Viral Susceptibility Range of the Fathead Minnow 
(Pimephales promelas) Poikilothermic Cell Line¹

JUAN SOLIS² AND EMILIO C. MORA

Department of Poultry Science, Auburn University, Auburn, Alabama 36830

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The viral susceptibility range of a poikilothermic cell line derived from the fathead minnow (Pimephales promelas) (FHM) to infection by a number of homioothermic viruses representing most of the presently recognized viral groups and a member of the psittacosis-lymphogranuloma-trachoma group of agents was studied. All infectious agents, except polyovirus types 1 and 3, infectious bursal agent, and an avian infectious bronchitis virus (IBV) strain, readily multiplied in the FHM cell culture system, producing a detectable cytopathic effect. Although inconclusive evidence was obtained with two other avian IBV strains, these results indicated the ability of the FHM cell culture system to readily support the propagation of a variety of cytopathogenic homioothermic viral agents.

During the past 2 years this laboratory has been engaged in the evaluation of established cell lines of various animal and tissue origin as host cells for the propagation of animal viruses. This report describes the susceptibility of a poikilothermic cell line derived from the fathead minnow (FHM) to infection by a variety of viral agents affecting homioothermic animals including human, bovine, canine, and avian species.

MATERIALS AND METHODS

Viruses. Table 1 enumerates the virus strains used and their sources.

Cell cultures. The FHM cell culture (8) was obtained from Nikolai Fijan, Fisheries Section, Zoology Department, Auburn University, Auburn, Ala. These cells were grown in Eagles minimum essential medium (MEM) with twice the normal concentration of vitamins, essential amino acids, and 1-glutamine, and supplemented with 10% heat-inactivated fetal bovine serum (FBS), plus 100 units of penicillin and 100 µg of streptomycin per ml. Cell cultures were maintained in an identical medium, except that the serum concentration was reduced to 2% and the antibiotics were deleted. The pH of the media was adjusted to 7.4 to 7.6 by the addition of a 7.5% solution of sodium bicarbonate. The heteroploid cell line derived from human embryonic intestine by Henle (9) was propagated and maintained in a similar culture medium except that FBS was replaced by calf serum. Both cell lines were grown in Leighton tubes and incubated at 36 C throughout the study.

¹ An abridgment of portions of a dissertation submitted by Juan Solis in partial fulfillment of the requisite for the degree of Doctor of Philosophy, Auburn University, Auburn, Ala.

² Present address: Poultry Science Department, Clemson University, Clemson, S.C. 29631.

Virus assay. The infectivity of all test viruses, except that of polioviruses and infectious bursal agent (IBA), was titrated in embryonated hens' eggs by using standard techniques (5) and expressed as 50% chicken embryo lethal dose (CELD₅₀) per ml. Henle's intestine cells were used to assay the polioviruses, whereas 4-week-old susceptible chickens were utilized to assay the IBA.

Experimental. Each viral agent was inoculated undiluted in 0.2-ml amounts into each of six culture tubes of FHM cells and allowed to adsorb for 3 to 4 hr at 36 C. Inoculated cultures were rinsed four times with warm Earles balanced salt solution to remove unadsorbed virus before the addition of 1.5 ml of maintenance medium per tube. Generally, serial passages were conducted at timed intervals corresponding with first appearance of viral cytopathic effect (CPE) (Table 2). Maintenance medium was replaced every 3 days with those agents that produced a delayed CPE. The culture tubes were quickly frozen and thawed three times, and the medium and cells were harvested for virus passage. At least three blind passages with undiluted cell culture fluid as inoculum were attempted before negative results were recorded.

The amount of virus present in the cell cultures at each passage level was titrated by inoculation of serial 10-fold dilutions into a susceptible host. For all titrations, five tube cultures or five eggs were used per dilution. The presence of mumps virus in the infected eggs was determined by hemagglutination with 1% chicken erythrocytes. Hemadsorption tests in the control noninoculated cell cultures were performed by the method of Shelokov et al. (16). Virus titers were calculated by the method of Reed and Muench (14).

RESULTS

Table 2 summarizes the results. Control noninoculated cultures were simultaneously observed
at each virus passage for spontaneous appearance of cytopathology, and each culture was tested for hemadsorption with chicken and human type "O" and "A" erythrocytes. In no case was there evidence of adventitious viral agents in the cell cultures.

