INFECTIOUS BOVINE RHINOTRACHEITIS (HERPESVIRUS BOVIS) INFECTION IN SWINE

by

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TABLE OF CONTENTS

INTRODUCTION

The history of the infectious bovine rhinotracheitis virus (IBR virus, Herpesvirus bovis) is a fascinating example of the ability of a virus to persist in an animal population for many years causing a single disease entity, and then to erupt into many syndromes when favorable circumstances are provided. The IBR virus is now recognized as a major disease-producing agent in cattle throughout the world, but the pathogenicity of the virus for hosts other than cattle and laboratory animals has not been intensively investigated. The isolation of IBR virus from naturally-infected swine by Saxegaard and Onstad (91) has shown the versatility of the virus and has raised the question of whether Herpesvirus bovis is responsible for clinical disease in swine.

In Iowa there are large numbers of cattle in which infectious bovine rhinotracheitis is enzootic and there are large numbers of swine in close association with the cattle. These would seem to be the ingredients necessary for the introduction of IBR virus into the swine population if this is possible. The purpose of this study was to investigate the possible presence of a strain of IBR virus in the swine population of Iowa as shown by the presence of IBR antibodies in swine sera, and to determine the pathogenicity of IBR virus for swine.

McKercher (59) has stated... "new viral isolates should be characterized fully so that their disease-producing potential may

be delineated fully. Provision should be made also for comparing new viral isolates with known viruses from the corresponding and related natural animal host species. In time, this comparison should be extended to include viruses considered to be of an exotic nature. Only through a better understanding of the behavior of viruses under different physiologic and environmental conditions can their role in disease be understood fully."

REVIEW OF THE LITERATURE

The disease known as "infectious bovine rhinotracheitis" or "IBR" is a relatively new disease of cattle which was recognized as a distinct disease entity only twenty years ago when Miller (69) described an acute upper-respiratory disease of cattle that appeared in 1950 in a feedlot in northern Colorado. The infection was characterized by high temperature and by localized or generalized areas of acute hyperemia of the respiratory tract that often became affected by caseation necrosis. The cause was presumed to be a virus. The disease persisted in the feedlot for several years and other veterinarians observed a similar disease in other feedlots in northern Colorado. For several years the disease appeared to be confined almost exclusively to beef cattle which had been on feed for at least three weeks. Changes in the nature of the disease were reported in 1954 by Miller (69) when it was recognized by veterinary practitioners in young calves and in dairy herds.

In October 1953, an upper respiratory disease appeared suddenly in a large dairy herd in Los Angeles County, California. This outbreak and subsequent outbreaks were described by Schroeder and Moys (92) and by McKercher, et al. (60). In the following four months the disease spread to 52 herds in the counties surrounding Los Angeles. Of the 13,108 cattle in these herds 1002 were infected and 30 deaths occurred. In January, 1954 the disease appeared in a herd 350 miles from Los Angeles. The disease reappeared in a feedlot of 10,000 cattle in this same area in September.

Chow, et al. (15) working in Colorado, transmitted the disease by inoculation of cattle with nasal and tracheal secretions and exudates and splenic tissue from field cases. They succeeded in reproducing the infection using bacteria-free inocula in susceptible cattle and concluded that the causative agent was a virus.

McIntyre (55) reproduced the California disease by inoculation of blood, feces, pooled saliva and nasal material from infected cows. McKercher, et al. (61) also demonstrated that the disease could be transmitted by intranasal inoculations of nasal washings from infected cattle. They suggested that the causative agent was a virus and that the disease was possibly identical with the Colorado disease. They named the disease infectious bovine rhinotracheitis (IBR).

Madin, York and McKercher (50) isolated the virus in bovine embryonic kidney cell cultures from nasal washings from naturally infected beef and dairy cattle in California and beef cattle in Colorado. They were successful in reproducing infectious bovine rhinotracheitis using tissue-culture-propagated virus. York, et al. (111) were successful in isolating a viral agent in bovine embryonic tissue-culture cells from upper respiratory tissues of cattle with infectious bovine rhinotracheitis. They were able to reproduce the disease with the tissue-culture-propagated virus. Brown and Cabasso (6) transmitted IBR to susceptible cattle using tissue-culture-

propagated virus administered intranasally, conjunctivally or intratracheally.

Immunological studies conducted by McKercher, et al. (62) established that the acute upper respiratory disease of California dairy cattle, the respiratory disease of California beef cattle and the rhinotracheitis of Colorado feeder cattle were one disease entity.

Descriptions of the disease in the late 1950's by McKercher (57), Brown, et al. (8) and Jensen, et al. (37) indicate that infectious bovine rhinotracheitis (IBR) was confined to concentrated cattle operations such as the large western feedlots and the large California dairies. The disease was characterized by a sudden onset and a high temperature of 103°F. to 108°F. Anorexia was often present. A profuse serous to mucoid nasal discharge developed and the visible nasal mucosa was hyperemic. Coughing and dyspnea occurred. The primary lesions were confined to the upper respiratory tract. The nasal mucosa was congested and swollen and in advanced cases there was mucopurulent and fibrinous exudate on the surface. Removal of the exudate sometimes revealed necrotic areas of the mucosa. The trachea had localized areas of inflammation and hemorrhage. Mucopurulent and fibrinous exudate was present in severe cases and areas of necrosis were present. In advanced cases a diphtheritic membrane covered the necrotic mucosa of the trachea and larynx. Chow, et al. (17)

conducted an epizootiological study in a large Colorado feedlot feeding 25,000 - 30,000 cattle annually. There were 3,204 cattle infected and 203 died. The infection became evident only after the cattle had been in the lot for at least 10 days and the clinical course of the disease lasted 3-7 days.

Early studies of the IBR virus by Cabasso and Brown (6, 10) and by York and Schwarz (110) showed that intramuscular inoculation of IBR virus did not produce illness in susceptible cattle. They also showed that these animals developed appreciable levels of antibodies and were immune to subsequent challenge by intranasal inoculation. There was no evidence of spread of the virus to susceptible contact cattle. All of these features were evidence that an effective vaccine could be developed. Subsequently Kendrick, et al. (46), Schwarz, et al. (93) and Brown and Chow (7) reported on the development and field trials of vaccines produced by modifying IBR virus by rapid passage in tissue culture. Rosner (84) reported in 1968 that 20 million doses of IBR vaccine are used annually.

Infectious bovine rhinotracheitis (IBR) virus was considered in the late 1950's to produce only a disease of the upper respiratory system as the name indicates. A seemingly incongruous relationship was demonstrated when Gillespie, Baker and Wagner (26) in 1958 and Gillespie, et al. (28) in 1959 reported that the virus isolated from a localized genital infection, infectious pustular vulvovaginitis

(IPV) was identical to the infectious bovine rhinotracheitis (IBR) virus. It was shown that the IPV virus produced no respiratory symptoms or very mild respiratory symptoms when inoculated intranasally although virus could be recovered from the nasal passages. The IBR virus produced lesions of a pustular vulvovaginitis when inoculated into the vagina. Immunological studies in cattle, reciprocal serum-neutralization tests and the growth characteristics of the viruses in tissue culture established that the IBR and IPV viruses were identical. McKercher, et al. (66) confirmed these results.

The relationship between the IBR and IPV viruses answered the perplexing questions raised by some of the early serological surveys. IBR was constantly associated with large concentrations of cattle as seen in the western feedlots and the California dairy herds. No reports of the disease in the eastern areas of the United States had been made, yet antibodies to the IBR virus were found in cattle in New York dairy herds by Gillespie, et al. (28) and Gillespie, Lee and Baker (27). In fact Gillespie, Lee and Baker (27) in 1957 found IBR antibodies in the serum obtained in 1941 from a calf in a dairy herd in Princeton, New Jersey.

Infectious pustular vulvovaginitis was not a new disease. It had been previously termed coital exanthema, vesicular venereal disease, vesicular vaginitis, coital vesicular exanthema and Bläschenausschlag before the name of infectious pustular vulvovaginitis (IPV)

was proposed by Kendrick, et al. (43). McKercher (59) compared three virus strains isolated from cattle affected with Bläschenausschlag in Austria, East Germany and Belgium with an IBR virus isolated from California dairy cattle affected with a respiratory infection and found the four isolates to be immunologically homogenous. Reisinger and Reimann (83) in 1928 reported on the experimental transmission of the disease by intravaginal inoculation of bacteria-free material. Greig, et al. (32) in Canada isolated a cytopathic agent in tissue culture from cows infected with coital exanthema and reproduced the infection by intravaginal inoculation of the tissue-culture-propagated agent. Kendrick, et al. (43) isolated a virus from an outbreak of IPV in a New York dairy herd and were able to reproduce the disease. They described the development of small areas of necrosis of the epithelium with an influx of neutrophils that result in the characteristic pustules. The pustules may coalesce to produce large ulcers covered with exudate. Both the vagina and vulva are involved but the lesions are usually more diffuse in the vagina. A febrile response and neutrophilia are present. An antibody response occurs in about two weeks.

Diseases clinically identical to infectious pustular vulvovaginitis have been reported from various areas of the country since the early part of the century. In fact the disease, Bläschenausschlag was reported in Europe in the 1880's. In 1933 Witte (107) reported

on the incidence of Bläschenausschlag which was first observed in Germany in 1886 in an epizootic in which there were approximately 4,000 reported cases. The highest incidence was in 1896 when 9,500 cases were reported. Jones and Little (40) in 1927 described cases of a granular vaginitis in cows originating in Ohio and Oregon that had been shipped to New Jersey. Mohler (70) described a disease clinically similar to IPV in a Special Report on Diseases of Cattle from the Bureau of Animal Industry, U.S.D.A. in 1923. Parker (77) in 1900 described an infectious pustular vulvar disease in cattle in Kansas.

Thus it becomes evident that infectious bovine rhinotracheitis was actually a new disease manifestation of a long-established virus. However, additional distinct clinical entities caused by the IBR-IPV virus have been observed. Conjunctivitis had been listed as a clinical sign in some of the early reports of the disease by Jensen, et al. (37) and McKercher, et al. (61). McKercher, et al. (64) reported in 1958 on the experimental infection of cattle by ocular instillation of IBR virus. A mild temperature increase, lacrimation and a slight nasal discharge were observed. Virus was recovered from the nasal and ocular secretions. Abinanti and Plummer (1) in 1961 isolated IBR virus from the ocular and nasal secretions of four animals in a group of yearling beef cattle affected with a conjunctivitis. The affected cattle had a slight to moderate clear nasal discharge but

no respiratory symptoms were evident. In 1961 Quin (81) also reported an outbreak of conjunctivitis in a group of feeder cattle in a public stock yard. About two-thirds of the animals developed a severe palpebro-conjunctivitis about two weeks after arrival and no other symptoms were detected. IBR virus was isolated in tissue culture from the ocular secretions of several of the affected eyes. Rosner (85) reported that feeder cattle coming into a terminal market for resale to cattle feeders became regularly affected with a conjunctivitis. He also reported an outbreak of conjunctivitis in a lot of feeder cattle on a farm. In both instances diagnosis was made by virus isolation from the ocular secretions. McKercher, et al. (68) have posulated that the conjunctiva may be a site where viral persistence and multiplication may occur in spite of circulating antibodies. Infection of the conjunctiva may occur by extension from the nasal passages by way of the lacrimal ducts. The virus may persist in the conjunctiva because the antibody level in the conjunctival secretions is very low since there is a continuous flushing of the ocular and conjunctival surfaces by the tears. Virus isolations from the nasal passages in the immune animal may be the result of virus being washed down the lacrimal ducts from the ocular tissues.

A more serious involvement of the eyes was reported by Hughes, et al. (36) and St. George (98) in which a keratitis followed the conjunctivitis associated with the respiratory syndrome of the IBR

virus. The corneal involvement ranged from corneal opacities of varying severity to ulceration and corneal cysts. IBR virus was isolated from nasal and conjunctival secretions. Hughes, et al. (36) reported isolating IBR virus from the aqueous humor of one eye. St. George (98) isolated IBR virus for 55 days from the conjunctival sac of one cow.

Involvement of the digestive tract by the IBR-IPV virus varies from severe gastro-intestinal lesions concurrent with the respiratory infection to symptoms and lesions identical to those of mucosal disease (BVD-MD). Baker, et al. (4) and McEntee (53) reported the effects of IBR virus on newborn calves. Administration of the virus by the intravenous route, by feeding or by contact exposure, resulted in a temperature elevation, excessive salivation and anorexia. Pustular lesions and areas of hyperemia were present in the oral cavity and on the tongue. Calves that survived the infection began eating and subsequently developed respiratory signs. Focal areas of necrosis, ulceration and neutrophilic infiltration of the mucosa and submucosa were present from the oral cavity through the rumen. McEntee (53) observed that many of the calves developed bronchopneumonia from inhalation of milk due to laryngeal edema. Thomson and Savan (102) reported on outbreaks in 1-2 week old calves in two herds in which there was epithelial necrosis involving the region of the oral cavity through the rumen in addition to respiratory involvement. York (109) also observed that IBR virus may produce lesions in the oral cavity or in the gastro-intestinal tract in very young calves. Gratzek,

et al. (29) isolated a strain of IBR virus from a Peyer's patch of a calf which had died with lesions typical of mucosal disease (BVD-MD). Another virus related to the bovine virus diarrhea (BVD-MD) group was also isolated from the calf. However, Peter, et al. (78) produced a disease similar to bovine virus diarrhea (BVD-MD) by experimental inoculation of calves with the IBR strain.

