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Characterization of a Bovine Parvovirus

Thomas J. Langpap

CHARACTERIZATION
OF A
BOVINE PARVOVIRUS

BY

THOMAS J. LANGPAP

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OF A
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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser _____ Date _____

Head, Microbiology Department _____ Date _____

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INTRODUCTION

Bovine parvovirus has been shown to produce calf scours (40). Although exact figures are unavailable, a sizeable economic loss is suffered annually by South Dakotans as a result of this disease. Bovine parvovirus has been isolated in South Dakota (7). However, the extent of exposure to the virus is unknown.

The object of this study is to characterize the South Dakota parvovirus isolate and determine by serological methods the extent of exposure in cattle. A preliminary study to determine the ability of parvovirus to cause abortion in cattle will also be undertaken.

LITERATURE REVIEW

Parvoviruses were classified in the following manner by the Provisional Committee for the Nomenclature of Viruses in 1966 (14). The classification is based on the system developed by Lwoff in 1966 (25).

Phylum: Vira
Subphylum: Deoxyvira
Class: Deoxycubica
Order: Haplovirales
Family: Parvoviridae

Further division into genera has not been completed at this time. The type species of this family is the Kilham Rat Virus (14).

A detailed list of the characteristics of the family Parvoviridae would include (45):

Nucleic acid - DNA
Capsid symmetry - icosahedral
Envelope - none
Site of maturation - nucleus
Ether sensitivity - resistant
Number of capsomeres - 32
Particle size - 18 to 24 nm
Molecular weight of the virus - 3.0 to 3.6 X 10^6 Daltons

Parvoviruses have been isolated from a number of host species including: rats, cats, mink, swine, dogs, humans and cattle (2). They have also been found associated with adenoviruses (2). A discussion of the parvoviruses isolated from these species follows.

Latent Rat Virus

This isolate, also known as the Kilham Rat Virus, was originally described by Kilham and Olivier in 1959 (21) and is the type species of the family Parvoviridae. The virus particle is 17 to 25 nm in diameter with a capsid of 32 capsomeres in icosahedral symmetry (2). The nucleic acid present in this virus is single stranded DNA with a

molecular weight of approximately 1.6×10^6 Daltons. This constitutes 34% of the weight of the virion (34). The virion has a buoyant density of 1.43 g/ml (2). Kilham Rat Virus agglutinates guinea pig, rat and human red blood cells (2). The hemagglutinin cannot be separated from the virus particle. Hemadsorption is possible on infected cell cultures indicating the formation of RBC adsorption sites on the cell surface (2). Antihemagglutinins and neutralizing antibodies are formed in the sera of infected animals (21). This would prompt the use of the hemagglutination inhibition (HI) and tissue culture serum neutralization (SN) tests for the diagnosis of Kilham Rat Virus infections.

The virus grows in rat, but not mouse, embryo cell cultures and in various cultures of primate and hamster origin (2). At nine days post infection, a characteristic cytopathogenic effect (CPE) can be seen in the cell cultures. This CPE is described as dense masses of cells with thin radiating extensions (2). The original isolation of this virus was from a rat tumor (21). Later isolations were also made from transplantable leukemias (21). The authors did not try to associate the virus with the etiology of either the tumors or the leukemias, but they did indicate that this isolate was a latent virus in rats (21). In the laboratory, suckling hamsters infected with small doses of Kilham Rat Virus display the osteolytic effects of dwarfism and pseudomongoloidism (2). Brain damage can also be observed in these animals (23, 27, 5).

Minute Virus of Mice

A parvovirus has also been isolated from mice. This virus has been named Minute Virus of Mice or MVM. The isolate was originally described by Crawford in 1966 (10). Morphologically, the virion exhibits icosahedral symmetry with a diameter in the range of 19 to 26 nm (10, 11). It is sometimes found in closely packed arrays within the nucleus (2). The core of the viral nucleocapsid contains single stranded DNA, and the virion has a buoyant density in cesium chloride of 1.43 g/ml (10). Hemagglutination of guinea pig, hamster,

rat and mouse erythrocytes was observed; and hemagglutination inhibition can be used as a diagnostic tool (10).

The virus can be cultivated in cultures of rat or mouse embryo cells, and a plaque assay has been developed (43). MVM replicates in many organs of baby mice and may cause runting and cerebral lesions (10). It is fatal to suckling rats (10). This virus was carried as latent infections in 79% of the mouse colonies studied by Parker et al. (32).

Feline Panleukopenia

Feline Panleukopenia was originally described by Hammon and Enders in 1939(15). They classified it as a viral disease mainly on the basis of filterability of the agent. The etiological agent of this disease has recently been classified as a parvovirus on the basis of its physical and chemical properties by Studdert et al. (42). The diameter of this virus is about 20 to 24 nm (2). It produces weak hemagglutinins for swine erythrocytes at 4°C, but the HI test is not a useful diagnostic tool (2). Neutralizing antibodies are produced in infected animals, and the fluorescent antibody test is used in diagnosis (2). The virus replicates in monolayers of feline embryo cells, and a distinct CPE is observed (42). Prominent intranuclear inclusions are observed in infected culture cells and in histologic sections of cat tissue (15, 24, 42). The disease mainly affects young cats; and the symptoms include fever, depression, dehydration, vomiting and bloody diarrhea (19). Mortality is usually between 65% and 90% (2), and all species of cats are susceptible (19). Mink enteritis is a similar disease, and the etiological agent is serologically related to feline panleukopenia virus (42).

Porcine Parvovirus

A parvovirus of swine was first described by Mayr et al. (28). The virion exhibits icosahedral symmetry with a diameter of 20 to 22 nm (28). Results of tests to determine the number of the DNA strands were inconclusive (2). The buoyant

density of the virion is 1.37 to 1.38 g/ml (28). The virus agglutinates chick, rat, guinea pig, cat, mouse and human "O" red cells (28). Hemagglutination inhibition (9, 20) and serum neutralization tests (29, 20) can be used diagnostically. Porcine parvovirus replicates in pig kidney cell cultures where it produces a distinct CPE and intranuclear inclusions (28). The virus was originally isolated from swine with infertility problems and from aborted piglets (2). It has also been isolated from cell cultures infected with hog cholera virus (28). Experimental infections in swine resulted in no clinical illness (20).

Canine Parvovirus

A canine isolate was described by Binn and co-workers in 1970 (8). It was isolated from canine feces and was cultivable only in the Walter Reed Canine Cell Culture (8).

Avian Parvovirus

Virus particles tentatively classified as parvovirus have been isolated from quail by Dutta and Pomeroy (12) and from geese by Schettler in Germany (35).

Adeno-Associated Virus

One of the most unusual groups of viruses known is the adeno-associated virus (AAV) group. This virus is of the parvovirus type and is found in some cultures of adenoviruses (4). It is theorized that the adeno-associated virus has a defective genome and cannot replicate in the absence of its adenovirus helper (2). Morphologically, the diameter of the virus is 18 to 24 nm; and it exhibits icosahedral symmetry (36). The buoyant density of the virion is 1.25 to 1.33 g/ml (36). Some adeno-associated viruses agglutinate human "O" erythrocytes at 4°C (2). At lease four serotypes exist and these can be differentiated by serum neutralization (2), complement fixation (36) and precipitin tests (2). Antibodies can be found in human sera indicating a possible association with human adenoviruses (2). Adeno-associated viruses can only replicate in the presence of

an actively replicating adenovirus genome (2, 4, 36). The AAV may reach higher titers in these cultures than the adenovirus (4). Some strains of AAV have been shown to depress replication of adenoviruses in concurrent infections (36). No pathogenicity for animal hosts has been observed with the adeno-associated viruses (2).

Bovine Parvovirus

Bovine parvovirus was first described by Abinanti and Warfield in 1961 (1). They originally isolated the virus from the feces of normal calves. Properties of the original isolate include ether resistance, thermal resistance and the ability to hemagglutinate and hemadsorb (1). Abinanti and Warfield referred to the virus as HADEN, which stands for hemadsorbing enteric (1). They considered the hemadsorption pattern of their parvovirus isolate to be rather unique. The erythrocytes had a tendency to adhere to the cell sheet in aggregates that varied from a few cells to large clumps (1). They stated that, in contrast, the myxoviruses hemadsorb individual erythrocytes diffusely over the entire cell sheet. The HADEN virus exhibits hemadsorption with both guinea pig and human "O" erythrocytes (1). By Gradocol filtration, Abinanti and Warfield found the size of the virus to be smaller than 30 nm (1). A serological survey conducted during this study showed significant HI and SN titers in 86% of the cattle tested.

Spahn, Mohanty and Hetrick, in 1966, experimentally infected calves with the HADEN virus and reported results of a serological survey in Maryland (37). The calves were infected both orally and by the intranasal route and were observed for twelve days. Those infected orally had diarrhea on days 4, 5 and 6; and the virus was isolated on days 2 through 7. Those infected intranasally experienced a mild respiratory illness on days 1 through 6 and diarrhea on day 6. The virus was recovered from these calves on days 2 through 7. In the serological survey, 83% of the cattle sampled had an HI titer of 20 or greater, further substantiating Abinanti and Warfield's observation of wide-spread exposure to the virus (1).

Stortz, Bates, Warren and Howard found significant HI titers in 65% of the cattle tested in thirty-five herds (41). Stortz and Warren, in 1969, cultivated the HADEN virus in the presence of different concentrations of 5-fluoro-2'-deoxyuridine and 5-bromo-2'-deoxyuridine and found that the multiplication of the parvovirus was inhibited at concentrations of 50 to 100 ug/ml (39). A study of the cell tropism of bovine parvoviruses by Bates and Stortz in 1973 indicated that the virus would replicate optimally in actively dividing bovine fetal lung and spleen cells (6). They further stated that other primary bovine fetal cells supported growth to a lesser extent, but bovine line cells and line cells of other animal species did not.

MATERIALS AND METHODS

Cell Cultures

Bovine fetal adrenal and bovine fetal lung cells in the fifth to the eighth passage were used in this study. The cells were initially obtained from bovine fetuses collected from a local abbatoir. Methods of propagation and subculture are as outlined (30). Stocks were stored frozen at -70°C in growth medium containing 7.5% dimethyl sulfoxide (22).