The FHM cell culture system was readily susceptible to infection by 13 of the 19 viruses tested. Each virus was propagated for at least five serial passages, with some undergoing as many as 10 to 20. Only Sindbis and vesicular stomatitis viruses showed a marked and rapid cytocidal property against the FHM cells. All other viruses produced at best only a moderate degree of cellular destruction even though most of them replicated to high titers. Without exception, every virus that successfully multiplied in the FHM cells induced a detectable CPE.

The replication of poliovirus types 1 and 3 as well as that of IBA was not supported by the FHM cell culture system. Two of the three strains of avian infectious bronchitis virus (IBV) multiplied only for three serial passages during which time they produced no visible CPE, but failed to replicate on subsequent passages.

**DISCUSSION**

Cell culture systems of different animal origin have been widely used in the last 15 years to propagate animal viruses. Although growth of fish viruses appears to be supported only by fish cell cultures (8, 10, 20, 21), some frog viruses are able to propagate in certain poikilothermic, mammalian, and chick cell culture systems (7, 4, 13). However, few cell lines (3, 8, 11; G. H. Waddel and M. M. Sigel, Bacteriol. Proc., p. 99, 1965) and primary cell cultures (6, 17, 18) derived from poikilothermic vertebrates support limited replication of some mammalian and avian viruses.

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**Table 1. Virus strains studied**

| Virus                        | Source                                    | Passage history, in our laboratory | Strain        | Virus group     |
|------------------------------|-------------------------------------------|------------------------------------|---------------|-----------------|
| Vaccinia                     | Commercial vaccine, Eli Lilly & Co.        | 6 CAM                              | GB Texas Calif. | Poxvirus        |
| Fowl pox                     | Commercial vaccine, Delaware Poultry Labs. Inc. | 10 CAM                             | Poxvirus      |
| Newcastle disease virus      | Field outbreak                            | TNTC allantoic                     | Myxovirus     |
| Newcastle disease virus TC   | Commercial vaccine, Elanco Products Co.    | 3 Allantoic                         | Myxovirus     |
| Mumps                        | ATCC VR 106                               | 5 Allantoic                         | Enders        |
| Avian infectious laryngotraceitis | ATCC VR 189     | 5 CAM                              | Myxovirus     |
| Avian infectious laryngotraceitis TC | ATCC VR 158 | 5 Allantoic                         | Herpesvirus   |
| Herpes simplex               | Clinical oral specimen                     | 6 CAM                              | Herpesvirus   |
| Herpes simplex               | Clinical oral specimen                     | 6 CAM                              | Herpesvirus   |
| Poliovirus 1                 | Commercial vaccine, Lederle Laboratories   | 10 HHEI                            | Picornavirus  |
| Poliovirus 3                 | Commercial vaccine, Lederle Laboratories   | 10 HHEI                            | Picornavirus  |
| Infectious bursal agent      | Roberts, Auburn, Ala.                     | 5 C                                | Beaudette     |
| Infectious bronchitis virus  | Cunningham, Mich. State Univ.             | 6 Allantoic                         | Unclassified  |
| Infectious bronchitis virus  | Cunningham, Mich. State Univ.             | 2 Allantoic                         | Unclassified  |
| Infectious bronchitis virus  | ATCC VR 68                                | 5 Allantoic                         | Beaudette     |
| Sindbis                      | ATCC VR 158                               | 5 Allantoic                         | AR-339        |
| Vesicular stomatitis virus   | ATCC VR 189                               | 6 Allantoic                         | Indiana       |
| Rabies                       | Commercial vaccine, American Cyanamid Co. | 2 Allantoic                         | Flury         |
| Sporadic bovine encephalo-  | ATCC VR 189                               | 5 CEYS                             | Rhabdovirus   |
| myelitis                     |                                           |                                    | Rhabdovirus   |

\[a\] Numbers represent number of passages. Abbreviations indicate: ATTC, American Type Culture Collection; CAM, chicken embryo chorioallantoic membrane; TNTC, too numerous to count; HHEI, Henle's Human Embryonic Intestine Cell Line; C, chicken; CEYS, chicken embryo yolk sac.