In December, 1960 several calves in a herd in Australia died after showing nervous symptoms and Johnston, et al. (39) investigated the epizootic. In a herd with about 100 calves, mostly 4-5 months old, about half of the calves died in a period of six weeks after showing nervous symptoms. Microscopic examination of the central nervous system showed the presence of a nonpurulent meningo-encephalitis. A virus was isolated from brain tissue of affected calves and was identified as IBR virus by French (23, 24). Experimental studies by French (23), Hall, et al. (34), and Johnston, et al. (38, 39) revealed that the virus had a predilection for the central nervous system. Intracerebral inoculation produced an encephalitis. Administration by the intranasal route resulted in a mild rhinitis and a meningo-encephalitis. Intravaginal administration produced lesions of infectious pustular vulvovaginitis (IPV) and the infection remained localized in the vagina. One calf acquired a rhinitis and subsequent meningo-encephalitis presumably from nasal contact with the infected vagina of another heifer. Intravenous inoculation of this strain of

IBR virus failed to elicit any symptoms. In 1963 Barenfus, et al. (5) described a sporadic epizootic of meningo-encephalitis in a large California dairy herd in which eleven 5-6 month old heifers died over a two year period. The heifers died about 3-4 days following the onset of nervous signs. IBR virus was isolated from brain tissue, cerebrospinal fluid and other tissues from five of the affected calves. There was no clinical evidence that other cattle in the herd had been affected with any of the IBR syndromes. Gardiner and Nairn (25) described a second outbreak of meningo-encephalitis in Australia in 1964. About a third of a group of 50 calves were affected following weaning. Most of the affected calves died several days after the onset of the disease and IBR virus was recovered from brain tissue. None of the IBR syndromes had been observed previously in the area.

In one of the early outbreaks of infectious bovine rhinotracheitis (1955) Jensen, et al. (37) observed abortions in some of the feedlot heifers. A similar observation was made by Brown, et al. (8) in 1957. Abortion did not attain real significance as a manifestation of IBR infection until the disease spread from the feedlots and became established in breeding herds.

A case history of IBR abortion in a large California dairy herd was described by Ormsbee (75), Kennedy and Richards (47) and McKercher and Wada (67). A respiratory infection and conjunctivitis developed in a group of calves. On the same premises 61 abortions occurred in 283 pregnant heifers that had shown no signs of previous illness.

The abortions occurred after the fifth month of pregnancy and IBR virus was isolated from seven fetuses.

Lukas, et al. (49) isolated IBR virus from 152 fetuses from 28 premises over a two-year period. Abortions were observed in herds with histories of IBR vaccination, upper respiratory infections, conjunctivitis alone, rhinitis alone or with no previous clinical signs of IBR. Sattar, et al. (88) reported on the isolation of IBR virus from six fetuses in three Ohio herds. One herd had a history of respiratory infection four weeks previously, one herd had been vaccinated for IBR and the third herd had no previous signs of illness. Kahrs and Smith (41) described abortion in a New York dairy herd with a previous history of upper respiratory infection. Two animals in the herd were affected with IPV infection without showing any evidence of respiratory infection. IBR virus was isolated from nasal discharge, vaginal mucosa and placenta.

IBR vaccine was incriminated as a cause of abortion and subsequently this was proven. McKercher (58) had reported in 1962 that over the previous 12-18 months abortions had occurred following vaccination of cattle with the modified live virus IBR vaccines. He suggested that pregnant animals not be vaccinated. Lukas, et al. (49) and McKercher and Wada (67) observed IBR abortions in herds with a history of IBR vaccination. Kennedy and Richards (47) described an abortion outbreak in a beef herd vaccinated for IBR.

The abortions occurred 23-52 days after vaccination and none of the cows under 5½ months aborted. Sattar, et al. (88) reported abortion 40-70 days following IBR vaccination. McFeely, et al. (54) described an abortion epizootic in a large herd in Pennsylvania that had been vaccinated with IBR vaccine. The first abortion occurred 13 days following vaccination and the last occurred 48 days post-vaccination. The stage of gestation ranged from 103-207 days.

The characteristics of IBR abortion epizootics under field conditions have been substantiated by experimental studies by McKercher and Wada (67), Kennedy and Richards (47), Owen, et al. (76), Chow, et al. (16), Molello, et al. (71), Sattar, et al. (89) and Kendrick and Straub (45). An incubation period varying from 13 to 64 days occurs before abortion is produced. Experimentally, abortion has been produced by intranasal, intravenous and intramuscular inoculation but not by intravaginal inoculation. There are no signs of impending abortion. Fetuses are most susceptible to infection during the first and third trimesters of pregnancy and most fetuses are aborted after the sixth month of gestation. Death of the fetus usually occurred in utero 24-48 hours prior to expulsion. Expulsion is the result of the death of the fetus rather than the effect of the virus on the uterus or placenta. The most characteristic lesions in the fetus are focal necrosis of the liver and spleen.

Maré and van Rensburg (52) in South Africa investigated herds affected with an infectious infertility involving both bulls and cows. Several viral agents were isolated and IBR-IPV virus was recovered from several herds. The bulls showed acute inflammation of the penis and preputial mucosa, seminal vesiculitis and orchitis. Spermatogenesis was affected and infertility persisted for several months. The cows had an acute cervico-vaginitis without the pustule formation seen in IPV infection. A mild vaginitis and infertility persisted for several months. Experimental inoculation of the IBR virus strain reproduced the natural diseases in both bulls and cows. Intranasal inoculation of this strain of IBR virus by Maré (51) resulted in a response very similar to that observed when the IPV strain is inoculated intranasally.

In 1964 Studdert, et al. (100) described infectious pustular vulvovaginitis (IPV) virus infection of the penile and preputial mucosa of bulls. The number of pustules observed were comparatively fewer than observed in the female and obvious signs of infection were not always observed unless the penis was exteriorized. Virus was isolated for 10-14 days following infection and in one case for as long as 26 days. The bulls were resistent to re-infection. In 1965 Hellig (35) reported an outbreak in South Africa in which a pustular vulvovaginitis was observed in the cows and a pustular balanoposthitis was observed in the bulls. Saxegaard (90) described a moderate purulent balanoposthitis in two bulls in a Norwegian artificial-breeding stud. Virus isolation attempts were made on all of 24 bulls

though none of the others showed lesions. IBR-IPV virus was isolated from each although all required extensive passages in tissue culture.

Since the IBR virus has become rather ubiquitous in the cattle population and since semen-processing methods are also excellent for virus preservation, the effect of using semen contaminated with IBR-IPV virus for artifical insemination was studied by Kendrick (42) and Kendrick and McEntee (44). Only one of 12 animals conceived from insemination with the contaminated semen. A necrotizing endometritis was observed and an abnormally short estrous cycle was consistently found. Cystic corpus lutea were observed in five heifers.

The effect of the IBR virus on the bovine udder was described by Greig and Bannister (31). The experimentally inoculated udders showed acute inflammation, swelling, reduced milk secretion and abnormal milk. Virus was isolated for 10-15 days following exposure.

Since the first reports of infectious bovine rhinotracheitis (IBR) in Colorado and California the distribution of the respiratory disease has become almost world wide. One of the first reports that infectious bovine rhinotracheitis was present in the less concentrated cattle operations was by McKercher and Straub (65) in 1960. They isolated IBR virus from a cow in a large herd of range cattle in Nevada. Chow (14) also reported the isolation of IBR virus from range cattle in Colorado in 1961. Antibodies to IBR virus were found in sera of cattle widely distributed in the range areas of Colorado. Clinical infectious bovine rhinotracheitis was first

reported in the eastern states by Van Kruiningen and Wolke (105) in New York in 1963. Newberne, et al. (72) conducted a serological survey and found IBR antibodies in bovine serum samples from 28 of 31 states. Of 2,190 samples tested, 759 or 35% had IBR antibodies. Studdert, et al. (101) in Ontario reported the isolation of IBR virus in 1961 from feedlot cattle affected with upper respiratory infection. Greig (30) in 1961 reported on a serological survey of 422 farms in southern Ontario in which 8.13% of the 1,365 serum samples were positive for IBR antibodies. He felt that the incidence of IPV was insufficient to account for the high incidence of IBR antibodies. Miilo, et al. (73) reported that 37% of the bovine serum samples tested in a study in Alberta were positive for IBR antibodies. In 1966 Curtis, et al. (19) reported an increased incidence of IBR from the case records at the Ontario Veterinary School since 1961.

In New Zealand the isolation of IBR virus from a cow with nasal catarrh and the experimental reproduction of the disease was reported by Webster and Manktelow (106) as early as 1959. In 1966 Fastier and Hansen (22) examined sera from 118 cows selected for a past history of nasal catarrh and found 81.6% possessed antibodies to IBR virus, 4% to BVD-MD virus and 98% to parainfluenza 3 virus. Snowdon (95) reported that the presence of disease conditions resembling clinical IBR and clinical IPV in dairy cattle in Australia were first observed by practitioners in 1959. He investigated outbreaks of IPV

in seven herds and IBR in two herds and recovered IBR-IPV virus from 33 vaginal, 7 preputial and 5 nasal specimens.

IBR virus was recovered from a bull affected with an upper respiratory infection by Liess, et al. (48) in Germany in 1960. In the United Kingdom Darbyshire, et al. (20) isolated IBR virus from conjunctival swabs obtained from two herds of cattle affected with conjunctivitis and rhinitis. The incidence of IBR does not appear to be high in the United Kingdom since Dawson and Darbyshire (21) in 1964 reported an incidence of IBR antibodies of only 2.1% in 2,000 bovine sera.

Electron-microscopic studies of the IBR-IPV virus particles by Tousimis, et al. (103), Armstrong, et al. (3) and Grinyer, et al. (33) show that several sizes of particles are present during viral replication. Viral replication begins in the nucleus of the cell. In the nucleus are found small particles 40-45 mμ in size which are the nucleoids and larger particles 110 mμ in size consisting of the nucleoid surrounded by a single membrane, the capsid. As the larger particles pass from the nucleus to the cytoplasm of the cell a second membrane or envelope is acquired from the nuclear membrane. The size of the mature viral particle is 136-150 mμ. The infectivity of the virus particle is believed to be related to the acquisition of the envelope.

In tissue culture the IBR-infected cells become separated from the adjacent cells and develop a round granular appearance. These

cells soon become detached from the surface. In 1963 Stevens and Groman (97) and Sabina and Parker (87) described a plaque-assay system for IBR virus in MDBK cells. In 1963 Rowhandeh and Werder (86) described plaque production by IBR virus in BEK cell cultures using Dulbecco's agar-overlay method and Postlethwaithe's liquid-overlay methods. In 1957 Cheatham and Crandell (13) described Cowdry Type A intranuclear inclusions in IBR-infected tissue cultures of human amnion cells. In 1959, Crandell, et al. (18) demonstrated intranuclear inclusions in the epithelial cells of the respiratory tract of calves inoculated with IBR virus. The inclusions occurred as early as 36 hours and persisted through 60 hours after inoculation.

Armstrong, et al. (3) suggested that the IBR-IPV virus is a bovine member of the Herpesvirus group on the basis of its cytopathic effect in tissue culture, its sensitivity to ether and its size, morphology and growth cycle as observed with electron microscopy. Andrewes (2) placed the IBR virus in the same group with the other Herpesviruses. Hall, et al. (34) suggested that the infectious bovine rhinotracheitis (IBR-IPV) virus be given the binomial name. Herpesvirus bovis.

Plummer (79) serologically compared members of the Herpesvirus group. Herpes simplex, B-virus, pseudorabies, IBR and equine Herpes virus types 1 and 2 were compared and the only cross neutralization that occurred was that of Herpes simplex by B-virus antiserum. He

also demonstrated, as had Carmichael and Barnes (12) that there was cross complement fixation between equine Herpesvirus type 1 and IBR virus.

The host cell range of IBR virus in tissue culture is extensive. Madin, et al. (50) propagated IBR virus on bovine embryonic kidney (BEK) cell cultures as well as bovine testicle and lung cell cultures. They were not able to propagate the virus on HeLa, KB, L or chick fibroblast cell cultures. Cabasso, et al. (10) were able to adapt IBR virus to HeLa cells by alternate passages between BEK and HeLa cell cultures. McKercher and Saito (63) reported a host cell range of IBR virus as kidney and testicle cell cultures of bovine, ovine and rabbit origin; kidney cell cultures of equine, porcine, caprine and feline kidney cells; bovine lung and lymph node; and HeLa and human amnion cells.

Early attempts at isolating the IBR virus by McKercher, et al. (60) and Chow, et al. (15) showed that mice of all ages, rabbits and chicken embryos were not susceptible to infection with the IBR virus. McKercher, et al. (64) in 1958 reported further on host range studies in which sheep, goats, swine and horses were inoculated intranasally or intravenously with IBR virus. Symptoms were observed only in the goats. A temperature increase and hyperemia of the nasal mucosa was observed in all of the goats. Several developed anorexia and one goat had a severe reaction characterized by depression, nasal discharge and respiratory distress. IBR virus was recovered on the

5th, 10th and 20th days post-inoculation. Antibody titers ranged from 1:7 to 1:26. The IBR antibody titers of the pooled sera of the animals of the other species were 1:8 in the swine, 1:10 in the horses and 1:11 in the sheep.

In 1966 Van Houweling (104) inoculated 14 goats intranasally and 3 goats intracerebrally with IBR virus. There was no clinical evidence of infection and serum antibodies to IBR virus were not detected. IBR virus was recovered from nasal washings of six goats on the fifth post-inoculation day.

Onstad and Saxegaard (74) in 1967 described outbreaks of apparently contagious vaginitis and balanitis in Norwegian swine herds. A purulent vaginal discharge was observed in the sows a few days after breeding and in the boars there was swelling of the prepuce with an intermittent purulent discharge. Saxegaard and Onstad (91) reported on the isolation of IBR virus from a boar affected with a balanitis and from three sows affected with vaginitis. In addition IBR virus was isolated from the vagina of a healthy sow from a herd that had never experienced the vaginitis-balanitis syndrome. Virus isolations were made on primary and secondary pig kidney cell cultures.

Woods, et al. (108) exposed pigs to IBR virus by various routes. The clinical response consisted of a temperature rise, depression and variable lymphocytosis. Virus was not recovered from any of the pigs. One pig developed an IBR antibody titer of 1:4.