Virus

The bovine parvovirus was isolated from the intestine of an eight month old Angus calf submitted to the Animal Disease Research and Diagnostic Laboratory as accession number 70-7012. The intestine of this animal was also found to be heavily infested with Eimeria sp. oocysts. The virus was isolated in bovine adrenal cell culture, plaque purified and identified as a parvovirus by methods previously reported (7). All subsequent virus passages were stored in ampules at -70°C. The virus used in this study was at the ninth passage level at a titer of 1.3×10^8 Plaque Forming Units per ml (PFU/ml).

Media

The growth medium used was the Minimal Essential Medium of Eagle (MEM)* supplemented with 10% fetal calf serum,** penicillin (100 units/ml), streptomycin (100 ugm/ml) and kanamycin (100 units/ml). In the maintenance medium, the concentration of serum was changed from 10% to 2%. The plaque medium consisted of double strength MEM stock solution mixed with an equal volume of

*Catalog No. F-15, Grand Island Biological Company, Grand Island, New York.

**Catalog No. 614, Grand Island Biological Company, Grand Island, New York.

melted 1.4% ion agar.* Before mixing, the melted agar and 2X plaque medium were brought to 45°C in a water bath.

Plaque Assay

Infectious virus was quantitated using the plaque assay method (6). Bovine fetal lung cells were propagated in 60 mm plastic petri dishes** until complete monolayers were formed. The monolayers were washed with maintenance medium, and 0.5 ml aliquots of virus dilutions were each inoculated onto duplicate plates and allowed to adsorb for one hour. After the adsorption period, excess inoculum was decanted; and the plates were overlaid with plaque medium. The cultures were incubated for seven days at 37°C in a humidified 5% CO_2 atmosphere. The agar overlay on the plates was removed, and the cells were fixed for one minute with 2 ml of 10% formalin (17). They were then stained with crystal violet (17). The stain was rinsed off with water, and the plaques were counted.

Serological Procedures

In the hemagglutination inhibition (HI) procedure, a micro-titration system was utilized. A 0.05 ml volume of veronal buffer pH 7.3 (16) supplemented with 0.5% bovine serum albumin was added to all wells in "U" bottom Microtiter plates.*** Equal volumes of the sera to be tested were added to the eight wells in row one of each eight by twelve row plate. A 0.05 ml diluter*** was used to make serial dilutions in rows one through eleven. Row twelve was used as a red blood cell control. The serial two-fold dilutions ranged from 1:2 through 1:2048 in all HI tests. A 0.05 ml volume

*Colab Laboratory Inc., Glenwood, Illinois.

**Falcon Plastics, Oxnard, California.

***Cooke Engineering Company, Alexandria, Virginia.

of virus diluted to contain four hemagglutination (HA) was added to all wells except those in row twelve. The test was allowed to stand for fifteen minutes at room temperature for the antigen-antibody reaction to occur. A 0.05 ml volume of 0.5% v/v washed guinea pig red blood cells was added to each well in the Microtiter plate. The test plate was then agitated, sealed with transparent tape and incubated for one hour at 25°C. The HI titer was read as the highest dilution with complete inhibition of red blood cell agglutination.

In the serum neutralization (SN) test, eight by twelve row flat bottom microculture plates* were used. One tenth ml of each serum sample was added to each well in the first row. Tissue culture maintenance medium was added to the remaining wells at a volume of 0.05 ml per well. Serial two-fold dilutions of the sera were made through eight wells, using 0.05 ml Microtiter diluters. Dilutions of 1:2 through 1:256 were obtained with this procedure. A 0.05 ml volume of parvovirus containing 6.5×10^2 PFU in maintenance medium was added to each well. The virus was also added to one row without serum and to one row containing a positive control serum. The plates were held at 37°C for one hour to allow the neutralization reaction to occur. A 0.05 ml volume of low passage bovine adrenal cells was added to each well, and the test was incubated at 37°C in a humidified chamber containing a 5% CO_2 atmosphere until a distinct cytopathogenic effect (CPE) was seen in the infected control cells. The antibody titer was read as the highest dilution showing complete inhibition of CPE.

Growth Curve

A one-step growth curve experiment was designed similar to that of Stevens and Groman (38) as modified by Bates and Storz (6). Forty tubes containing two-day monolayers were inoculated with 0.2

*Falcon Plastics, Oxnard, California

ml of 1:20 stock virus (1.3×10^6 PFU) and adsorbed for one hour at 37°C. One tube containing medium with no cells was also inoculated and incubated at 37°C for one hour. This tube would later determine the thermal inactivation of the parvovirus. After one hour of adsorption, the inoculum was decanted. The monolayers were washed once with 1.0 ml of medium, and 1.0 ml of fresh medium was added. Washings were saved to determine the unadsorbed virus in a later titration. Two tubes were harvested every four hours during a seventy-two hour period. For each sampling, the medium in two tubes was removed; and the monolayers were washed twice with 2 ml volumes of maintenance medium. The original medium and washings were pooled and saved for determination of extracellular virus, and 1.0 ml of medium was replaced on the monolayers. Both tubes were frozen at -70°C for determination of intracellular virus. The experiment was completed after seventy-two hours, and the samples were assayed. Samples were frozen and thawed four times to release intracellular virus particles. The plaque assay procedure was used to determine the virus content of all the cell and supernatant samples.

Hemagglutination Assay

The samples taken in the one-step growth curve were also examined for viral hemagglutinins. In this experiment, guinea pig red blood cells were collected by cardiac puncture into Alsever's solution (13). The cells were sedimented by centrifugation and washed three times using three five-volume washes of 0.01 M phosphate buffered physiological saline pH 7.2. After the third wash, the sedimented cells were resuspended to 10% v/v in Alsever's solution. A working red blood cell stock of 0.5% v/v was prepared in veronal buffer. All growth curve samples were diluted 1:5, and 0.05 ml of each diluted sample was placed in the first row of a Microtiter "U" bottom plate. Serial two-fold dilutions were made in veronal buffer through well number eleven, yielding a dilution series of 1:10 through 1:10,240. Row number twelve did not contain virus and was left as a red blood cell control. A 0.05 ml volume

of the 0.5% red blood cell suspension was added to each well in the plate, and the plate was incubated at 25°C and observed for hemagglutination during a one hour period. The HA titer was read as the highest dilution exhibiting complete hemagglutination.

Slide Culture Method

Thirty-six double chamber tissue culture slides* were seeded with 2.0 ml of growth medium containing a sufficient number of cells to form a monolayer in twenty-four hours. They were incubated at 37°C in a humidified chamber containing a 5% CO_2 atmosphere. The growth medium was decanted. The monolayers were washed with maintenance medium, and 1.0 ml of an inoculum containing 1.3×10^6 PFU was placed in one chamber of the slide. In the other chamber, 1.0 ml of maintenance medium was placed. The virus was allowed to adsorb for one hour, and the inoculum was decanted. The monolayers were rinsed with 2.0 ml of maintenance medium, and fresh maintenance medium was added. Two slides were removed every four hours for seventy-two hours, fixed in Carnoy's fixative (30) and stored at -70°C. The slides were later examined with an indirect fluorescent antibody test, an acridine orange stain and a hematoxylin and eosin stain.

Staining Procedures

Nucleic acid stain: Parvovirus infected and non-infected bovine adrenal cell cultures were established in chambered slides and stained with Schiff reagent (30). At forty-eight hours after infection, the monolayers were fixed with Carnoy's fixative for twenty minutes and then rinsed in absolute ethanol for three minutes. The monolayers were then hydrated by successive dips in 95% and 70% ethanol. This was followed by a rinse in distilled water and an incubation in 1 N HCl solution at 60°C for ten minutes. The acid was removed by a rinse

*Lab Tek Model 4804 Double Chamber Slides, Lab Tek Products Division, Miles Laboratories Inc., Westmont, Illinois.

in distilled water, and the monolayers were stained in Schiff reagent for ten minutes. Three two-minute rinses in sodium metabisulfite wash solution and a fifteen minute wash in running tap water followed. The slides were then rinsed in 70% ethanol. A counter-stain of Fast Green FCF* solution was applied to the slides for five seconds, and the monolayers were dehydrated in absolute ethanol and were cleared in xylene. Coverslips were applied, and the slides were examined microscopically.

Fluorescent antibody stain: Four guinea pigs were hyperimmunized with five 1.0 ml intraperitoneal injections of parvovirus stock at four day intervals. The guinea pigs were exsanguinated seven days after the last injection, and antibody levels in the sera were determined by the SN test. Two sera having SN titers of 1:3200 were pooled for use in the indirect fluorescent antibody test. A commercial fluorescein isothiocyanate, labeled rabbit anti-guinea pig globulin, ** was used at the recommended dilution of 1:10. In the indirect fluorescent antibody test, infected and non-infected cell cultures grown on chambered slides were fixed by dehydration in acetone for two minutes followed by a rehydration in 0.01 M PBS pH 7.2. Dilutions of guinea pig anti-parvovirus antiserum were applied, and the slides were incubated at 37°C for thirty minutes. The slides were then dipped in 0.01 M PBS pH 7.2 followed by a fifteen minute soak in the same solution. The dilute, commercial rabbit anti-guinea pig conjugate was applied; and the slides were incubated for an additional thirty minutes. The wash step was repeated, and the slides were dipped in distilled water and mounted in Bacto FA mounting fluid pH 9.0. *** The slides were examined and photographed with a fluorescence microscope and automatic

*Allied Chemical and Dye Corp., New York, New York.
**Custom Reagent Laboratory Inc., San Diego, California.
***Difco Laboratories, Detroit, Michigan.

camera.*

Acridine orange stain: The number of strands of nucleic acid in the parvovirus was determined by acridine orange staining of infected cell cultures (26). Chambered slides of bovine adrenal cells were fixed in Carnoy's fluid for twenty minutes and rehydrated by respective dips in 80%, 70% and 50% ethanol. The slides were soaked in McIlvaine's buffer (26) at pH 3.8 for ten minutes. They were then stained in 0.01% w/v acridine orange solution. The monolayers were rinsed for two minutes in two changes of fresh McIlvaine's buffer, blotted and mounted in fresh buffer. The staining reactions were examined immediately with the fluorescence microscope, and photographs were taken.