\[b\] These viruses were not propagated in our laboratory.
Table 2. Results of propagation of various viral agents in fathead minnow cell culture

| Virus                               | Virus passage | Infectivity titer (log$_{10}$ CELD$_{50}$/ml) | Passage interval (hr) |
|-------------------------------------|---------------|-----------------------------------------------|-----------------------|
| Vaccinia                            | TC 1          | 5.5                                           | 72                    |
|                                     | TC 10         | 6.7                                           |                       |
| Fowl pox                            | TC 1          | 5.2                                           | 48–72                 |
|                                     | TC 10         | 6.1                                           |                       |
| Newcastle disease virus GB          | TC 1          | 6.6                                           | 72                    |
|                                     | TC 20         | 6.7                                           |                       |
| Newcastle disease virus TC          | TC 1          | 4.1                                           | 72                    |
|                                     | TC 10         | 7.7                                           |                       |
| Mumps                               | TC 1          | 3.8                                           | 24–30                 |
|                                     | TC 5          | 6.4                                           |                       |
| Avian infectious laryngotracheitis TC | TC 1        | 5.8                                           | 48                    |
|                                     | TC 10         | 6.9                                           |                       |
| Avian infectious laryngotracheitis TC | TC 1        | 5.5                                           | 48                    |
|                                     | TC 7          | 6.7                                           |                       |
| Herpes simplex                      | TC 1          | 4.5                                           | 96                    |
|                                     | TC 5          | 5.2                                           |                       |
| Herpes simplex TC                  | TC 1          | 3.7                                           | 72                    |
|                                     | TC 6          | 4.2                                           |                       |
| Poliovirus 1                        | TC 1          | Negative$^a$                                  |                       |
| Poliovirus 3                        | TC 1          | Negative$^b$                                  |                       |
| Infectious bursal agent            | TC 1          | Negative$^a$                                  |                       |
| Infectious bronchitis virus (Roberts) | TC 1        | 5.4                                           | 48–72                 |
|                                     | TC 3          | 1.7                                           |                       |
| Infectious bronchitis virus (Beaudette) | TC 1        | 5.7                                           | 48–72                 |
|                                     | TC 3          | 1.4                                           |                       |
| Infectious bronchitis virus (Beaudette TC) | TC 1    | Negative$^b$                                  |                       |
| Sindbis                             | TC 1          | 7.0                                           | 24                    |
|                                     | TC 7          | 7.4                                           |                       |
| Vesicular stomatitis virus          | TC 1          | 7.5                                           | 12–24                 |
|                                     | TC 5          | 6.9                                           |                       |
| Rabies                              | TC 1          | 7.5                                           | 72                    |
|                                     | TC 5          | 5.2                                           |                       |
| Sporadic bovine encephalomyelitis   | TC 1          | 4.5                                           | 168                   |
|                                     | TC 5          | 7.2                                           |                       |

$^a$ Infectivity titer expressed as TCID$_{50}$.
$^b$ Infectivity titer expressed as chicken infective dose (CID$_{50}$).

To our knowledge, this is the first comprehensive report on the potential use of a poikilothermic cell line for homiothermic animal viral research. The data obtained in this study indicated that the FHM cell culture system possesses a fairly broad spectrum of virus susceptibility. Thirteen of 19 viruses readily replicated in this host cell system, including representative members of the myxo-, arbo-, herpes-, pox-, and rhabdovirus groups and a member of the psittacosis-lymphogranulomatous chlamydia (PLT) group of agents, and to a limited extent two other strains of an as yet unclassified avian virus.

The failure of poliovirus types 1 and 3 to propagate in FHM cell cultures was probably due to the operation of an intrinsic cellular mechanism regulating the early stages of the virus-host cell interaction, possibly at the receptor site level. This hypothesis is supported by the work of Plotkin et al. (12) who reported that only cells derived from primate tissues possess enterovirus receptors and are generally susceptible to infection by poliovirus, whereas nonprimate cells lack these receptors and are normally resistant to infection. The reported susceptibility of FHM cells to poliovirus type 1 (19) should be considered with caution in lieu of the inadequate evidence presented to substantiate this contention. The resistance shown by FHM cells to infection by IBA, which may tentatively be classified as an avian enterovirus on the basis of its suggested ribonucleic acid (RNA) core (Solis, unpublished data) and its physicochemical and epidemiological features (1, 2), may be similarly explained by drawing a parallel with poliovirus and other enteroviruses of different animal species (15), all of which are highly specific for their host cell and require homologous cell cultures with their associated specific cellular receptors to establish infection.

The results obtained with the IBV strains are inconclusive and are presently being studied further. It was tentatively postulated that the observed outcome of virus propagation was influenced by the accumulation of an interferon-like substance in the infective cell culture fluids.

This study represented a preliminary evaluation of the FHM cell culture system for animal viral research. This poikilothermic cell line may offer an unusual opportunity to study the behavior of homoiothermic viruses in a host cell phylogenetically far removed from the normal host. Also, since this host cell system possesses many attributes not found in cells derived from warm-blooded vertebrates and since it is apparently free of viral contaminants and is readily available, it is felt that it may represent a valuable addition.
to the virologist's armamentarium in his never ending search for disease control and prevention.

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