The time of the initial appearance of the IBR-IPV virus as a disease-producing agent in cattle is unknown. However, the history of the virus indicates that virus caused a localized genital infection for over sixty years before conditions produced an alteration in the virus and the appearance of new disease syndromes. An explanation of the factors involved in the alteration in the virulence of the IBR-IPV virus has been proposed in reports by McKercher, et al. (66) and McKercher (56, 59). The cattle herds in Europe for the most part are small and relatively segregated. The genital infection was often spread between herds by the use of a common bull. Since the herds were small the opportunity for the virus to acquire an affinity for the respiratory tract was very limited and if a respiratory infection were to occur the development of antibodies would tend to maintain the infection within the isolated herd and prevent the spread to other herds. The virus was probably transported to the United States from Europe by genitally-infected cattle. The genital infection persisted in the eastern United States for many years until cattle husbandry underwent drastic changes after World War II. The appearance of huge feedlots and large confinement dairy operations provided large concentrations of cattle favorable for the establishment of severe disease problems. It is speculated that the IBR-IPV virus was introduced into these operations by genitally-infected cattle, but since

the feedlot cattle were reproductively inactive the opportunity for the maintenance of the genital infection was not present. However, the respiratory system proved to be an effective portal of entry, and it is possible that by the rapid passage of the virus through a large number of animals and by the selection of the viral population which had a predilection for the epithelium of the respiratory tract a new disease entity was developed. Later the virus acquired the ability to invade the bloodstream and other tissues and new syndromes appeared.

MATERIALS AND METHODS

Cell lines

MDBK: The Madin-Darby bovine-kidney cell line (MDBK) was used in the propagation of infectious bovine rhinotracheitis virus strains, in the serum-virus-neutralization tests and in the virus isolation procedures.

The cells were propagated in 250 milliliter plastic tissue culture flasks[a] with the growth medium of Minimum Essential Medium (Eagle) with Earle's salts[b] (MEM) and 5% newborn calf serum[b]. After trypsinization with a 0.2% trypsin-versene solution the cells were diluted 1:3 in growth medium and one milliliter was inoculated into disposable glass tissue-culture tubes[c], five milliliters were inoculated into 35x10 mm. plastic tissue-culture plates[a] or 20 milliliters were inoculated into 250 milliliter plastic tissue-culture flasks. Monolayers usually developed in 48-72 hours.

ST: A swine-testicle cell line (ST) developed by Dr. A. W. McClurkin, National Animal Disease Laboratory, U.S.D.A., Ames, Iowa, was obtained after the Pregnant Sow Pathogenicity Experiment (Experiment No. 3) was concluded. A number of selected samples from Experiment No. 3 were inoculated onto ST cells in an attempt to re-isolate virus.

The cells were propagated in 250 milliliter plastic tissue-culture flasks with the growth medium of MEM with 10% newborn-calf serum. After

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[a] Falcon Plastics, Los Angeles, Calif.

[b] Grand Island Biological Company, New York, N.Y.

[c] Kimble Glass Company.

trypsinization with trypsin-versene solution the cells were diluted 1:2 in growth medium and one milliliter was inoculated into disposable glass tissue-culture tubes for use in virus isolation procedures. Monolayers developed in 72-96 hours.

Trypsin-versene solution

A trypsin-versene solution was used to remove the cells from the surface of the flasks after propagation of the MDBK and ST cell lines. The formulation was as follows:

Trypsin[a]	2 g.
NaCl	8 g.
KH_2PO_4	0.2 g.
Na_2HPO_4	1.15 g.
Ethylenediamine tetraacetic acid, disodium salt (EDTA)[a]	1 g.
H_2O	q.s. 1 liter

Growth medium

Minimum Essential Medium (Eagle) with Earle's salts (MEM) was used as growth medium for both the MDBK cell line and the ST cell line. Five per cent newborn-calf serum was added to the medium used in propagating the MDBK cells and 10% newborn-calf serum was used for the ST cells. No antibiotics were added to the growth medium.

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[a]Difco Laboratories, Inc., Detroit, Mich.

Maintenance medium

Minimum Essential Medium (Eagle) with Earle's salts were used without the addition of serum for the maintenance of the cell monolayers. The maintenance medium used in the virus isolation procedures contained 200 units of penicillin and 200 micrograms of streptomycin per milliliter of medium.

Agar-overlay medium

A double concentration of Minimum Essential Medium (Eagle) with Earle's salts was combined with an equal volume of 1.8% Noble-agar[a]. This was used as an agar-overlay medium giving a final concentration of 0.9% agar.

Neutral red agar-overlay medium

A second agar overlay containing 1:10,000 neutral red was added to the cell monolayer in the 35x10 mm. plastic tissue-culture plates after three days to differentiate the virus plaques more clearly. The following formulation was used:

Neutral red	0.1 g.
Agar	10 g.
H_2O	q.s. 1 liter

[a]Difco Laboratories, Inc., Detroit, Mich.

Saline solution G (Puck, et al., 80)

H_2O	1 liter
Glucose	1.1 g.
Phenol red	5 mg.
NaCl	8 g.
KCL	0.4 g.
$Na_2HPO_4 \cdot 7\ H_2O$	0.29 g.
KH_2PO_4	0.15 g.
$MgSO_4 \cdot 7\ H_2O$	0.15 g.
$CaCl_2 \cdot 2\ H_2O$	16 mg.

Cleaning fluid (antibiotic solution)

Immediately after collection the nasal, conjunctival and genital tract swabs were placed into three milliliters of Earle's Balanced Salt Solution[a] containing 5% fetal calf serum[a] and 1,000 units of penicillin, 1,000 micrograms of streptomycin and 20 milligrams of Fungizone[b] per milliliter. A serum-virus-neutralization test was conducted on each lot of fetal-calf serum in order to determine that infectious bovine rhinotracheitis virus antibodies were absent.

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[a]Grand Island Biological Company, New York, N.Y.

[b]E. R. Squibb and Sons, Inc., New Brunswick, New Jersey.

Virus titration

Tenfold dilutions of the virus were made in Earle's salt solution. Each dilution of virus was inoculated onto two tubes of MDBK cell monolayers, 0.2 milliliter of virus dilution to each tube. After an adsorption period of one hour at 37°C. the virus solution was poured off and the tubes were washed twice with 1.5 milliliters of Earle's salt solution. Then 1.5 milliliters of maintenance medium was added and the tubes were incubated at 37°C. for four days. The tubes were examined microscopically and the cytopathic effect was classified. The titer of the virus was determined by the method of Reed and Muench (82).

Virus strain

The strain of infectious bovine rhinotracheitis virus (Herpesvirus bovis) used in this study was isolated from the pooled livers of two aborted bovine fetuses in March, 1965. Nineteen Aberdeen Angus cows experienced abortion in a group of 33 cows, the abortions occurring at 7½-8½ months of pregnancy. The cows had been vaccinated for brucellosis and leptospirosis. Two aborted fetuses were submitted to the Iowa Veterinary Diagnostic Laboratory. No gross lesions were observed in one fetus and a mild icterus was seen in the other. Bacteriological cultures of the stomach contents and livers were negative. Infectious bovine rhinotracheitis virus was isolated on tissue cultures of primary bovine-testicle cells from the pooled livers.

Virus purification procedures

The original isolate of infectious bovine rhinotracheitis virus (Herpesvirus bovis) designated as IBR-Fetus was stored at -90°C. until used in this study. After two passages on MDBK cell cultures the virus was cloned three times for purification using the following procedure each time.

Tenfold serial dilutions of the virus were made in saline solution G. One milliliter of each dilution was inoculated onto MDBK cell monolayers grown in 35x10 mm. plastic tissue-culture plates. The cells had been washed with five milliliters of saline solution G prior to inoculation. After an adsorption period of one hour the plates were washed twice with five milliliters of saline solution G. The monolayers were then overlayed with five milliliters of agar-overlay medium. The plates were incubated at 37°C. in a humidified atmosphere containing 5% CO_2. After 3-4 days of incubation a suitably isolated plaque, at least 10 mm. distant from the nearest plaque, was removed with a pipette and placed in one milliliter of saline solution G. This was then inoculated onto a monolayer of MDBK cells in a 30 milliliter plastic tissue culture flask[a]. After an adsorption period of one hour, five milliliters of maintenance medium was pipetted into the flasks and they were incubated at 37°C. The flasks were examined daily and when 80% or more of the cells showed a cytopathic effect (CPE) the virus was released by alternately freezing and thawing the infected cells three times. The fluid was removed and centrifuged

[a]Falcon Plastics, Los Angeles, Calif.

at 2500 r.p.m. for 15 minutes to remove the cellular debris. The supernatant fluid was used in tenfold serial dilutions as the inoculum for the subsequent cloning procedures.

After being cloned three times the virus had undergone eight passages. A large volume of virus was produced on the ninth passage for use in animal inoculation. After clarification by centrifugation for 15 minutes at 2500 r.p.m. and testing for bacterial sterility the virus was dispensed into vials and stored at -90°C. until used. Additional samples were taken for sterility testing at this time.

Samples obtained for sterility testing were inoculated onto blood-agar plates, Sabouraud dextrose-agar plates and tryptose broth. The blood agar plates and the tryptose broth were incubated for 72 hours at 37°C. and the Sabouraud agar plates were incubated at 25°C. for one week. All samples were negative for bacteria and fungi.

To confirm that the virus strain being used was Herpesvirus bovis, a serum-virus-neutralization test was done using the ninth passage level of the IBR-Fetus strain of virus and anti-serum to the Los Angeles strain of IBR virus (IBR-LA). The concentrated IBR-LA anti-serum completely neutralized the IBR-Fetus virus with a titer of 1.5×10^7 $TCID_{50}$ per milliliter.

Preparation of IBR-Fetus virus and cell controls for the intracerebral inoculation experiment (Experiment No. 2)

Forty-eight hour old tube cultures of MDBK cells were used to prepare the virus and control inoculums. A 10^{-1} dilution of 9th passage

IBR-Fetus in saline solution G was used to inoculate 12 tubes, 0.1 milliliter per tube. The control tubes were inoculated with 0.1 milliliter of saline solution G. After a one hour incubation period the tubes were washed once with 1.5 milliliters of saline solution G and then 1.5 milliliters of maintenance medium was added. After 48 hours incubation at 37°C. the virus was harvested as described previously. The cell controls were treated in an identical fashion. The fluids from the infected and non-infected cells were centrifuged at 2500 r.p.m. for 10 minutes and the supernatant fluid was removed for use in inoculation. Sterility testing of the final material was done as previously described and the prepared inoculums were stored at -90°C. until used.

Preparation of IBR-Fetus anti-serum

Five rabbits were bled and serum-neutralization tests were conducted to confirm the absence of antibodies to IBR virus prior to inoculation of the IBR-Fetus virus. The rabbits were then inoculated with ninth passage IBR-Fetus virus with a titer of 1.6×10^7 $TCID_{50}$ per milliliter using the following schedule.

Day No. 1	1 ml. intravenous (i.v.) + 1 ml. intramuscular (i.m.)
3	1 ml. i.v. + 1 ml. i.m.
5	1 ml. i.v. + 1 ml. i.m.
7	1 ml. i.v.
26	1 ml. i.v.
40	1 ml. i.v.

Blood was collected aseptically on day no. 50. The blood was allowed to clot at room temperature and then centrifuged. The serum was inactivated at 56°C. for 30 minutes. Serum-virus-neutralization tests were conducted on the rabbit serum and it was determined that the neutralization index (NI) was $1x10^3$.

Experimental animals

Pathogenicity experiment (Experiment No. 1):

Eight Yorkshire pigs were obtained from the closed swine herd at the Iowa State University Veterinary Medical Research Institute. This herd is free of known swine pathogens. The pigs were weaned at three weeks of age and moved into the isolation facilities. Each of the animals was identified with a tattoo number in the right ear.

Pig No. 1 - male
2 - female
3 - female
4 - male
5 - male
6 - male
7 - female
8 - male

The pigs were allowed to adjust to their new environment for 15 days and pre-inoculation samples were collected for bacteriological, virological and hematological examination. Nasal swabs were obtained and examined for

bacteria, particularly of the genera Pasteurella, Bordetella and Mycoplasma. An Aerobacter species was isolated from pig no. 2 and a Klebsiella species was isolated from pig no. 7.

Nasal swabs, conjunctival swabs and vaginal or preputial swabs were obtained for virus isolation 11 days, 5 days and 1 day prior to inoculation. Blood samples were collected in heparin tubes for virus-isolation attempts from the buffy coat. Virus-isolation attempts were made on MDBK cell cultures in tubes. No viruses were isolated from the pre-inoculation samples.

Blood samples were also collected for the determination of total leucocyte (WBC) counts, differential leucocyte counts and packed cell volume (PCV).

Serum samples were obtained one day prior to inoculation for antibody titration.

Rectal temperatures were taken twice daily.

Two male Holstein calves, two months old, were purchased from a private source. These animals were used to determine that the IBR-Fetus strain of virus was in fact pathogenic to calves. They were moved into the isolation quarters 12 days prior to inoculation with virus. Rectal temperatures were taken twice daily. Blood samples were collected every second day of the pre-inoculation period for the determination of WBC counts, differential leucocyte counts and PCV. A serum sample was obtained and tested for the presence of infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD) and parainfluenza -3 (PI_3) antibodies.

An IBR titer of less than 1:4, a BVD titer of 1:16 and a PI_3 titer of 1:32 were found.

Nasal, conjunctival and preputial swabs, and buffy coat samples were collected from the calves 11 days, 5 days and 1 day prior to inoculation for virus isolation attempts on MDBK cell monolayers. No virus isolations were made. Nasal swabs were also collected for bacteriological examination and Pasteurella multocida was isolated from both calves.

Intracerebral inoculation experiment (Experiment No. 2)

Four pigs that had been maintained under conditions of isolation since weaning at three weeks of age were placed into the isolation facilities five days prior to inoculation. The pigs were 6-8 weeks old.