Hematoxylin and eosin stain: After the acridine orange stain, the coverslips were removed. The slides were then dipped in distilled water and were stained with hematoxylin and eosin (3).

Buoyant Density Determination

A modified buoyant density procedure of Phillips et al. (33) was used in this study. Sufficient virus stock was obtained by cultivating the virus on low passage bovine adrenal cells in a 75 cm^2 tissue culture flask** The virus was harvested forty-eight hours after infection by four cycles of freeze-thaw. Cell debris was removed by centrifugation at 3020g for fifteen minutes at 4°C. The resulting virus suspension was concentrated fifteen-fold by osmotically-forced dialysis in Carbowax*** (polyethylene glycol 20,000). The virus suspension was placed in 22 mm dialysis tubing and dialyzed against 500 ml of Carbowax (20% w/v) in 0.01 M phosphate buffered physiological saline (PBS) pH 7.2 at 4°C for

*E. Leitz, Rockleigh, New Jersey.

**Falcon Plastics, Oxnard, California.

***Fisher Scientific Company, Fairlawn, New Jersey.

twenty-four hours. Dialysis against 0.01 M PBS pH 7.2 for twenty-four hours at 4°C followed. A solution of cesium chloride was prepared in double distilled water to a density of 1.45 g/ml. Three ml of this solution were placed in a 4 ml plastic ultracentrifuge tube, and 0.5 ml of the concentrated virus suspension was layered on top followed by 0.1 ml of heavy mineral oil. The tubes were placed in an International Preparative Ultracentrifuge Model B60 with an SB-405 rotor* and were centrifuged at 40,000 rpm (135,000 g at the center of the tube) for twenty-four hours at 7°C. After centrifugation, the tubes were punctured with a hypodermic needle; and twenty-three 8-drop fractions were collected. All densities were determined by weighing 0.1 ml samples in tared Oxford** sampler tips and by dividing the weights of the samples by the weight of an equal volume of water at the same temperature. After the density of each fraction was measured, the 0.1 ml aliquots were reclaimed and diluted with 1.0 ml of maintenance medium. This was followed by dialysis for seventy-two hours against 0.01 M PBS pH 7.2 to remove the cesium chloride. Two methods, including a hemagglutination assay and an infectivity assay, were used to locate the viral material in the gradient fractions. The hemagglutination assay was similar to that previously described. The infectivity assay employed serial two-fold dilutions of each fraction in flat bottom microculture plates. A 0.05 ml volume of maintenance medium was added to all wells in the plate. An equal volume (0.05 ml) of each fraction was added to each well in the first row. A 0.05 ml diluter was used to make serial dilutions in rows one through eleven. Row twelve was used as an uninfected control. A 0.05 ml volume of bovine adrenal cells was added to all wells, and the plates were incubated at 37°C in a humidified 5% CO_2 atmosphere.

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*International Equipment Company, Needham Heights, Mass.

**Oxford Laboratories, Foster City, California.

The infectivity titer was read as the highest dilution showing CPE.

Heat Sensitivity

A 1.0 ml aliquot of parvovirus stock was incubated in a 56°C water bath while an identical aliquot was maintained at room temperature. After a thirty-minute incubation period, the contents of both tubes were diluted 10^{-1} through 10^{-7} and were assayed for infectivity by the plaque method.

Chloroform Sensitivity

One-half ml of parvovirus stock was added to two sterile test tubes. Chloroform (0.05 ml) was added to one tube, and both tubes were shaken for ten minutes. A control consisting of chloroform-sensitive, infectious bovine rhinotracheitis (IBR) virus (44) was treated in the same manner. The tubes were centrifuged for five minutes (170 g), and the supernatant fluids were assayed for infectivity by the plaque method.

Acid Sensitivity

A pH 3.0 buffer was prepared by mixing 1.7 ml of 0.1 M sodium citrate with 3.3 ml of 0.1 M citric acid. A buffer at pH 7.2 was prepared by mixing 7.2 ml of 0.5 M dibasic potassium phosphate with 2.8 ml of 0.5 M monobasic potassium phosphate. Both solutions were added to identical aliquots of parvovirus and IBR virus (44) at the rate of 0.5 ml of virus to 0.5 ml of each buffer. All treated and control virus suspensions were incubated for one hour at 37°C in a water bath. The suspensions were neutralized by the addition of 5.0 ml of the phosphate buffer prepared above and were assayed by the plaque method.

BUDR Sensitivity

A 5-bromodesoxyuridine* (BUDR) sensitivity was conducted on

*K and K Laboratories, Hollywood, California.

the parvovirus and a picornavirus control. The picornavirus used in this experiment was the bovine enterovirus LCR-4 (31), which is resistant to BUDR. Solutions of 50 and 100 ug/ml BUDR were prepared in tissue culture maintenance medium. Tubes were seeded with a sufficient quantity of bovine adrenal cells to form a monolayer in twenty-four hours. The growth medium was decanted and replaced with 1.0 ml of either 50 or 100 ug/ml BUDR medium. Incubation was continued for an additional twenty-four hours to allow for the inhibition of DNA replication in the cells. The media were decanted from the monolayers, and the viruses were added to the cells as 10^{-1} dilutions (1.3×10^{7} PFU/ml) in 50 or 100 ug/ml BUDR medium. The viruses were allowed to adsorb for one hour and were then decanted. The monolayers were washed with ten volumes of their respective media. Untreated viruses and monolayers were handled in the same manner. The inoculated tubes were observed for cytopathogenic effect, and all tubes were frozen when CPE became evident in the untreated controls. All tubes were assayed by the plaque method.

Hemagglutination Spectrum

The ability of the bovine parvovirus to agglutinate various species of red blood cells was determined. Sources of red blood cells included sheep, rat, goose, cattle, chicken, pig, horse, human "O", guinea pig, monkey and rabbit. Hemagglutination tests were conducted with each species of red blood cell at 4°C, 25°C and 37°C. The procedure used has been described under hemagglutination assay. The tests were incubated at their respective temperatures for one hour, during which they were observed for the presence of hemagglutination.

Animal Inoculations

Five adult mice* were inoculated intracerebrally with 0.02 ml

*Webster Swiss SPF mice, National Laboratory Animal Company, Creve Coeur, Missouri.

of the parvovirus stock suspension (2.6×10^7 PFU). A separate group of five adult mice were exposed by aerosol. In this procedure, 1.0 ml of stock virus diluted 1:3 (4.3×10^7 PFU) was atomized* into a beaker containing the mice. Two pregnant females received tail vein injections of 0.01 ml of stock virus (1.3×10^7 PFU), and seven suckling mice were inoculated intracerebrally with the same volume. The mice were observed for signs of infection and abortion of fetuses for periods ranging from fourteen to fifty-one days. The newborn mice from inoculated dams were examined for birth defects.

A colostrum-deprived, newborn calf was obtained and was inoculated orally with 2.0 ml of the bovine parvovirus (37). The virus inoculum contained 2.6×10^8 PFU. A pre-inoculation serum sample was tested for antibody to parvovirus by the SN test and found to be negative. The rectal temperature of the calf was observed at the time of inoculation and for seven days thereafter. The calf was observed for signs of diarrhea; and when blood and mucus was observed in the fecal material, a virus isolation from it was attempted. The calf was sacrificed, and an autopsy was performed seven days after infection. Specimens of brain, liver, spleen, kidney, adrenal, stomach, duodenum, ilium, jejunum, spiral colon and descending colon were taken. These specimens were subjected to virus isolation and fluorescent antibody examination.

A seven-month bovine fetus was exposed by laparotomy and was injected intramuscularly with 2 ml of bovine parvovirus containing 2.6×10^8 PFU, and a normal birth was allowed. Fecal material from the dam was cultured for parvovirus. Pre-colostral serum from the calf was examined for parvovirus antibodies with the SN test.

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*DeVilbiss Atomizer, RALS Laboratories, Cleveland, Ohio.

RESULTS

Serological Survey

Specimen sources and results of the serological survey are summarized in Tables 1, 2, 3 and 4. The sources of the various types of serum specimens tested are listed in Table 1. Results of hemagglutination inhibition (HI) tests on these sera are summarized in Table 2. Results of serum neutralization (SN) tests are recorded in Table 3. Serological test totals indicated that 50.7% of the sera were positive by the HI test, and 88.1% were positive by the SN test. In the HI and SN comparison study (Table 4), only the results of tests on paired pre and post-colostral sera and thoracic fluids were compared. The results of SN tests on thoracic fluids were not placed in the SN results table because the number of successful tests was insufficient. Most of the thoracic fluids contained a substance, possibly of bacterial origin, which was toxic to the bovine adrenal cells used in the test.

Growth Curve

Results of the growth curve can be found in Figures 1 and 2. The sequence of events in the development of viral antigen and nucleic acid, as detected by three staining methods, is listed in Table 5. Representative photographs at zero, twenty-four, forty-eight and seventy-two hours are shown in Figures 4, 5, 6 and 7.

Schiff reagent staining of infected and non-infected cell cultures at forty-eight hours yielded the characteristic dark red reactions of DNA in intranuclear inclusions in the infected cells. RNA in the nucleoli and cytoplasm of the infected and control cells stained green. Diffuse nuclear chromatin stained pink.

Buoyant Density Determination

The density gradient obtained is represented in Figure 3. A straight line was calculated for the data by the method of least squares (45). The hemagglutination (HA) and cell culture infectivity peaks are shown in Figure 3.

Chemical and Physical Agents

The effect of various chemical and physical agents upon the titer of parvovirus 70-7012 and control viruses is summarized in Table 6.

Hemagglutination Spectrum

Results of tests to determine the ability of parvovirus 70-7012 to agglutinate erythrocytes from various species are listed in Table 7.

