

Hornell and Southwell[1] and Chhapgar[2] have described and figured *Pinnotheres placunae*. Silas and Alagarswamy[3] have reviewed the available literature on the systematics, ecology, biology and ethology of the pea-crab of the genus *Pinnotheres* (Latreille) while giving an instance of parasitisation by the pea-crab *Pinnotheres* sp. on the backwater clam *Meretrix casta*. The species identification of the pea-crab presently reported is under scrutiny. The frequency of its occurrence in eighty forms of *Placenta placenta* collected from Kakinada Bay, on the east coast of India during December, 1973 is reported here. Based on the existing literature on the subject it can be said that this is the first report on *Pinnotheres* sp. in *Placenta placenta*.

A total of sixty-four out of the eighty window-pane oysters (80·0%) examined were infested with one or more pea-crabs. An analysis of the frequency of occurrence of the pea-crabs in the infested forms showed the following position :

	No.	%
Number of infested forms out of eighty examined ..	64	80·0
Number of forms with single crab each ..	63	79·0
Number of forms with two crabs each ..	1	1·2

For sex-wise occurrence and stage of development of crabs, the window-pane oysters were specially examined. The examination has revealed that there were sixty-one females and four males as follows :

Female		No.
Stage I (Hard-shelled stage)	..	Nil
Stage II (Soft-shelled stage)	..	1
Stage IV (Adult, Non-ovigerous)	..	13
Stage V (Adult, Ovigerous)	..	47
Total	..	61

Male		
Stage I (Hard-shelled stage)	..	1
Stage II (Soft-shelled stage)	..	3
Total	..	4

A further analysis of the single and multiple infestations by the pea-crabs in the sixty-four infested window-pane oysters examined shows the following :

Single infestation		No.
Total Number	..	63
Female-stage I (Hard shelled stage)	..	Nil