Pig no. 11 - male - 8 weeks old
12 - male - 7 weeks old
13 - female- 7 weeks old
14 - male - 5 weeks old

Samples were taken one day prior to inoculation for bacteriological, virological and hematological examination as done on the pigs in experiment no. 1. No viruses or pathogenic bacteria were found on the pre-inoculation samples. Rectal temperatures were taken twice daily. A pre-inoculation serum sample was obtained for IBR antibody titration.

Pregnant sow pathogenicity experiment (Experiment No. 3):

A Hampshire sow (no. 15) and a Yorkshire sow (no. 16), both approximately one year old, were obtained from the SPF herd maintained at the Veterinary Medical Research Institute at Iowa State University. The Hampshire sow was 34 days pregnant and the Yorkshire sow was 23 days pregnant.

The sows were maintained 24 days in the isolation area prior to inoculation. Nasal, conjunctival and vaginal swabs were obtained 12 days, 9 days, 6 days and 1 day prior to inoculation of the virus. No viruses were isolated from the pre-inoculation swabs. Rectal temperatures were taken daily.

Animal isolation facilities

The isolation facilities used in Experiments no. 1 and no. 2 consisted of a small building containing two rooms, each approximately 16 feet by 20 feet in size.

The outer room was entered through a door from the outside and the inner room could only be entered through a door from the first room. Each room had its own air exhaust system. The pen walls were made of smooth concrete and were four feet high. The floors were concrete.

The control pigs were kept in the outer room. The infected pigs and calves were kept in the inner room. The two pigs receiving the same injection were kept together in the same pen as were the two calves. No bedding was used and the pens were washed down with water twice daily.

Protective clothing and boots were put on immediately after entering the building. Hands and boots were disinfected in a solution of Nolvasan[a]. The control pigs were examined and the samples were taken after the pen had been washed down. The pigs were then given feed and water. Hands and boots were disinfected again and the protective clothing and boots were removed. The inner room was entered immediately. After entering the inner room protective clothing and boots were put on. The animals in each pen were examined and samples were collected after the pen had been washed down. Before each pen was entered, hands and boots were disinfected in a Nolvasan solution. After all animals had been examined and sampled they were given feed and water. The protective clothing and boots were removed and left in the room. Exit was made through the outer room to the outside in a direct manner.

The sows used in Experiment no. 3 were kept in an isolation wing of a barn. Each sow was kept in a pen approximately four feet by ten feet in size. The wooden walls of the pens were three feet high. Straw bedding was used in the pens. The sows were examined and the samples were collected each afternoon. They were given feed and water twice daily.

IBR-Fetus virus inoculation of experimental animals

Experiment no. 1: The pigs were allotted to each group by random selection except that the stipulation was made that one male and one female were to be in the group to receive the intracerebral and genital inoculations.

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[a]Fort Dodge Laboratories, Fort Dodge, Iowa.

Pigs no. 8 (male) and no. 3 (female) were allotted to the control group and did not receive any virus.

Pigs no. 6 (male) and no. 2 (female) received one milliliter of IBR-Fetus virus by the intravenous route.

Pigs no. 5 (male) and no. 1 (male) were inoculated with one milliliter of IBR-Fetus virus into the right nostril.

Pigs no. 4 (male) and no. 7 (female) received IBR-Fetus virus by intracerebral inoculation and by inoculation into the genital tract. One milliliter of virus was placed into the prepuce or into the vagina. For the intracerebral inoculation the frontal area was shaved, scrubbed with a surgical soap and then swabbed with alcohol. Xylocaine[a] was used for local anesthesia by making a subcutaneous injection in the area of the inoculation site. A skin incision, 1-1.5 cm. in length was made 1-1.5 cm. to the right of the midline with a scalpel. An intramedullany pin drill with a 1/8 inch intramedullany pin exposed 1/2 inch was used to drill through the cranial wall. A 22 gauge one inch needle was inserted into the hole to a depth of one inch and one milliliter of IBR-Fetus virus was inoculated. The skin incision was closed with a single suture of Supramid[a] synthetic suture material.

Both calves (no. 9 and no. 10) were inoculated with one milliliter of IBR-Fetus virus into the right nostril, 0.1 milliliter into the right conjunctival sac and one milliliter into the preputial sheath as a control on the pathogenicity of the IBR virus strain being used.

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[a]Jensen-Salsbury Laboratories, Kansas City, Mo.

One milliliter of IBR-Fetus virus used in the inoculation of the pigs and one milliliter of the virus used in the calf inoculation were returned to the laboratory and titrations were done on MDBK cells to determine the minimum titer of the virus used in this experiment. The MDBK cells were inoculated with the virus within 3-4 hours after the virus had been used in the animal inoculations and the titer was 1.4×10^6 $TCID_{50}$ per milliliter for the virus used in the pigs and 4.4×10^5 $TCID_{50}$ per milliliter for the virus used in the calves. Prior to use in the experiment the titer of the IBR-Fetus virus had been determined as 1.6×10^7 $TCID_{50}$ per milliliter.

Experiment no. 2: The pigs were divided into two groups by the method of random selection. The control pigs were designated as no. 11 (male) and no. 12 (male) and the pigs destined to receive the IBR-Fetus virus were no. 13 (female) and no. 14 (male).

The methods of trephining and intracerebral inoculation were as described in Experiment no. 1 except that the volume of the inoculum was reduced to 0.5 milliliter. The control pigs were inoculated with 0.5 milliliter of inoculum prepared from un-infected MDBK cells. The other two pigs were inoculated with 0.5 milliliter of tenth passage IBR-Fetus virus.

Experiment no. 3: The sows were placed into separate pens in the isolation facilities. The Hampshire (no. 15) was 57 days pregnant at the time of inoculation and the Yorkshire sow (no. 16) was 43 days pregnant. Four milliliters of 12th passage IBR-Fetus virus was inoculated into the ear vein. A sample of the virus was retained from the inoculum and the titer was determined to be 5×10^4 $TCID_{50}$ per milliliter.

Sampling methods

Nasal swabs were obtained by inserting a cotton-tipped applicator into the nostril to a depth of 2-3 inches and applying a rotary motion before withdrawing it. Both nostrils were swabbed with the same applicator. The nostrils were cleaned with a piece of cotton dampened with 70% ethyl alcohol prior to swabbing.

Conjunctival swabs were obtained by inserting the cotton-tipped applicator into the conjunctival sac behind the lower eyelid. A rotary motion was applied to the applicator before it was removed from the conjunctival sac.

The preputial opening was cleaned with an alcohol swab and a cotton-tipped applicator was inserted and rubbed over the surface of the preputial cavity. The vulva was cleansed with an alcohol swab. The labia were parted and the cotton-tipped applicator was inserted to a depth of 3-4 inches and rotated.

A five milliliter blood sample was obtained by bleeding from the anterior vena cava. A portion of the blood sample was placed into a tube containing EDTA for hematological procedures. The remainder (three milliliters) was placed into a tube containing heparin for separation of the buffy coat. The heparinized sample was drawn into a syringe and the syringe was placed in a refrigerator in an upright position for four hours. A U-shaped needle was placed on the tip and the upper layer of serum in

the syringe was forced out. The buffy coat was collected in a vial containing one milliliter of cleaning fluid.

The swabs were placed in vials containing three milliliters of cleaning fluid immediately after they were collected and held in the fluid for approximately one hour before being removed and discarded.

If tubes of cell cultures were available, the samples were often inoculated onto cell cultures within several hours after collection. If cells were unavailable, the samples were frozen and held at -20°C.

Tubes of MDBK cell cultures were inoculated with 0.2 milliliters of the sample fluid. Two tubes were inoculated with each sample. After an adsorption period of one hour 1.5 milliliters of maintenance medium was added to each tube after the inoculum was poured off. The tubes were incubated at 37°C. and were examined microscopically each day.

After one week all of the tubes were freeze-thawed three times and the fluid was inoculated onto fresh cell cultures in tubes. All samples were carried through four passages on MDBK cells before being considered as negative.

After completion of Experiment no. 3 a swine testicle cell line (ST) was obtained. A selected number of samples from the sows in Experiment no. 3 were inoculated onto tube cultures of ST cells. The tubes were incubated for ten days at 37°C. The samples were passaged twice on ST cells.

Serum-virus-neutralization tests

Pre-inoculation serum samples were obtained from all animals. Post-inoculation serum samples were obtained at weekly intervals from the pigs and calves in Experiment no. 1 and at monthly intervals from the sows in Experiment no. 3. The serum was inactivated for 30 minutes at 56°C. Two-fold dilutions of serum were made in Earle's salt solution. Approximately 200 pfu of IBR-Fetus virus was added to an equal volume of the serum dilution and allowed to incubate at room temperature for one hour. The final result was a fourfold dilution of the serum beginning with a 1:4 dilution and continuing through a series of nine dilutions with a final dilution of 1:1024. One-half milliliter of the serum and virus mixture was inoculated onto each of two 35x10 mm. plastic tissue culture plates of MDBK cell monolayers. The plates were incubated for one hour at 37°C. Five milliliters of the agar-overlay medium was added after the adsorption period. The plates were incubated at 37°C. in a humidified atmosphere containing 5% CO_2.

The plates were overlayed with five milliliters of the neutral red agar-overlay medium after 72 hours of incubation and the plates were kept in the dark for 8-12 hours. The plaques were then counted and an average for the two plates in each dilution was calculated. The dilution of serum which produced a reduction nearest to 50% in the average number of plaques was considered to be the titer for the sample. This method was used in order to aid comparisons between samples and between animals.

All of the serum samples obtained from one individual animal were tested simultaneously.

Serological survey

Most of the swine serum samples submitted to the Iowa Veterinary Medical Diagnostic Laboratory for leptospirosis testing during the period of 29 November 1967 to 19 December 1967 were retained to test for the presence of IBR antibodies. A total of 1220 swine serum samples were examined.

The serum was removed from the blood samples and frozen at -20°C. Prior to testing the serum specimens were thawed and inactivated at 56°C. for 30 minutes.

Initially all of the samples were screened at a dilution of 1:4 using the procedures described previously. If there was a 50% or greater reduction in the number of plaques at the 1:4 dilution, the samples were tested at higher dilutions in order to determine an end point. The titration was done as described previously for the serum-virus-neutralization test.

Post mortem samples

The pigs were destroyed by electrocution. At the time the gross pathological examination was conducted, 1-2 gram samples of tissue were obtained aseptically and placed into three milliliters of cleaning fluid. The specimens were then frozen at -20°C. The following specimens were collected: lung; bronchial lymph node; tracheal swab; tonsil; brain; kidney; liver; spleen; testicle or uterus and ovary; adrenal; mesenteric lymph node; conjunctiva.

Tissue specimens were also collected and placed into 10% formalin solution for preparation for microscopic examination.

The animals were necropsied on the following schedule.

Experiment No. 1.

Pig no. 1	(intranasal)	18 days post-inoculation
3	(control)	31 days " "
4	(intracerebral)	11 days " "
5	(intranasal)	31 days " "
6	(intravenous)	18 days " "
8	(control)	17 days " "

Experiment No. 2

Pig no. 11	(cell culture)	24 hours post-inoculation
12	(cell culture)	72 hours " "
13	(virus-infected)	72 hours " "
14	(virus-infected)	24 hours " "

Experiment No. 3

Sow no. 16	86 days post-inoculation
16 I (piglet)	Stillborn
16 J "	Stillborn
16 K "	Stillborn
16 L "	24 hours post partum
16 A "	8 days post partum
16 F "	8 days post partum
15 A "	6 hours post partum
15 B "	6 hours post partum

The frozen tissue specimens were thawed and macerated in a tissue grinder. After the tissue debris had settled, the supernatant fluid from each specimen was inoculated into two tubes of MDBK cell cultures. After an adsorption period of one hour the cell cultures were washed twice with Earle's balanced salt solution and maintenance medium was added. The tubes were incubated at 37°C. for one week and were examined daily. Each tissue specimen was passaged three times in MDBK cells before being considered negative.

RESULTS

Serological survey

A total of 1220 samples of swine serum consisting of 123 accessions from 108 Iowa swine herds were tested for the presence of IBR antibodies. The number of samples tested in an individual herd ranged from one to 124. There were 478 males, 499 females and 243 animals of unknown sex. There were 454 animals under one year of age and 417 animals over one year of age. The age was unknown in 349 animals.

There were 32 herds which had one or more animals (Table 1) with an IBR antibody titer of 1:4 or higher. A total of 773 animals were tested in the 32 herds and 139 animals had antibodies to IBR virus. There were 76 herds in which no animals with IBR antibody titers were detected. A total of 447 animals were tested in the 76 negative herds.

The 139 positive animals had IBR antibody titers ranging from 1:4 to over 1:1024.

1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024
107	22	5	0	1	0	1	1	1	1

Since the test records accompanying the blood samples to the laboratory were often illegible or incomplete, the age and sex could not be determined in almost one-third of the animals. Any trend relating to a particular age or sex incidence would be difficult to determine and would not be highly reliable. As seen in Table 2 the incidence of positive animals

Table 1. Summary of 32 Iowa Swine herds having animals with IBR serum antibody titers.