Animal Inoculations

The two groups of five adult mice injected intracerebrally and exposed by aerosol were observed for fifty-one days. No evidence of clinical illness was detected. Normal litters were delivered by the two pregnant mice. The seven suckling mice injected intracerebrally were observed for twenty-three days and exhibited no signs of illness.

The colostrom-deprived calf exhibited a 2°F increase in temperature and diarrhea four days after inoculation. Six days after inoculation, diarrhea was severe; and the fecal material contained considerable mucus and some blood. At post-mortem examination, enteritis was observed, primarily in the large intestine. The large intestine contained yellow fluid and gas, and the colon was devoid of solid feces. A positive fluorescent antibody reaction was noted in frozen sections of jejunum and adrenal stained with parvovirus conjugate. The virus was isolated from specimens of intestinal contents.

Intramuscular inoculation of a seven-month fetus, exposed by laparotomy, resulted in no evidence of disease in the dam or fetus. Fecal material from the dam was cultured on bovine adrenal cells and found to be free of parvovirus. A clinically normal calf was delivered, and a serum sample from this calf exhibited an SN titer greater than 1:256.

Table 1--Sources of Serological Specimens.

Specimen	Source
Thoracic fluids	Aborted fetuses submitted to Animal Disease Research and Diagnostic Laboratory
Arizona Pre and Post-colostral and dams	University of Arizona
SDSU Pre and Post-colostral	SDSU dairy herd
Abortion	Dams of aborted fetuses submitted to Animal Disease Research and Diagnostic Laboratory
Healthy cattle	Routine submissions in the State-Federal Brucella Eradication Program
Deer	Arizona Game and Fish Department

Table 2--Serological Survey: HI Titers of 398 Sera Tested With Parvovirus Isolate 70-7012.

Serological Specimen	Antibody Titer*									
	Negative	16	32	64	128	256	512	1024	2048	Total
Thoracic fluid	76/47.8**	22/13.8	16/10.1	14/8.8	12/7.5	9/5.7	7/4.4	2/1.3	1/0.6	159
Deer serums	81/93.1	3/3.4	1/1.1	1/1.1	0/0	1/1.1	0/0	0/0	0/0	87
Pre-colostral calf serum (Arizona)	25/55.6	7/15.6	9/20.0	2/4.4	0/0	2/4.4	0/0	0/0	0/0	45
Post-colostral calf serum (Arizona)	8/26.7	2/6.7	4/13.3	7/23.3	5/16.7	1/3.3	2/6.7	1/3.3	0/0	30
Dams of Pre and Post (Arizona)	6/14.6	0/0	2/4.9	3/7.3	4/9.8	12/29.3	9/21.9	5/12.2	0/0	41
SDSU pre-colostral	0/0	1/5.6	0/0	1/5.6	1/5.6	5/27.8	6/33.3	3/16.7	1.5.6	18
SDSU post-colostral	0/0	0/0	0/0	0/0	0/0	1/5.9	6/35.3	0/0	11/61.1	18
Totals	196/49.3***	35/8.8	32/8.0	28/7.0	22/5.5	31/7.8	30/7.5	11/2.8	13/3.3	398

*reciprocal of highest dilution showing complete hemagglutination inhibition

**number / corresponding percent of total of each specimen type

***total of titer group / percent of total tested

Table 3--Serological Survey: SN Titers of 477 Sera Tested With Parvovirus Isolate 70-7012.

Serological Specimen	Antibody Titer*						
	Negative	16	32	64	128	256	Total
Pre-colostral calf serum (Arizona)	0/0**	13/35.1	4/10.8	2/5.4	7/18.9	11/29.7	37
Post-colostral calf serum (Arizona)	0/0	1/3.3	3/10.0	5/16.7	11/36.7	10/33.3	30
SDSU pre-colostral	33/78.6	7/16.7	1/2.4	0/0	1/2.4	0/0	42
SDSU post-colostral	0/0	2/3.4	6/10.2	12/20.3	18/30.5	21/35.6	59
Abortion	14/10.4	23/17.2	29/21.5	35/25.9	23/17.0	11/8.1	135
Healthy cattle	10/5.7	23/13.2	42/24.1	46/26.4	29/16.7	24/13.8	174
Totals	57/11.9***	69/14.5	85/17.8	100/21.0	89/18.7	77/16.1	477

*reciprocal of highest dilution showing complete inhibition of CPE

**number/ corresponding percent of total of each specimen type

***total of titer group/ percent of total tested

Table 4--Serological Survey: Comparison of Results Obtained by HI and SN Methods.

Serological Specimen	Comparison of Results			
	HI positive and SN positive	HI negative and SN positive	HI positive and SN negative	HI negative and SN negative
Thoracic fluid	3	0	8	8
Pre-colostral calf serum (Arizona)	18	19	2	8
Post-colostral calf serum (Arizona)	23	6	0	1
SDSU pre-colostral	2	0	14	0
SDSU post-colostral	16	0	0	0
Number/Total	62/128	25/128	24/128	17/128
Percent	48.0	19.5	18.8	13.3

Table 5--Sequence of Events in the Development of Viral Antigen and Nucleic Acid

Time	Acridine Orange	Fluorescent Antibody	Hematoxylin and Eosin
(hours)	Red Inclusions	Fluorescence	Intranuclear Inclusions
0	-	D*	-
4	-	-	-
8	-	-	-
12	-	SI**	-
16	-	I***	-
20	+	PIC****	+
24	+	PIC	+
28	+	PIC	+
32	+	PIC	+
36	+	PIC	+
40	+	PIC	+
44	+	PIC	+
48	+	PIC	+
52	+	PIC	+
56	+	PIC	+
60	+	PIC	+
64	cell destruction	D	cell destruction
68	cell destruction	D	cell destruction
72	cell destruction	D	cell destruction

*diffuse

**scattered intranuclear

***intranuclear

****perinuclear, intranuclear and cytoplasmic

Table 6--Chemical and Physical Agents: Effect of Chemical and Physical Agents on Parvovirus Isolate 70-7012 and Control Viruses.

Virus	Untreated	Heat	Chloroform	Acid	BUDR
Parvovirus 70-7012	1.3×10^{8}*	9.4×10^{7}	1.2×10^{8}	9.6×10^{8}	1.3×10^{3}
IBR	3.0×10^{7}	N. T.**	1×10^{1}	1×10^{1}	N. T.
Enterovirus LCR-4	2.7×10^{5}	N. T.	N. T.	N. T.	8.9×10^{6}

*virus titer in PFU/ml

** not tested

Table 7--Hemagglutination Spectrum: Ability of Parvovirus 70-7012 to Agglutinate Erythrocytes from Various Sources.

Erythrocyte Source	Incubation Temperature		
	4°C	25°C	37°C
Sheep	negative	negative	negative
Rat	negative	negative	negative
Goose	negative	negative	negative
Cattle	negative	negative	negative
Chicken	negative	negative	negative
Pig	negative	negative	negative
Horse	negative	negative	negative
Human "O"	positive	positive	positive
Guinea pig	positive	positive	negative
Monkey	negative	negative	negative
Rabbit	negative	negative	negative

Figure 1--One-Step Growth Curve of Parvovirus 70-7012 in Bovine Adrenal Cells: Assay of Infectivity in Bovine Lung Cells.

The virus titer of the supernatant (solid line) and the cell lysate (dotted line) are plotted as functions of the time, after inoculation, at which the sample was taken.

Figure 1--One-Step Growth Curve: Assay of Infectivity.

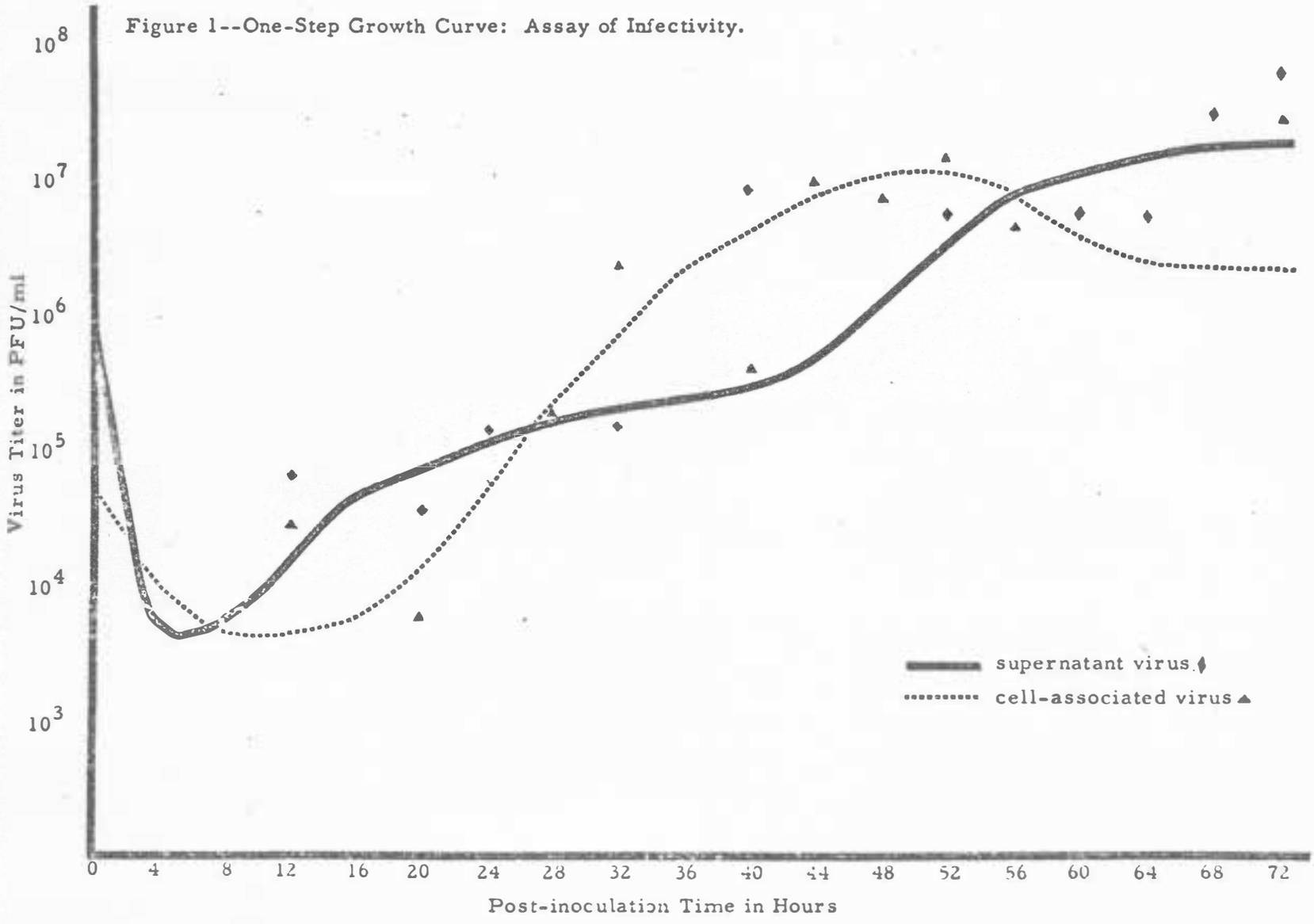


Figure 2--One-Step Growth Curve of Parvovirus 70-7012 in Bovine Adrenal Cells: Assay of Hemagglutinins.

The hemagglutination titer (reciprocal of highest dilution showing complete hemagglutination) of the supernatant (solid line) and the cell lysate (dotted line) are plotted as functions of the time, after inoculation, at which the sample was taken.

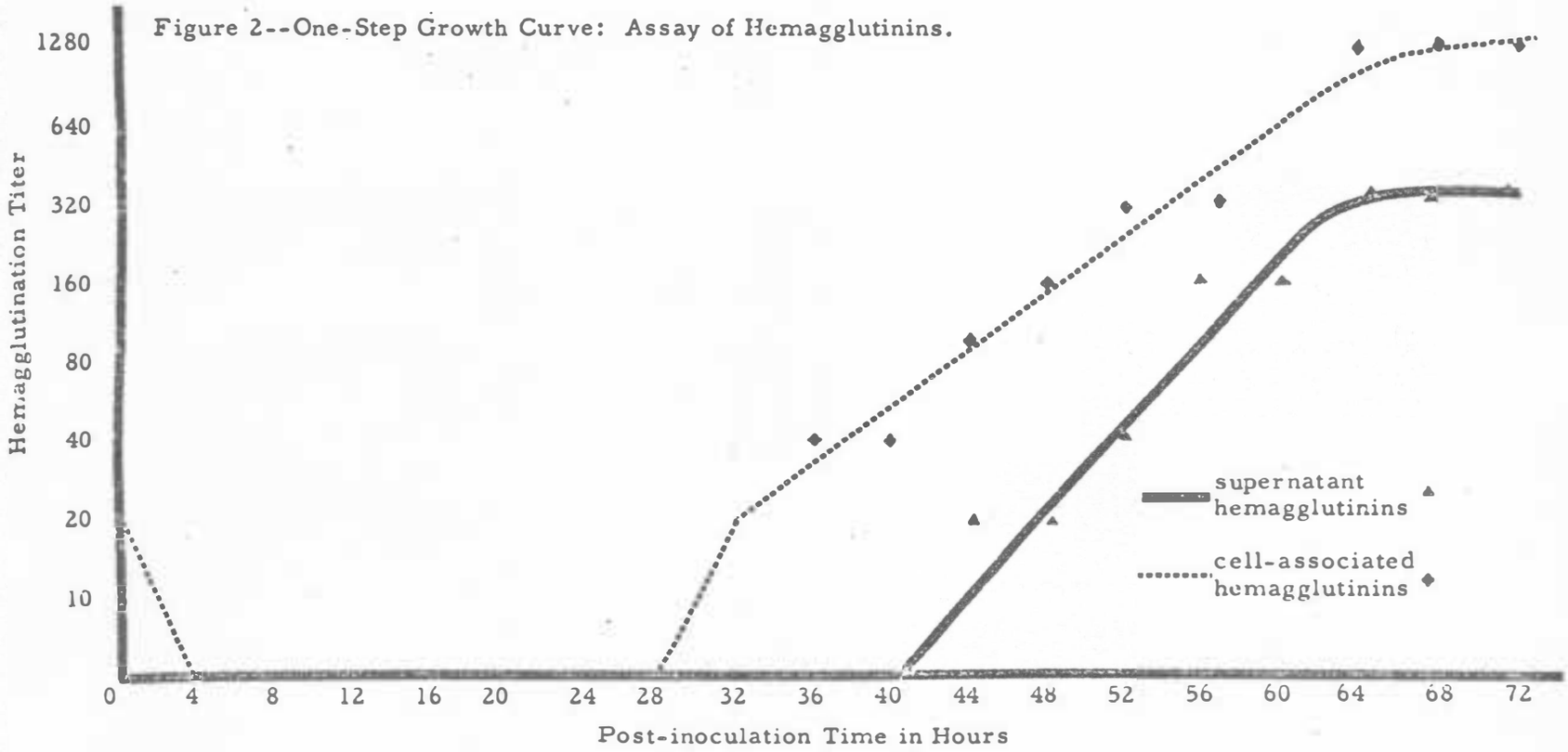


Figure 2--One-Step Growth Curve: Assay of Hemagglutinins.

Figure 3--Equilibrium Density Gradient Centrifugation of Parvovirus 70-7012.

The hemagglutination (dotted line) and infectivity (solid line) titers and the densities (dot-dash line) are plotted as functions of the gradient fraction number. The formula for the gradient line, as determined by the method of least squares, is recorded above that line.

Figure 3--Equilibrium Density Gradient Centrifugation of Parvovirus 70-7012.

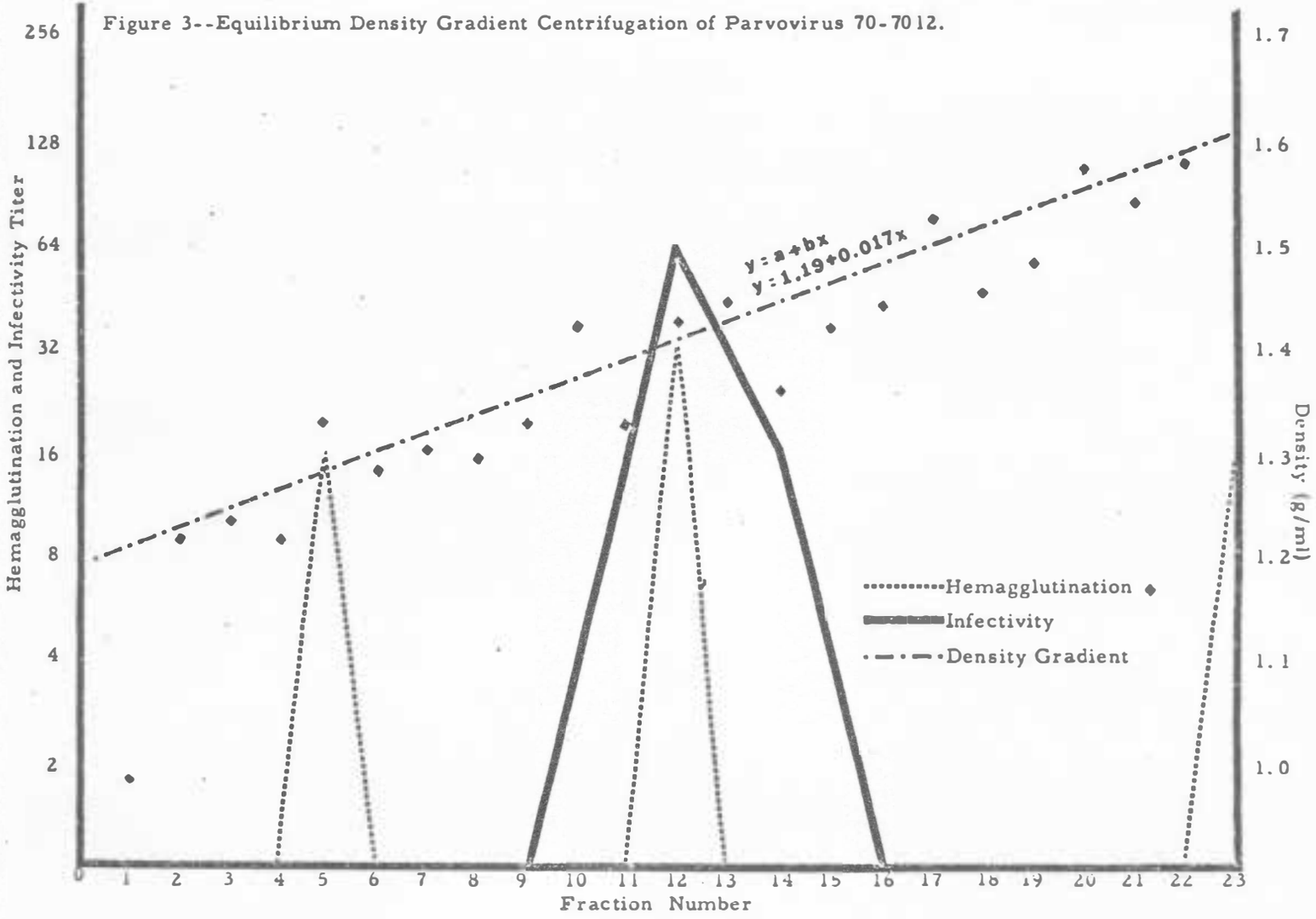
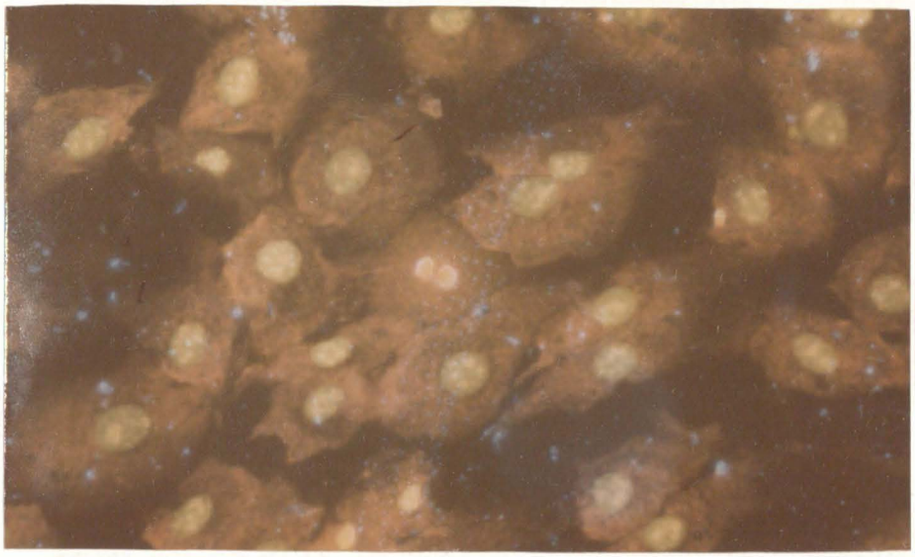