Herd No.	No. Tested	Age: under 1 year	Age: 1 year or over	Age: unknown	Sex: M	Sex: F	Sex: unknown	1:4	1:8
1	2		2			2			
4	8	4	4		2	6			
5	4			4			4		2
6	39			39			39		2
	5			5			5		
8	28	28			13	15		1(F)	2(M)
13	5		5		1	4			1(M)
24	10		10			10		2	2
25	32			32			32	2	
	8			8			8	3	
26	24	24			6	18		1(F)	
29	47	47			47				
	5			5			5		
	2			2			2		
	26			26			26	6	1
40	53	11	42		11	42			
42	7	7			7				
	7	7				7		1	
51	20	20			12	8		1(M)	
54	24	24			24				
	22	22				22		1	
60	29		29		1	28		1(M) 4	4
62	9			9	4	5		1(F)	
	11			11	5	6		1(M) 2(F)	
67	21	21			21			1	
74	3		3			3		1	
75	47	47			2	45		1(M) 10(F)	
77	2			2			2	1	
79	77	77			56	21		15(M) 7(F)	1(M)
	47			47			47	8	5
81	13			13			13	6	
84	4		4		4			3	
88	25			25		25		7	
90	1		1		1			1	
91	32		32		3	29		1(M) 8(F)	
93	2		2		2			2	
94	18		18		18			3	1
98	7		7		1	6		1(M)	
102	1		1			1		1	
107	34	34				34		2	
108	24	24			24			1	

1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024
				1			
2(1 yr. F)							
2							
1(F)							
		1					
1(8 mo. M)					1(2 yr. F)		
							1
						1(M)	

Table 2. Summary of serological survey of 1220 Iowa swine. Animals with IBR serum antibody titers classified by age and sex.

	under 1 year	1 year or over	unknown	M	F	unknown	Total
1:4	42	28	37	38	48	21	107
1:8	4	8	10	6	7	9	22
1:16	1	2	2	1	2	2	5
1:32							
1:64	1			1			1
1:128							
1:256		1			1		1
1:512		1			1		1
1:1024	1			1			1
>1:1024		1			1		1
Total	49	41	49	47	60	32	139
Total no. Tested	454	417	349	478	499	243	1220

in the age and sex groupings appear to correspond fairly well to the age and sex groups in the total test population.

All blood samples of satisfactory quality were selected from those received at the Diagnostic Laboratory over a period of approximately one month. This resulted in a good geographic representation of the state except that possibly northeast Iowa and south-central Iowa were not well represented (Figure 1). Herd no. 79 in the north-central area is a breeding herd for a firm engaged in producing hybrid hogs as is herd no. 54 in the northwest area. It is interesting to note that herd no. 8 in close proximity to herd no. 79 has the same breed of hybrid hogs. Herds no. 51, 67 and 108 in northwest Iowa also have these hybrid hogs. One might speculate that there would be traffic of hogs between some of these farms.

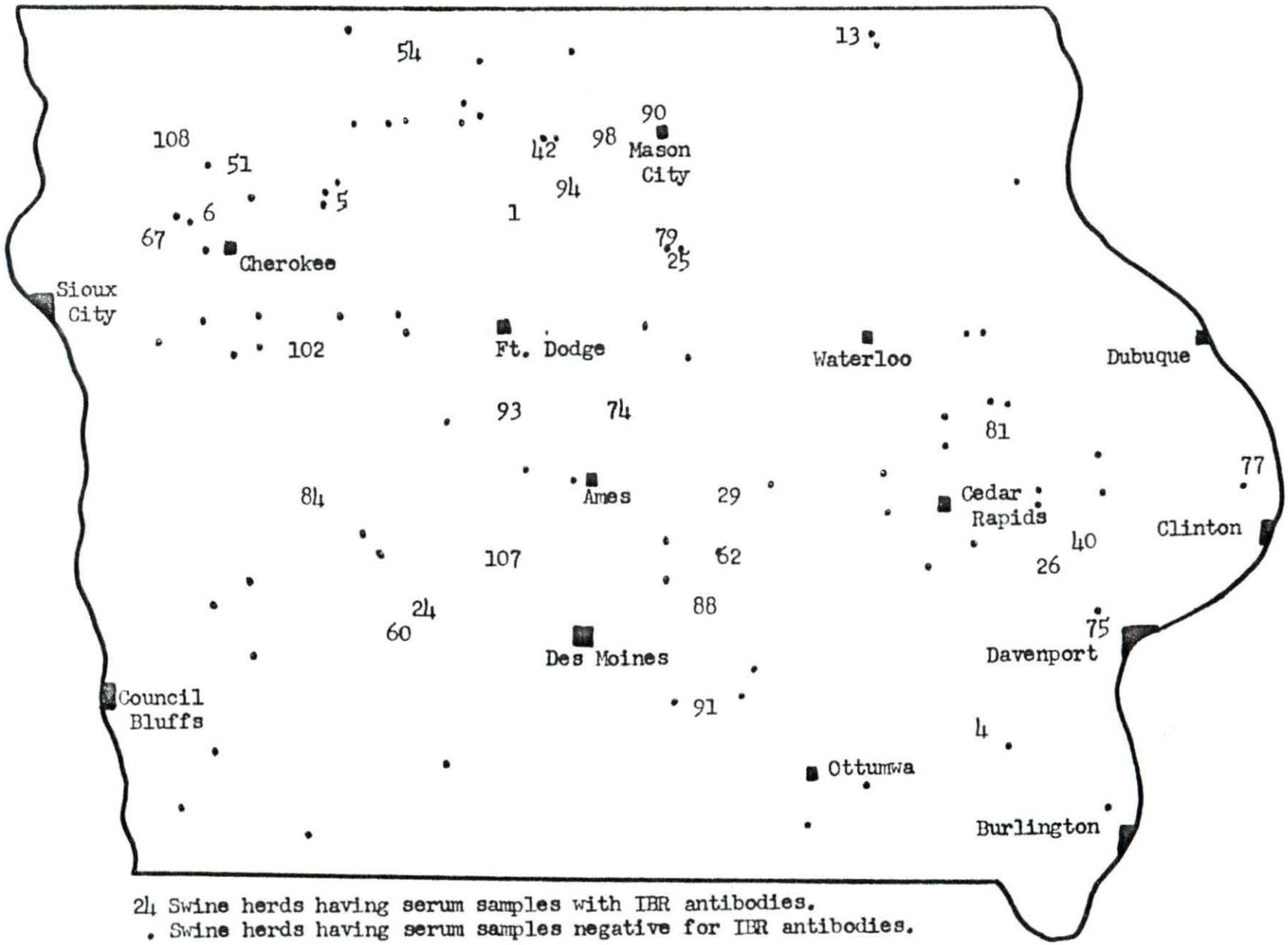
Seven of the herds have two or more separate submissions of samples (Table 1). Herd no. 29 had four separate accessions with positive samples found on two of the submissions. Eighty animals were tested in this herd and there were eight animals with IBR antibodies. Herds no. 25, 62 and 79 had positive samples on both submissions. There were 43 positive samples among the 184 that were tested in these three herds. Herds no. 6, 42 and 54 had positive samples on only one of the two submissions.

Experiment No. 1: Pathogenicity of the IBR-Fetus virus

Clinical signs

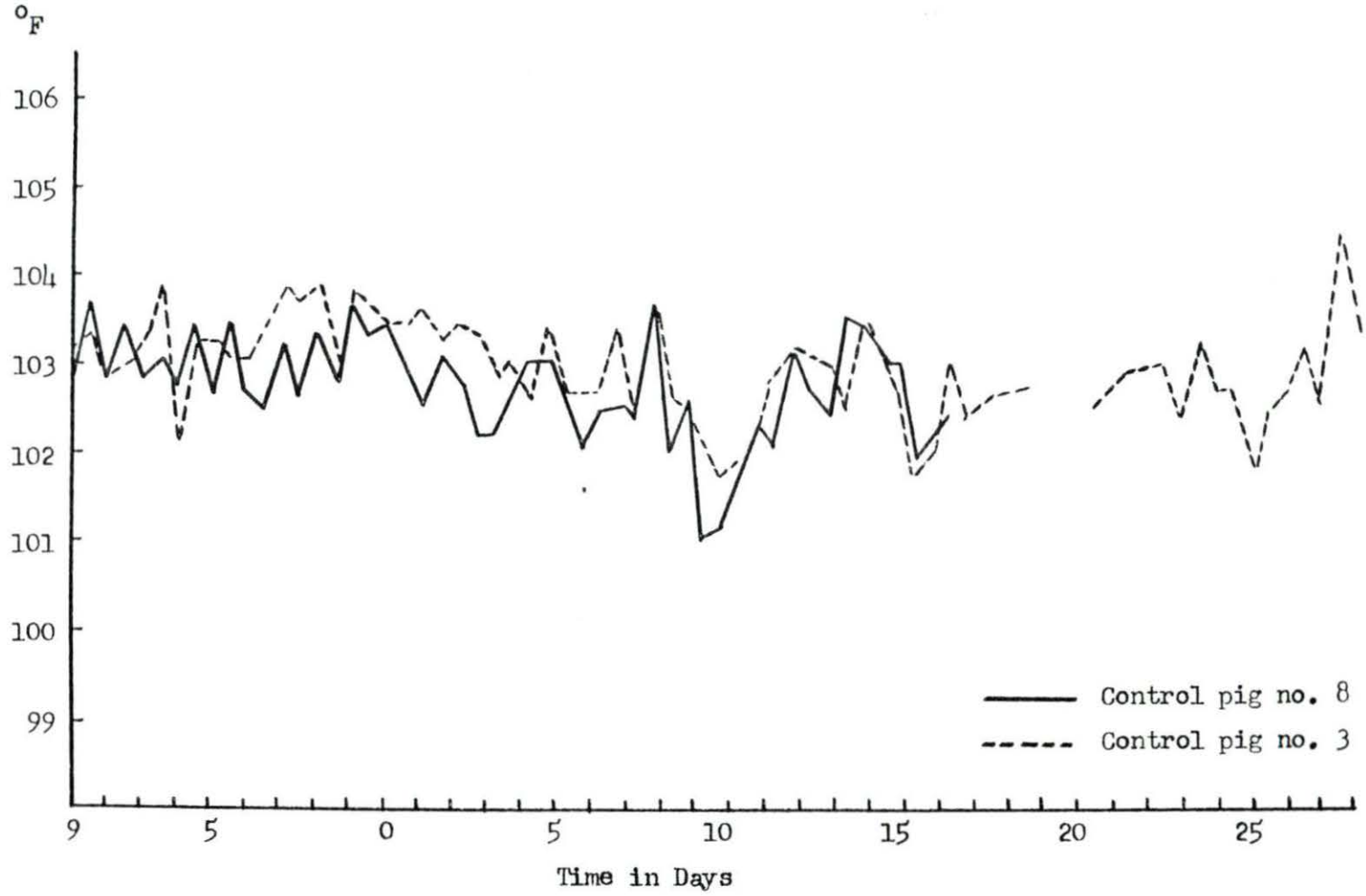
Pigs no. 3 and no. 8 which were maintained as uninoculated controls remained clinically normal throughout the period of observation. Body

Figure 1. Locations of the Iowa swine herds surveyed for the presence of IBR antibodies.



24 Swine herds having serum samples with IBR antibodies.
. Swine herds having serum samples negative for IBR antibodies.

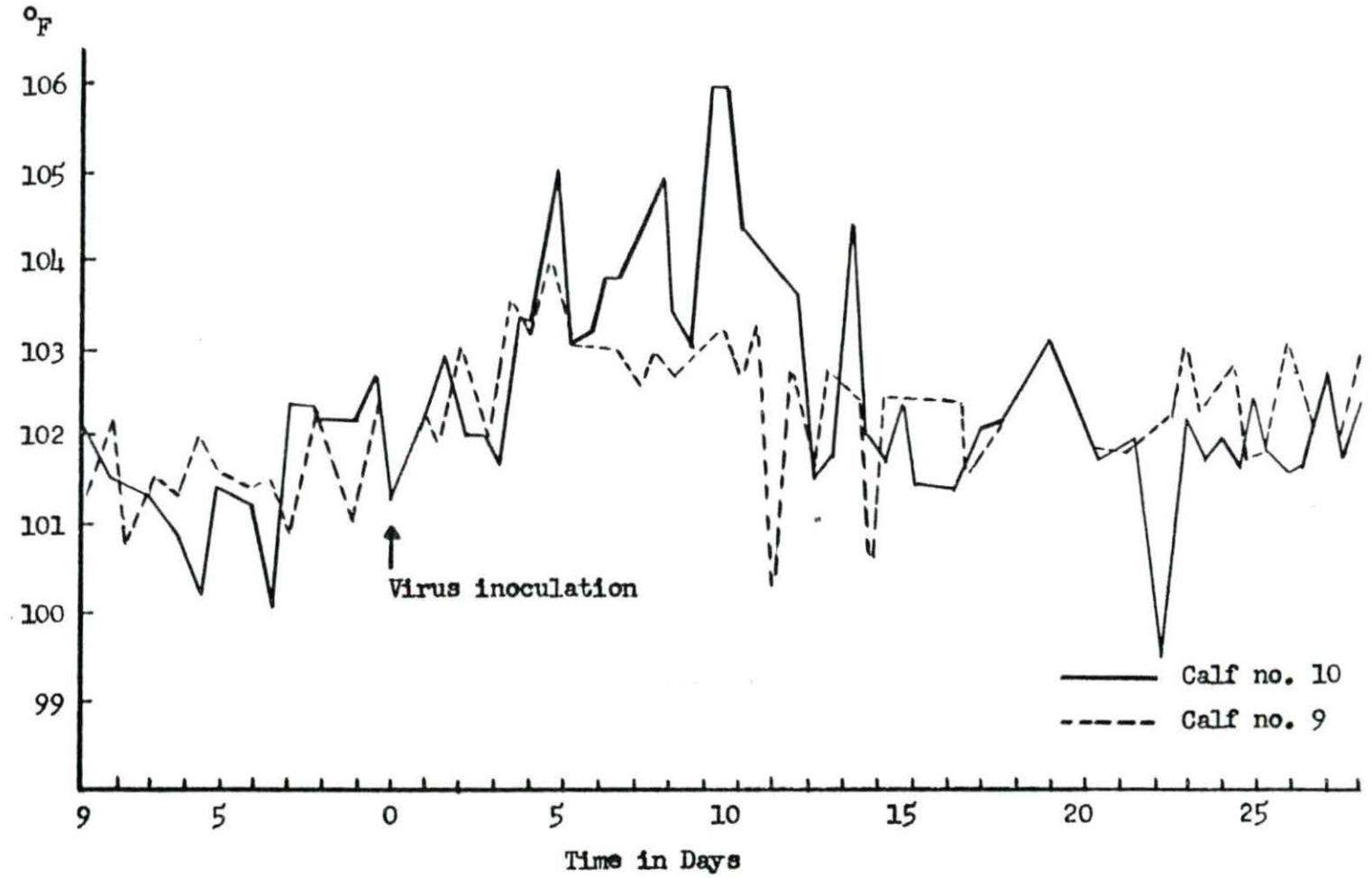
Figure 2. Morning and evening body temperatures of pigs no. 3 and no. 8 which were maintained as uninoculated controls.