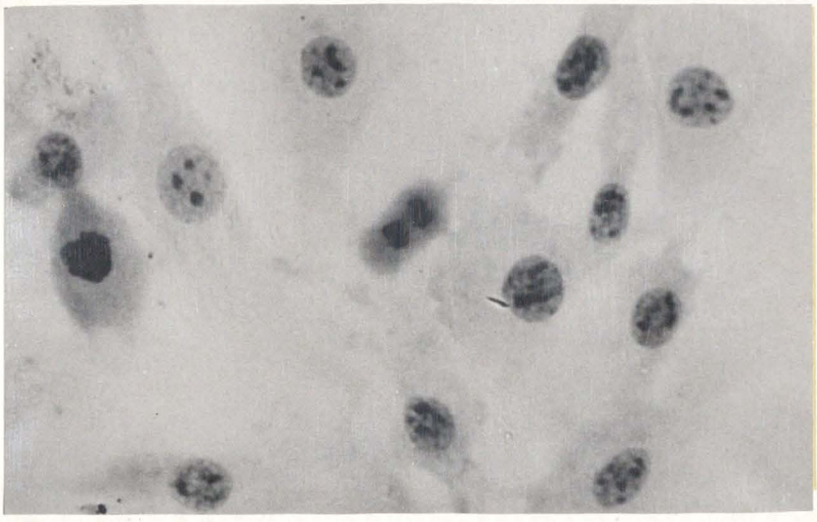
Figure 4--Parvovirus Infected Bovine Adrenal Cells at Zero-Time.

Acridine orange stain: No cell changes were evident. X 840

Hematoxylin and eosin stain: No evidence of cell change was observed when the cells were stained with this preparation. X 1200

Fluorescent antibody stain: Green-staining, specific antigen appeared to be diffusely distributed upon the monolayer. X 840





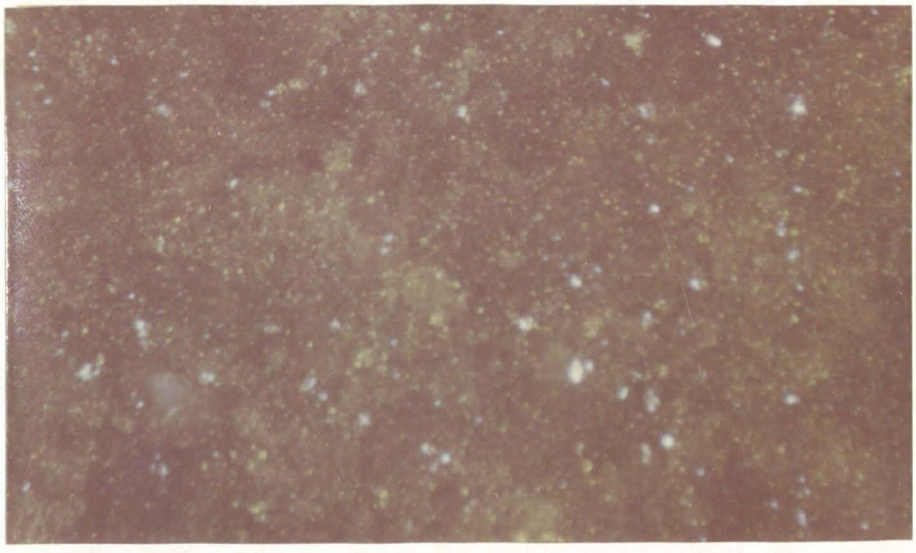
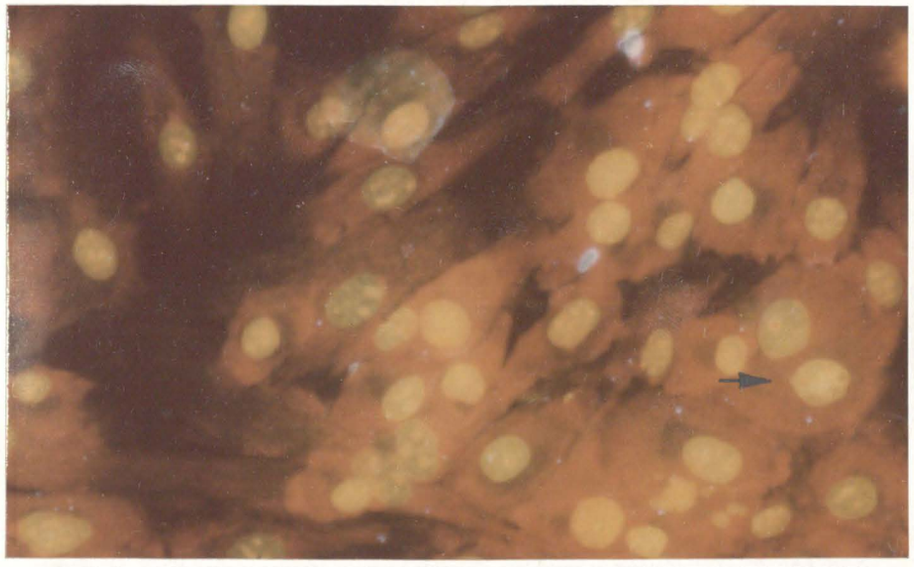


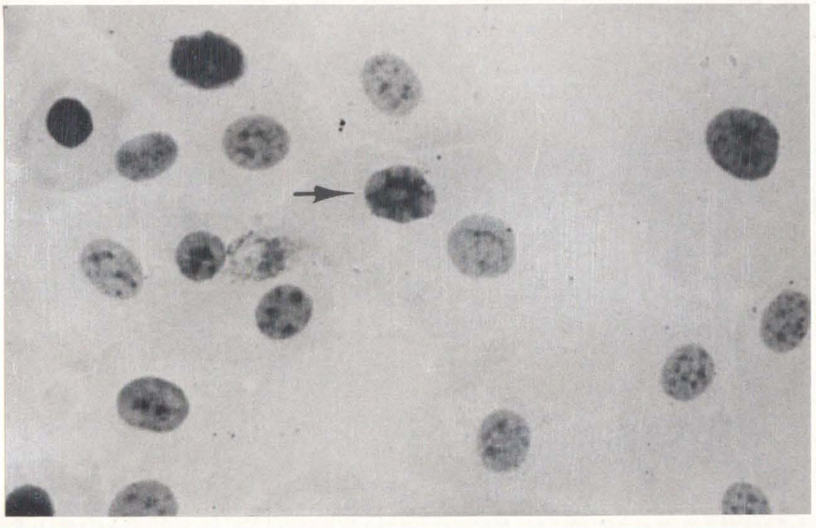
Figure 5--Parvovirus Infected Bovine Adrenal Cells at Twenty-Four Hours After Inoculation.

Acridine orange stain: A few scattered nuclei contained inclusion bodies (arrow). X 840

Hematoxylin and eosin stain: The inclusion bodies appeared light in color indicating an eosinophillic reaction (arrow). X1200

Fluorescent antibody stain: Nuclei exhibiting nuclear and perinuclear fluorescence were evident (arrow). X 840





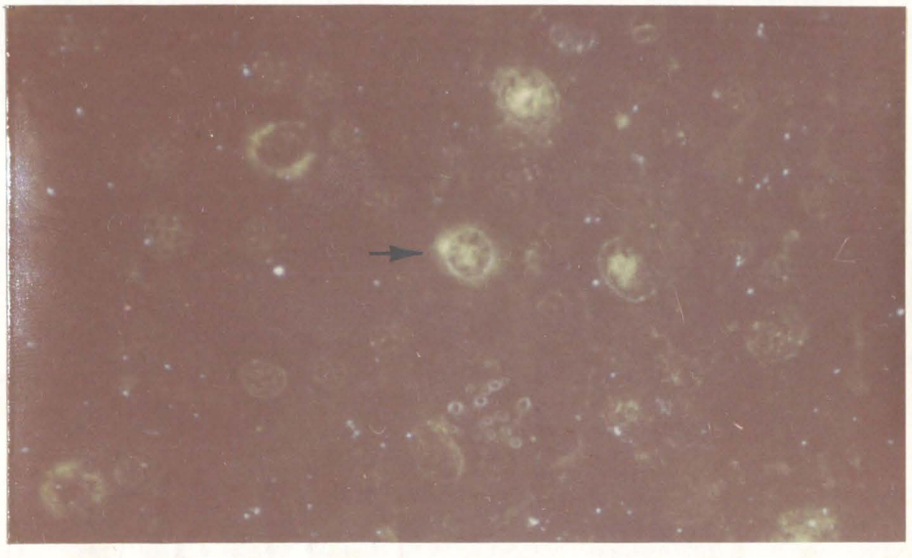
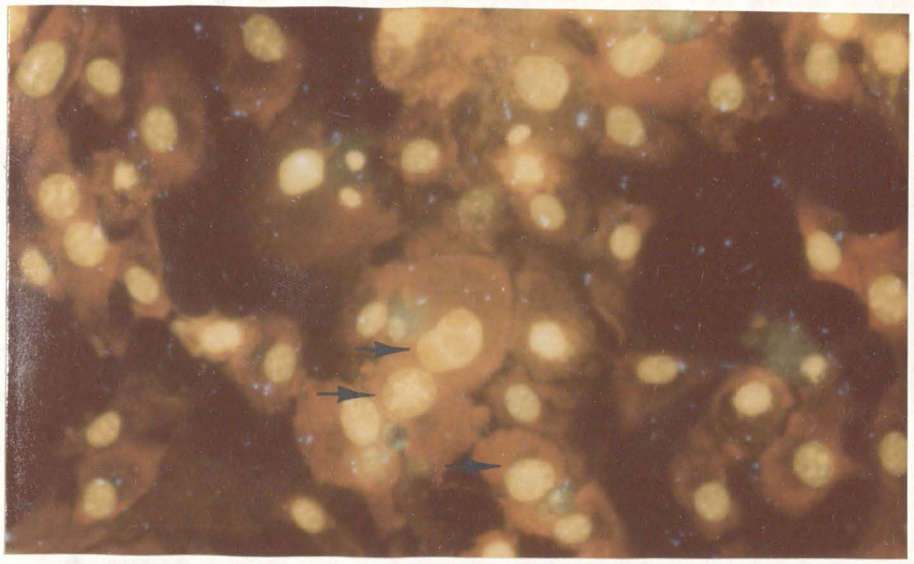