°F
106
105
104
103
102
101
100
99
9
5
0
5
10
15
20
25
Time in Days
Control pig no. 8
Control pig no. 3

temperatures remained within the normal range (Figure 2). Pig no. 3 was observed for the entire 31 day period of the experiment and pig no. 8 was observed for 17 days before it was killed and necropsied.

Both calves that were inoculated to prove the pathogenicity of the IBR-Fetus strain of virus developed clinical manifestations of an acute infectious bovine rhinotracheitis infection. Calf no. 9 developed anorexia 24 hours after inoculation and its appetite remained poor for eight days. A slight mucous nasal discharge developed in the right nostril on day no. 1 and by the fourth day the nasal discharge was very pronounced, bilateral and mucopurulent. The nasal discharge persisted until the twelfth day. Erosions appeared on the nasal mucosa and on the external nares on the fifth post-inoculation day. These erosions had disappeared by day no. 12. No erosions were visible in the mouth. Dyspnea was present on days no. 7 and 8 and auscultation of the lungs revealed mild moist rales. A slight serous discharge of the left eye and a mucous discharge of the right eye appeared on the third day. Hyperemia and swelling of the conjunctiva developed in the right eye and the ocular discharge became purulent. By the tenth post-inoculation day the ocular discharge had subsided and the eyes appeared grossly normal except for a slight hyperemia of the conjunctiva of the right eye. The body temperature rose to 103°F. at 48 hours, returned to normal at 60 hours and then remained above 103°F. from the third until the eleventh post-inoculation day with a maximum rise to 104°F. on the evening of the fourth day (Figure 3).

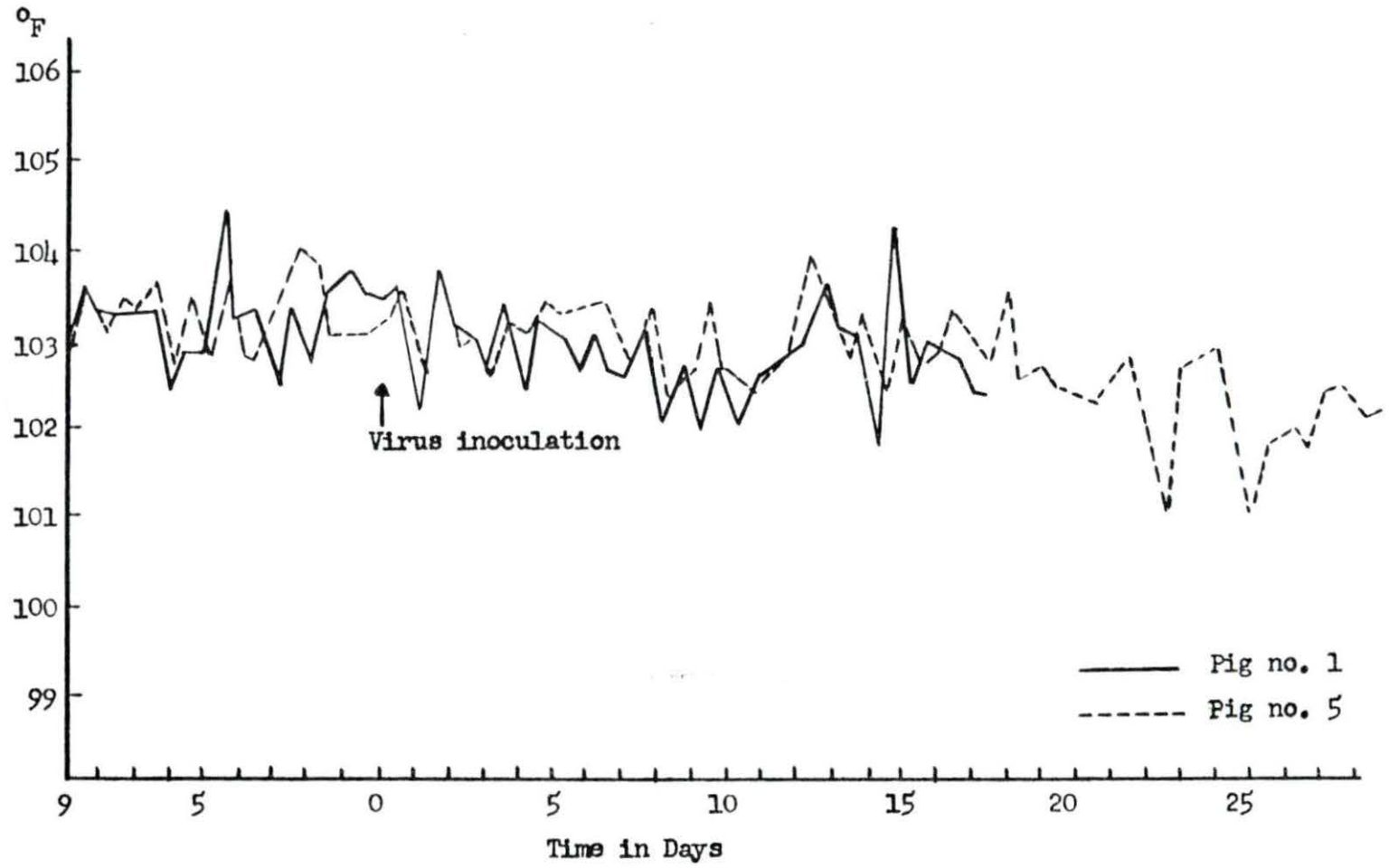
Figure 3. Morning and evening body temperatures of calves nos. 9 and 10 which were inoculated as controls for the pathogenicity of the IBR-Fetus virus.


°F
106
105
104
103
102
101
100
99
Virus inoculation
Calf no. 10
Calf no. 9
9
5
0
5
10
15
20
25
Time in Days

A slight mucous discharge from both nostrils developed in calf no. 10 on the day following inoculation. By the fifth post-inoculation day there was a moderate amount of mucopurulent discharge which decreased to only a slight amount by day no. 8, but which persisted for six more days. The nasal mucosa was congested and erosions appeared on the fifth day. By the tenth day the nasal hyperemia had subsided and the erosions had healed. Three days after inoculation a mucous discharge developed in the right eye and the serous secretion was increased in the left eye. The conjunctiva of the right eye developed a marked hyperemia and the discharge became purulent in character. The hyperemia and exudation had subsided from both eyes by the tenth day, however a slight mucopurulent discharge continued in the right eye for another week. From the fifth to the eighth post-inoculation day the calf exhibited lethargy and depression. The appetite was markedly depressed on the second day and it remained poor for eleven days. The body temperature was 103°F. on the first day, then rose to 105°F. and remained above 103°F. until the twelfth day (Figure 3).

Pig no. 1 which received the intranasal inoculation of the IBR-Fetus virus remained clinically normal throughout the period of observation. The body temperature remained in the normal range except for a rise to 104.2°F. on the evening of the 14th day of the post-inoculation period (Figure 4). Pig no. 1 was destroyed on the 18th day and a necropsy was performed.

Figure 4. Morning and evening body temperatures of pigs no. 1 and no. 5 which received the IBR-Fetus virus by intranasal inoculation.


°F
106
105
104
103
102
101
100
99
Virus inoculation
Pig no. 1
Pig no. 5
9
5
0
5
10
15
20
25
Time in Days

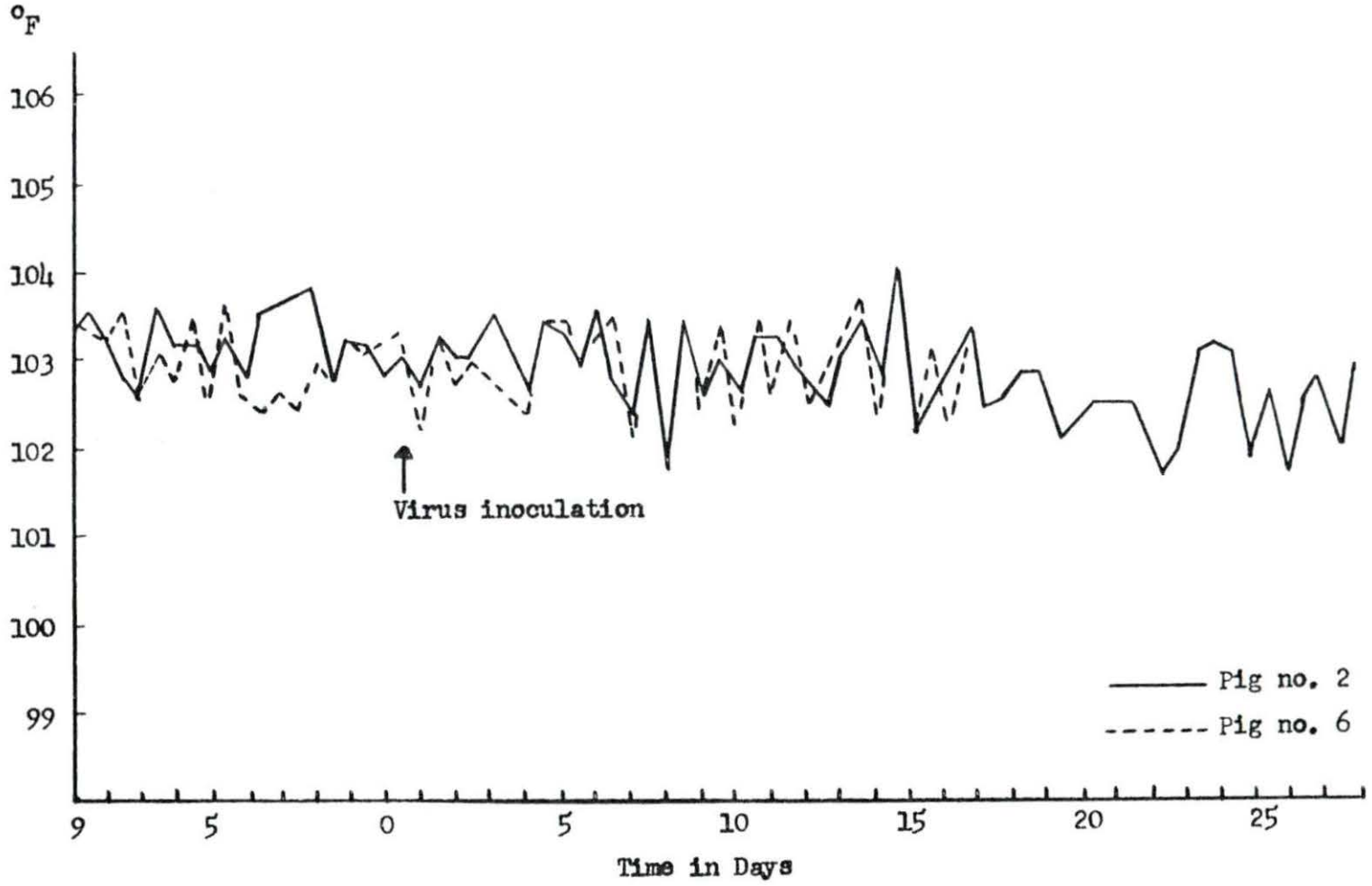
Pig no. 5 which also received the intranasal inoculation, developed hyperemia and swelling of the conjunctiva of both eyes on the fifth day after inoculation and again on the 14th day. The body temperature remained within normal limits (Figure 4). The pig was destroyed and necropsied after 31 days.

Pig no. 2 received the virus by the intravenous route. This pig was clinically normal throughout the period except for anorexia on the 14th day. The body temperature remained within the normal limits except on the evening of the 14th day when it was 104°F. (Figure 5). The pig was observed for the 31-day period of the experiment and was not destroyed.

Pig no. 6 also received the intravenous inoculation of the virus. A mucopurulent nasal discharge was present on the 13th and 14th days. The body temperature was 104°F. on the evening of the 14th day (Figure 5). No other clinical signs were observed over the 18-day period of observation. A post mortem examination was conducted at the end of this period.

Pigs no. 4 and no. 7 which received the virus by the intracerebral and genital routes exhibited evidence of central nervous system involvement. Twelve hours after the inoculation pig no. 4 was hypersensitive to stimulation. The pig did not eat and was lethargic and depressed, preferring to lie down. There was a bilateral mucopurulent nasal discharge and a yellowish mucous exudate in the prepuce. On the second day the appetite was improving and the pig was more alert and less sensitive to stimuli. The rhinitis had subsided but the posthitis was still evident. The appetite was good on the third day but an increased thirst was evident in the evening. On day no. 4 the appetite remained fairly good. The bilateral

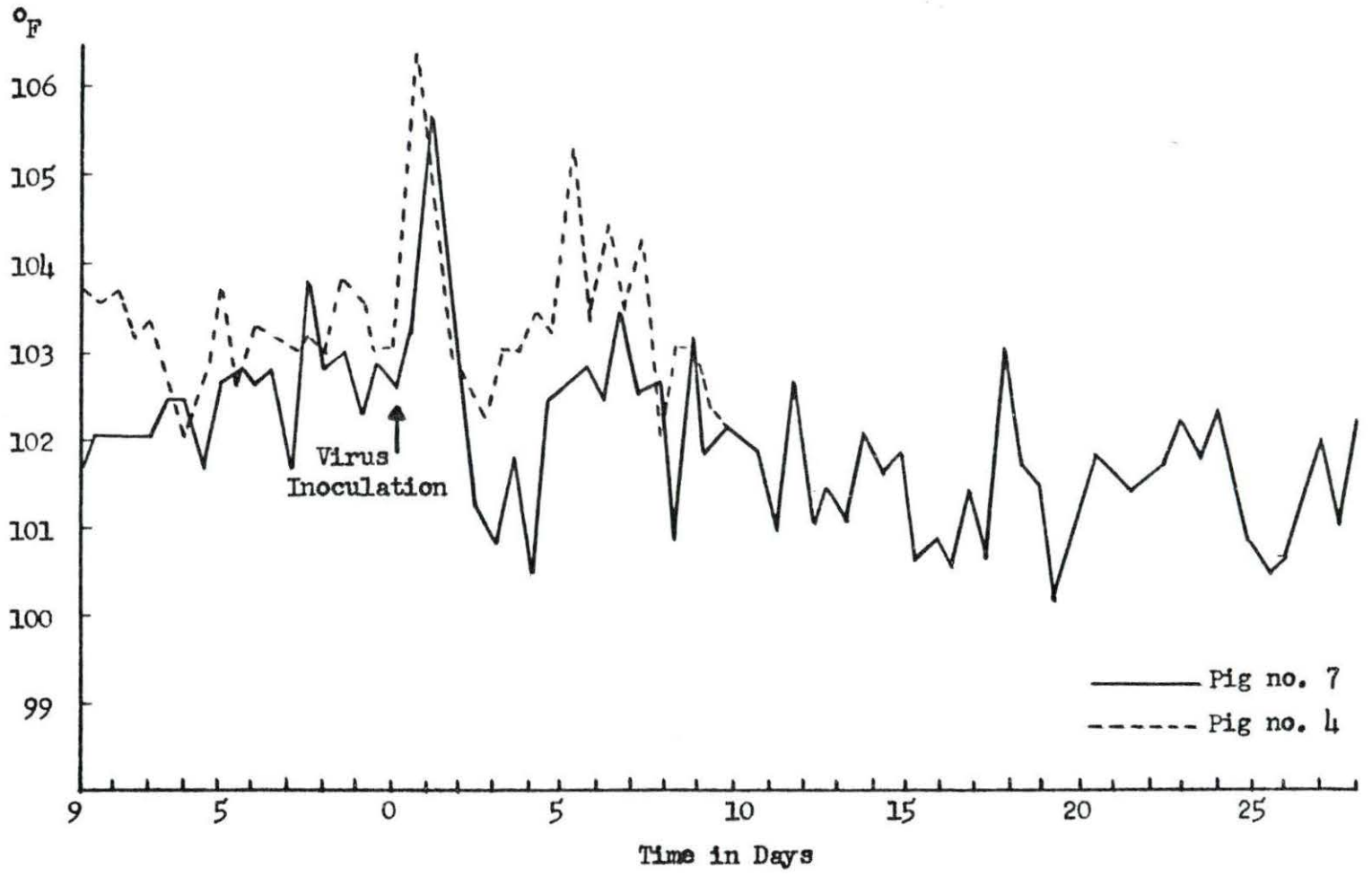
Figure 5. Morning and evening body temperatures of pigs no. 2 and no. 6 which received the IBR-Fetus virus by intravenous inoculation.


°F
106
105
104
103
102
101
100
99
Virus inoculation
Pig no. 2
Pig no. 6
9
5
0
5
10
15
20
25
Time in Days

mucopurulent nasal discharge became pronounced. The nasal mucosa was congested and it bled readily when the nasal swabs were inserted. The pig was alert and active on the fifth day and the appetite was good. However, there were signs of hyperpnea and posterior incoordination was evident. On day no. 6 the respiration rate was still rapid. Posterior incoordination was more pronounced and the pig had a tendency to "knuckle" over at the fetlock joints of the hind legs. The tail was frequently held arched over the back. The rectum appeared to be very relaxed and flaccid and the animal tended to defecate small amounts of feces frequently. The posterior incoordination had subsided by day no. 8. The pig appeared to be clinically normal when it was destroyed and necropsied on the eleventh day following inoculation. The body temperature rose to 106.5°F. twelve hours after inoculation but returned to the normal range after 36 hours. From the fourth through the eighth day the body temperature remained above 103°F. reaching a maximum of 105.2°F. on day no. 5 (Figure 6).

Twelve hours after the intracerebral inoculation pig no. 7 was very lethargic and depressed but was hypersensitive to physical stimulation. The pig did not eat and its temperature was 105.8°F. (Figure 6). On the second day a slight vaginal discharge was evident and the body temperature had returned to normal. The pig was eating well on the morning of the third day but by evening it would not eat and vomiting was observed. The pig showed posterior incoordination and had a tendency to circle when moving with its head inclined towards the right. Twenty-four hours later, on the fourth day, it was improved and the appetite was better.

Figure 6. Morning and evening body temperatures of pigs no. 4 and no. 7 which received the IBR-Fetus virus by intracerebral and genital inoculations.


°F
106
105
104
103
102
101
100
99
Virus
Inoculation
Pig no. 7
Pig no. 4
9
5
0
5
10
15
20
25
Time in Days

On the fifth day the pig appeared normal and was eating well. The pig was observed for the 31 days of the experiment and was not destroyed.

Hematology

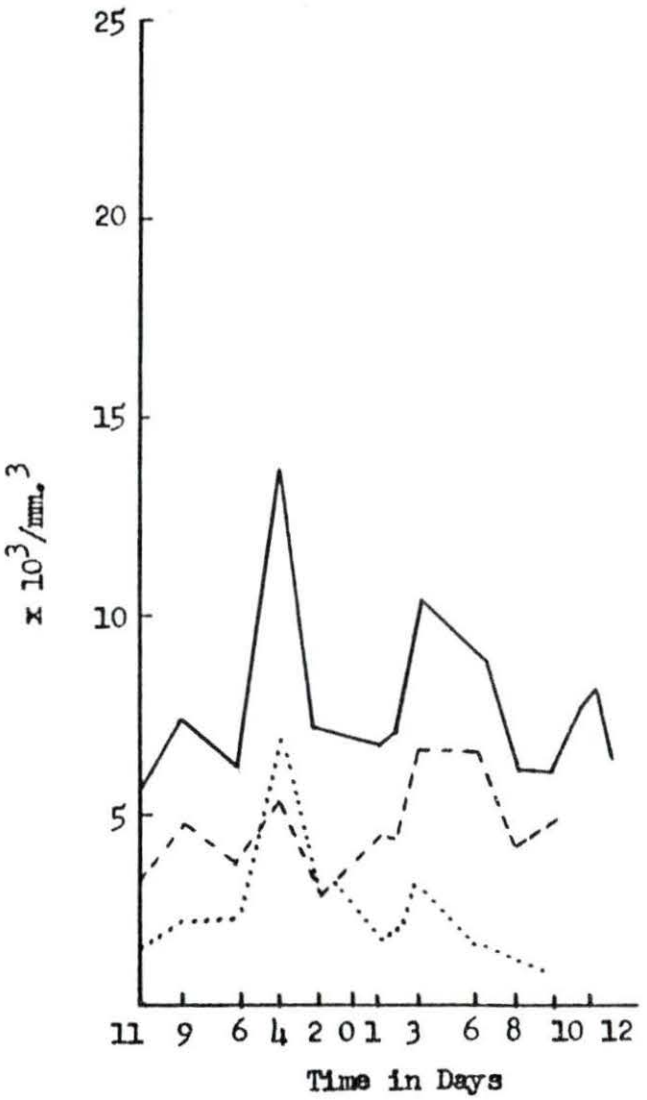
Calf no. 9 had a brief period of leucocytosis accompanied by a marked neutrophilia during the pre-inoculation period. Following inoculation of the virus the blood parameters remained fairly stable until an increased leucocyte count was evident on day no. 3. Both neutrophils and lymphocytes were increased. All three of the blood parameters had decreased by the eighth post-inoculation day (Figure 7). Calf no. 10 also had a period of leucocytosis early in the pre-inoculation period. On the second day after inoculation there was a marked decrease in the total WBC count, lymphocyte count and neutrophil count (Figure 8).

The total leucocyte count in the control pigs no. 3 and no. 8 ranged between 7100 and 18,000 (Figures 9 & 10). The lymphocyte count paralleled the total leucocyte count while there was a gradual decrease in the neutrophils during the 25 days of the pre-inoculation and post-inoculation periods. This resulted in an increase in the percentage of lymphocytes as well as an increased number of lymphocytes.

There was a precipitous drop in the three blood parameters in pig no. 5, 48 hours following the intranasal inoculation (Figure 12). In pig no. 1 the decrease in the total leucocyte count and the lymphocyte count at 48 hours post-inoculation was not as marked as in pig no. 5 and a slight neutrophilia was present (Figure 11). In both pigs the

Figure 7. Hemogram of calf no. 9 which was inoculated as a control for the pathogenicity of the IBR-Fetus virus.

Figure 8. Hemogram of calf no. 10 which was inoculated as a control for the pathogenicity of the IBR-Fetus virus.


25
20
15
10
5
x 10³/mm.³
11 9 6 4 2 0 1 3 6 8 10 12
Time in Days

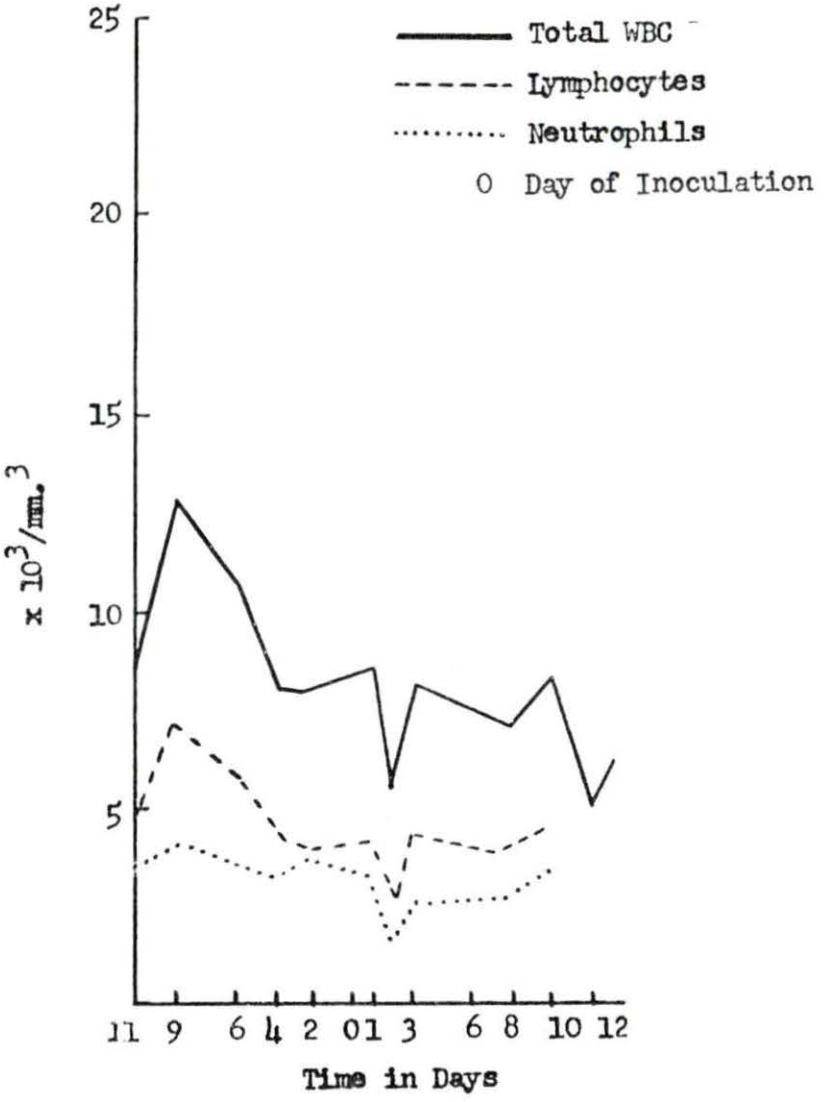
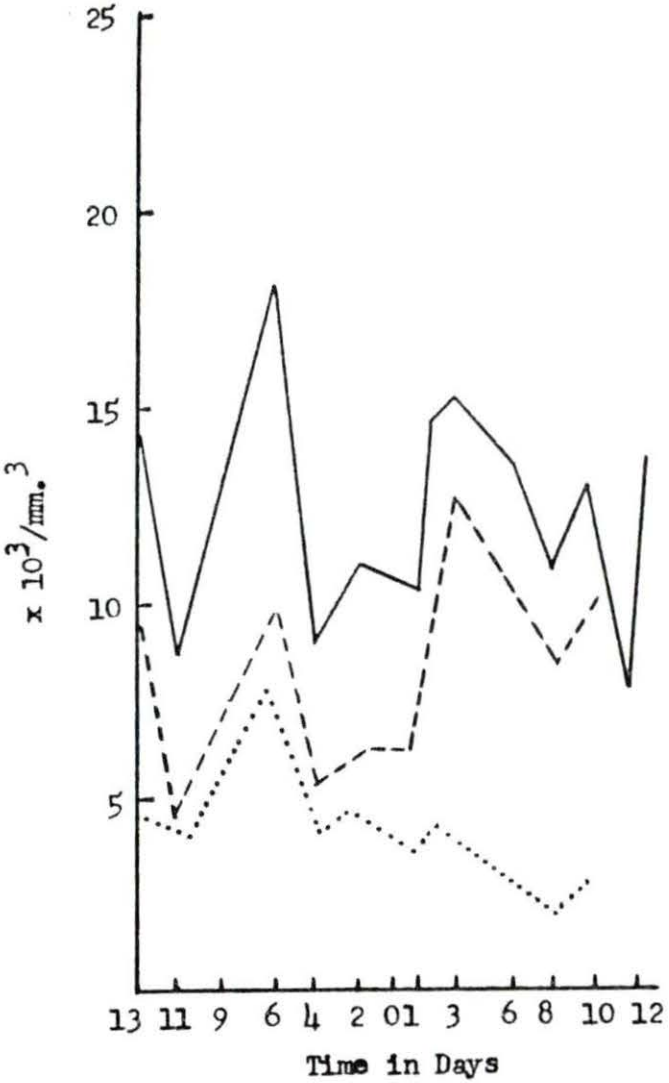

Total WBC
Lymphocytes
Neutrophils
0 Day of Inoculation
25
20
15
10
5
x 10³/mm.³
11 9 6 4 2 0 1 3 6 8 10 12
Time in Days

Figure 9. Hemogram of pig no. 3 which was maintained as an uninoculated control.