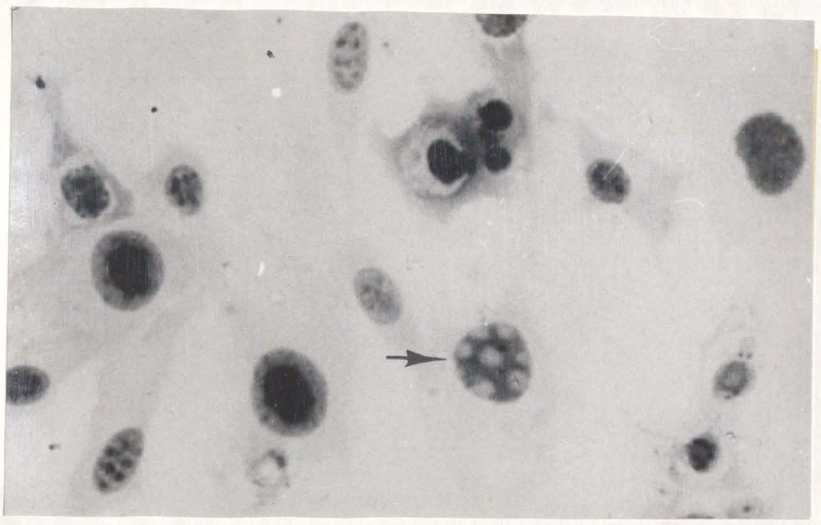
Figure 6--Parvovirus Infected Bovine Adrenal Cells at Forty-Eight Hours After Inoculation.

Acridine orange stain: An increased number of nuclei containing inclusion bodies (arrows) were observed . X 840

Hematoxylin and eosin stain: A nucleus swollen with eosinophillic inclusion bodies (arrow) was observed. X 1200

Fluorescent antibody stain: Cells were observed which exhibited nuclear, perinuclear and cytoplasmic fluorescence (arrow). X 840





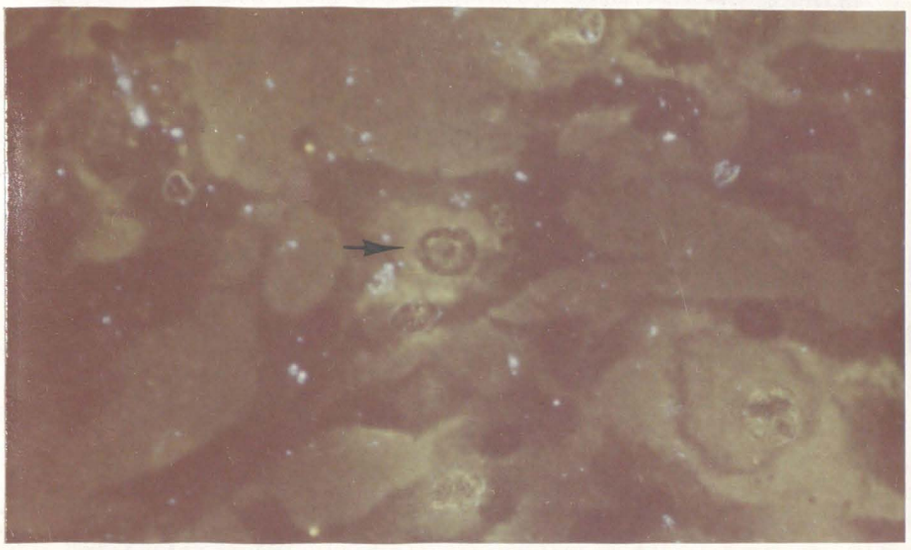
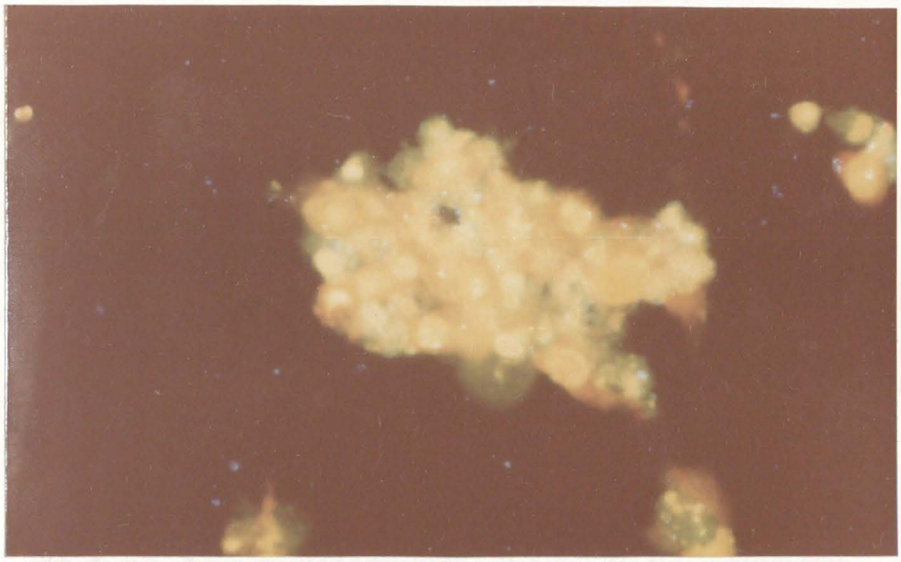


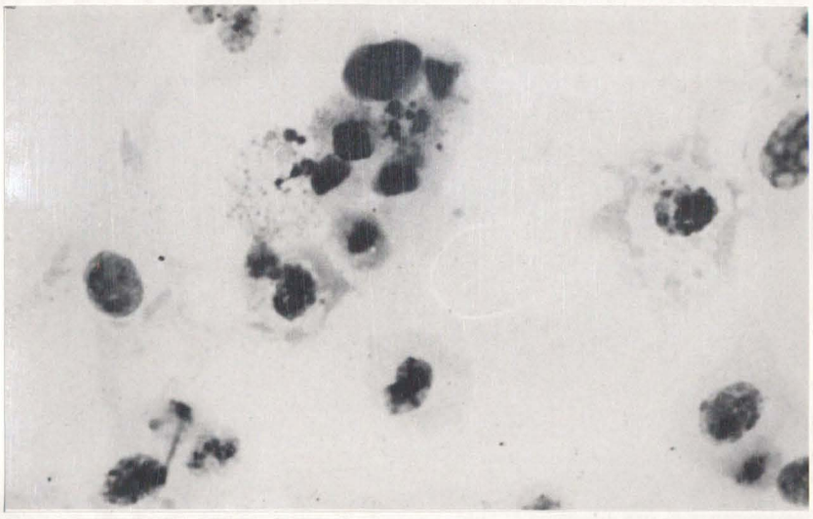
Figure 7--Parvovirus Infected Bovine Adrenal Cells at Seventy-Two Hours After Inoculation.

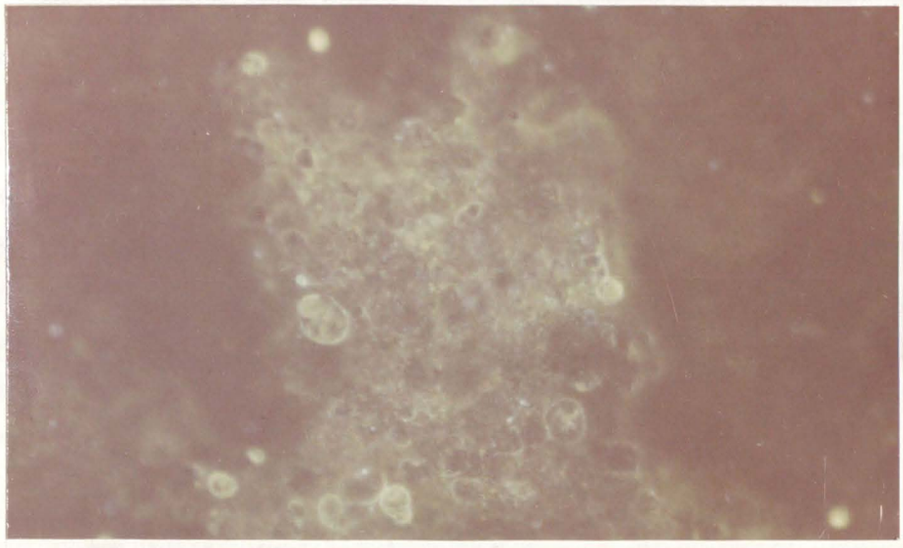
Acridine orange stain: A high degree of cell destruction was observed. X 840

Hematoxylin and eosin stain: Cell destruction was highly evident. X 1200

Fluorescent antibody stain: Diffuse fluorescence in clumps of aggregated cell debris was observed. X 840







DISCUSSION

Serological Survey

In the serological survey, results of the HI tests on thoracic fluids from aborted fetuses did not differ greatly from those of the Arizona pre-colostral calf sera. The percentages positive were 52.2% and 44.4% respectively. Pre-colostral calf sera from the SDSU dairy herd were 100% HI positive. The results are indicative of fetal antibody production, because transplacental transfer of antibody from dam to fetus does not occur in cattle (18). Antibody production, in this case, is suggestive of viral invasion from dam to fetus with the production of a subclinical infection in the fetus.

In the pre and post-colostral sera from Arizona, a distinct increase in percent positive was noted after the calves had received colostrum milk. Of the pre-colostral sera, 44.4% were HI positive; whereas, 73.3% of the post-colostral sera were positive. This increase might be attributed to the fact that 85.4% of the dams were positive. The difference in percent positive between the dams and pre-colostral calves might be explained by infection and recovery by the dam prior to conception.

Of the eighty-one deer sera tested, 6.9% were positive by the HI test. This might be explained by the fact that deer and cattle often feed on the same range. Under these conditions, it is possible for the deer to come in contact with infected bovine fecal material.

The HI test results indicated that 50.7% of the 398 sera tested were positive. A fairly even distribution of antibody levels was found throughout the diverse group of serum sources.

When the SN titers of the Arizona pre-colostral sera are compared with those from the SDSU dairy herd, a large difference is observed. One hundred percent of the pre-colostral sera from Arizona were positive, whereas, only 21.4% of the SDSU dairy herd sera were positive. This contradicts the HI results in which 100%

of the SDSU dairy herd was positive. Although both the HI and SN tests detect antibody, they do not detect the same antibody. The HI test detects masking, by antibody, of the erythrocyte receptor sites; and the SN test detects an actual neutralization of viral infectivity (14). Production of the different types of antibody by the immature immune system of the pre-colostral animal might explain this phenomenon. A comparison of post-colostral percent positives from Arizona and the SDSU dairy herd shows both to be at 100%. This could be indicative of the introduction of a homogenous mixture of HI and SN antibodies produced by the maternal immune systems. Both Arizona and SDSU sera show a high incidence of parvovirus-specific SN antibody.

A comparison of sera from adult animals, including those listed as healthy cattle and the specimens from cows that experienced abortion, indicated a minimal difference in percent positive. This evidence would help to disprove involvement of parvovirus in bovine abortion.

In the HI and SN comparison study (Table 4), the greatest number of positive agreements was found among the post-colostral sera. The greatest number of disagreements, in which the sera were found positive by one test and negative by the other, occurred among the pre-colostral sera. A discernable pattern could not be detected among the sera exhibiting a negative agreement (both tests negative). Of the total tested, 48.0% yielded a positive agreement, while 13.3% gave a negative agreement. A disagreement was noted in 38.3% of the sera.

Results of the serological survey indicated a high incidence of parvovirus antibody. The HI and SN tests showed significant titers in 50.7% and 88.1% of the sera respectively. These percentages agree favorably with those of Abinanti and Warfield, who found significant titers in 86% of the cattle tested (1). Further evidence of wide-spread exposure has been provided by Spahn, Mohanti and Hetrick, whose survey indicated 83% positive (37) and by Storz,

Bates, Warren and Howard, who found significant titers in 65% of the cattle tested (41).

A comparison of the two serological test methods used in this serological survey indicates that the SN test yields the higher number of positive results. The percent positive obtained with the SN test compares favorably with those obtained by other workers in the field. Although more difficult and more expensive to perform, the SN test has been documented to be more sensitive for most virus-antibody systems (14).

Growth Curve

In the one-step growth experiment (Figures 1 and 2), exponential increase of the extracellular virus started eight hours after inoculation and continued until a titer of 2.8×10^7 PFU/ml was reached at seventy-two hours after inoculation. Exponential increase of cell-associated virus started sixteen hours after inoculation and continued until a titer of 1.0×10^7 PFU/ml was reached at forty-eight hours. The increase of cell-associated hemagglutination began at twenty-eight hours after inoculation and continued until a titer of 1:1280 was reached sixty-eight hours after inoculation. Extracellular hemagglutinins started increasing at forty hours and continued until sixty-four hours after inoculation, when a titer of 1:320 was reached. The curve obtained in that experiment is different from that of Bates and Storz (6), especially in the development of extracellular virus. It can be seen in Figure 1 that the extracellular and cell-associated virus development graph lines cross in two places, with the higher titer being reached and maintained by the extracellular virus. In the Bates and Storz growth curve, the cell-associated virus titer was consistently higher than that of the extracellular virus. A possible explanation for this difference can be found in comparing the ages of the cell cultures used in these growth curves. Bates and Storz used a twenty-four hour cell culture, whereas the cell culture used in this study was forty-eight hours old. Cells undergoing rapid cell division are more

susceptible to parvovirus (6). The rapidly dividing, younger cells, as used by Bates and Storz, would promote a more even rate of exponential virus development than forty-eight hour cells. Another possible explanation might be that parvovirus 70-7012 is more cytolytic in bovine adrenal cells than the Bates and Storz isolate was in bovine lung cells. A cytolytic cell-virus combination would produce high levels of extracellular virus. The development of hemagglutinins is more closely parallel to the observations of Bates and Storz (6). The level of cell-associated hemagglutinins (Figure 2) started increasing twelve hours before the extracellular hemagglutinins and was consistently higher throughout the study.

Development of viral nucleic acid, as detected by acridine orange and hematoxylin and eosin stains (Table 5) became evident at twenty hours post inoculation. Red, intranuclear inclusions were observed in the infected cells stained with acridine orange. A red stain reaction with acridine orange indicates the presence of single-stranded nucleic acid (26). Intranuclear inclusions were also observed at twenty hours in hematoxylin and eosin stained, infected cell cultures. The staining reaction observed was eosinophillic, indicating a high level of protein in the inclusion bodies. The location of viral protein was determined by use of the fluorescent antibody (FA) test in Table 5. The diffuse fluorescence observed at zero time would tend to indicate adsorption of the virus to the cell surface. The lack of fluorescence at four and eight hours is indicative of an "eclipse phase", during which intact viral particles are absent. The presence of scattered intranuclear fluorescence at twelve hours indicates the beginning of progeny virus formation. At twenty hours, the fluorescence was brighter and had moved to the nuclear membrane. This could indicate a period of highly active virus production and is coincidental with the first appearance of inclusion bodies in the nuclei. Fluorescence was observed in the cytoplasm at twenty-eight hours, and this coincides with the approach of the midpoint in the cell-associated virus growth curve. Little change was noted in

the distribution of fluorescence in the cells until sixty-four hours after inoculation, when the fluorescence became diffuse throughout the remaining cells. Observation of acridine orange and hematoxylin and eosin stained, infected cultures indicated a high level of cell destruction at sixty-four hours. No changes were noted in the infected cells from sixty-four hours to the end of the experiment.

The nature of the viral nucleic acid was studied at forty-eight hours by Schiff reagent staining. The dark red staining inclusion bodies indicated that the nucleic acid of the parvovirus was composed of DNA (26). The acridine orange results coupled with the Schiff reagent results tend to support reports that the nucleic acid of parvovirus is single-stranded DNA (45). Additional support is found in the sensitivity of parvovirus 70-7012 to BUDR. These results agree with those presented by Wilner (45) and Storz and Bates (40).

Buoyant Density

In the buoyant density study, three hemagglutinin peaks were detected. These peaks occurred at densities of 1.28, 1.41 and 1.61 g/ml. One cell culture infectivity peak was observed at a density of 1.41 g/ml. The buoyant density of the whole, infective virus particle, according to this data, would be 1.41 g/ml. The hemagglutination peak found in the fraction at density 1.28 g/ml might consist of viral protein and empty viral capsids. The hemagglutination activity found at a density of 1.61 g/ml is probably due to the presence of heavier viral aggregates and viruses adsorbed to cell fragments. The buoyant density of 1.41 g/ml agrees well with the 1.42 g/ml figure presented by Storz and Bates (40). The range of buoyant densities of parvoviruses isolated from various species ranges from 1.25 g/ml to 1.43 g/ml (2).

Hemagglutination Spectrum

The bovine parvovirus under study appeared to be capable of agglutinating human "O" erythrocytes at 4°C, 25°C and 37°C.

Guinea pig erythrocytes were agglutinated at 4°C and 25°C. These results are in agreement with those presented by Bates, Storz and Reed (7).

Animal Infectivity Studies

Preliminary infectivity studies in mice were negative. The colostrum-deprived calf exhibited a slight rise in temperature and severe diarrhea. The virus was reisolated from post-mortem intestinal contents. These results agree with those reported by Storz and Bates (40). The fetus injected with parvovirus *in utero* was born normally and did not exhibit clinical illness. A pre-colostral serum sample from this calf possessed an SN titer greater than 1:256. When compared to the titers observed in the pre-colostral calves in the serological survey, the data suggests that parvovirus may not be an abortifacient. The animal infectivity studies are incomplete. Before any firm conclusions can be drawn on the possible role of parvovirus in bovine abortion, additional bovine fetuses at various stages of gestation should be infected and studied.

SUMMARY

In summary, wide-spread exposure of cattle to parvovirus has been determined in South Dakota by serological methods. Preliminary studies indicated that a similar problem could also exist in Arizona. A one-step growth curve was conducted, and the sequence of events in the development of viral protein and nucleic acid was studied. The buoyant density of the virus was also determined. The results of the buoyant density agreed with results reported by Storz and Bates (40). Parvovirus 70-7012 was found to be heat, chloroform and acid resistant. The virus was also found to be BUDR sensitive. These results supported those presented by Wilner (45) and Storz and Warren (39).

Mice were found to be resistant to bovine parvovirus. Symptoms similar to those observed by Storz and Bates (40) were produced by infecting a pre-colostral calf. A high serological titer without disease symptoms was produced in a calf infected in utero. A small amount of information was obtained which would lead one to question the ability of parvovirus to cause abortion in cattle.

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