Figure 10. Hemogram of pig no. 8 which was maintained as an uninoculated control.


25
20
15
10
5
x 10³/mm.³
13 11 9 6 4 2 0 1 3 6 8 10 12
Time in Days

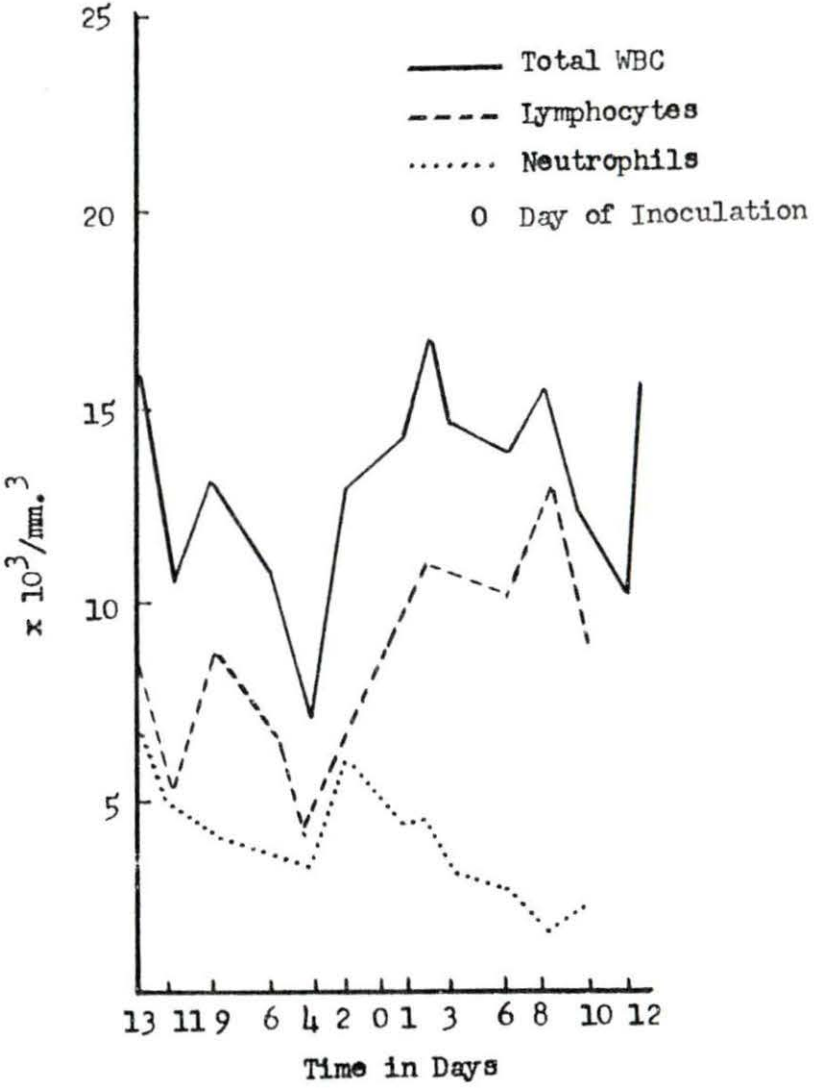
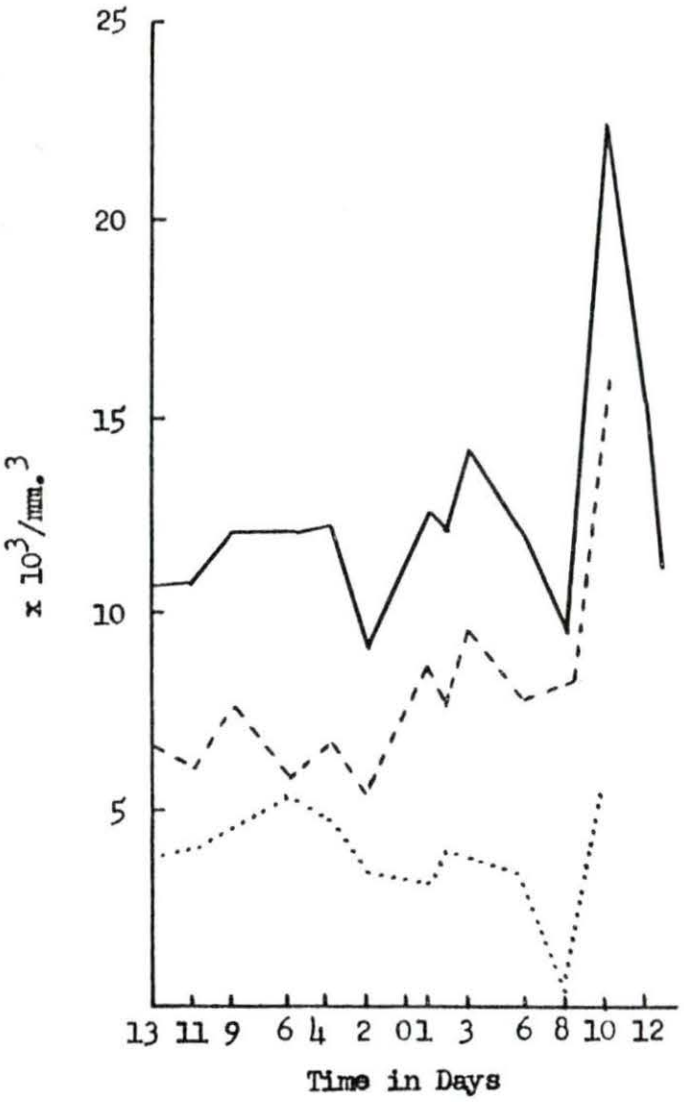
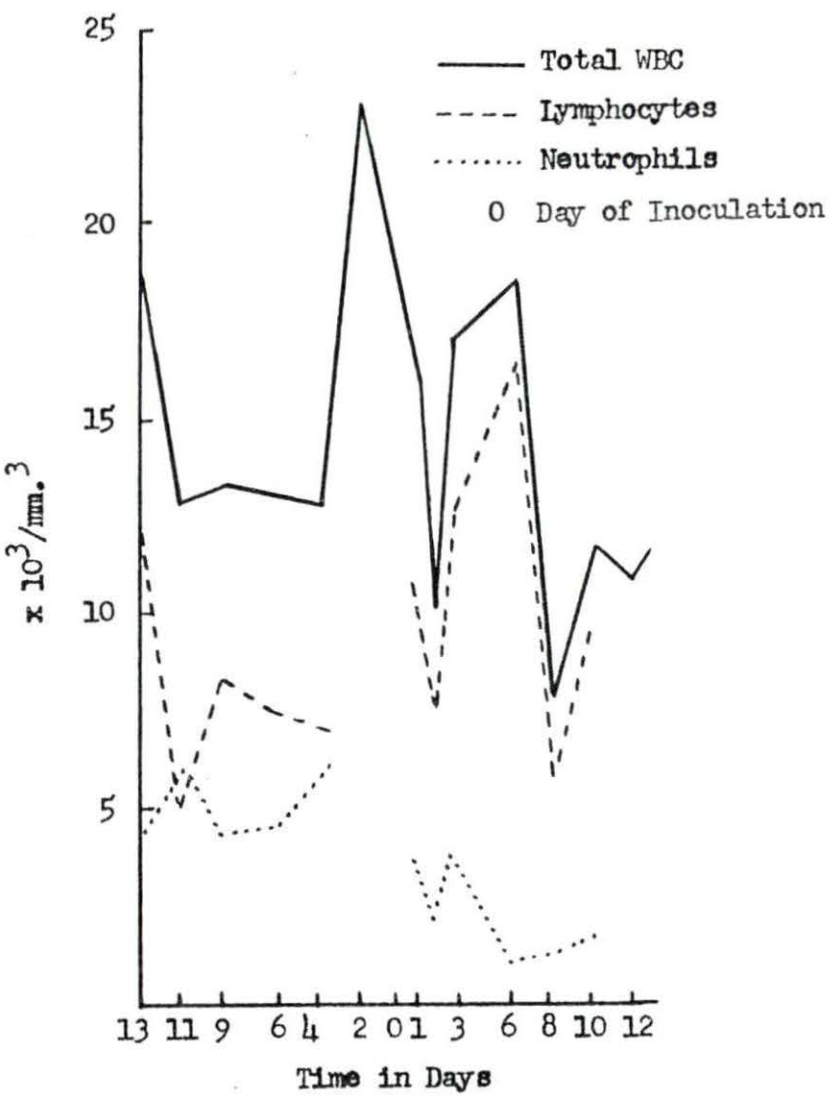

Total WBC
Lymphocytes
Neutrophils
0 Day of Inoculation
25
20
15
10
5
x 10³/mm.³
13 11 9 6 4 2 0 1 3 6 8 10 12
Time in Days

Figure 11. Hemogram of pig no. 1 which received the IBR-Fetus virus by intranasal inoculation.

Figure 12. Hemogram of pig no. 5 which received the IBR-Fetus virus by intranasal inoculation.


25
20
15
10
5
x 10³/mm.³
13 11 9 6 4 2 0 1 3 6 8 10 12
Time in Days


Total WBC
Lymphocytes
Neutrophils
0 Day of Inoculation
25
20
15
10
5
x 10³/mm.³
13 11 9 6 4 2 0 1 3 6 8 10 12
Time in Days

three values increased until the eighth day after infection when all of the counts were decreased except that the lymphocyte count in pig no. 1 increased slightly. The neutrophil counts in both pigs gradually decreased over the 25-day period.

There was a diphasic depression of total leucocyte numbers, lymphocytes and neutrophils on the second and eighth days after intravenous inoculation in pig no. 2 (Figure 13). The neutrophils and the total leucocyte numbers were decreased twelve hours after inoculation in pig no. 6. On the eighth day there was a second decrease in the total leucocyte count and in the lymphocyte count. The neutrophils gradually decreased over the 25 day period (Figure 14).

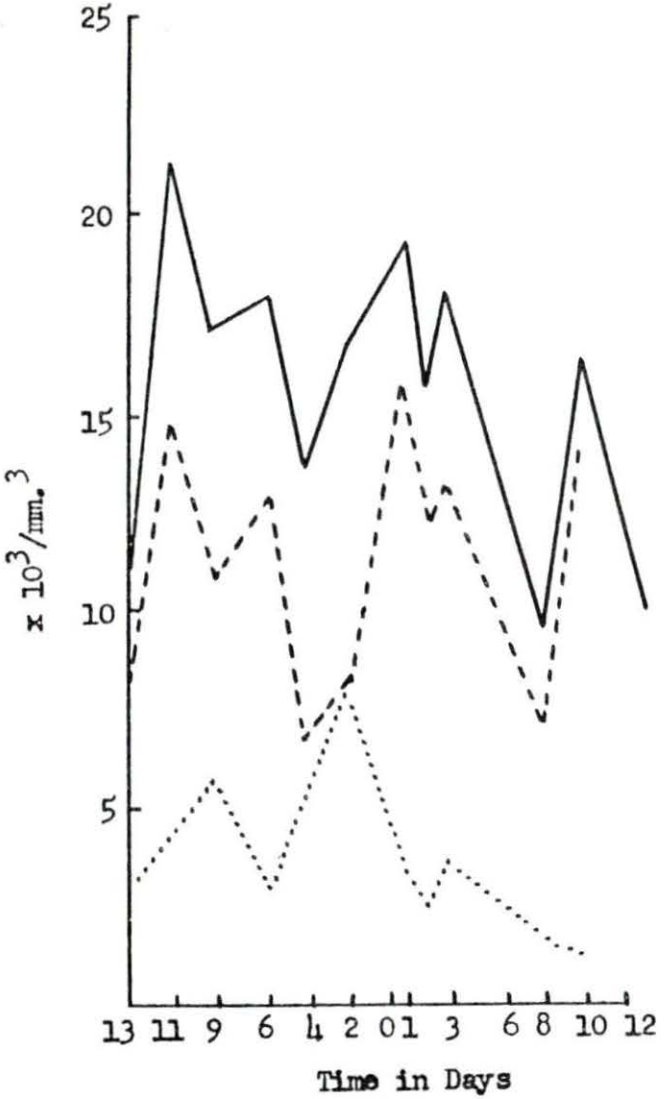
In pig no. 4 there was a marked decrease in neutrophil, lymphocyte and total leucocyte numbers 48 hours after the intracerebral inoculation of virus (Figure 15). A leucocytosis followed with a neutrophilia and a stable lymphocyte count. On the eighth post-inoculation day the neutrophil and total WBC counts were lowered but a lymphocytosis was present. In pig no. 7 there were sharp drops in the three parameters on the second, sixth and tenth post-inoculation days with sharp increases in the counts in between (Figure 16).

Necropsy findings

Post mortem examination of the control pigs after 17 days revealed no lesions in pig no. 8 either on gross examination or microscopic examination. Pig no. 3 was examined after 31 days and two foci of

Figure 13. Hemogram of pig no. 2 which received the IBR-Fetus virus by intravenous inoculation.

Figure 14. Hemogram of pig no. 6 which received the IBR-Fetus virus by intravenous inoculation.


25
20
15
10
5
x 10³/mm.³
13 11 9 6 4 2 0 1 3 6 8 10 12
Time in Days

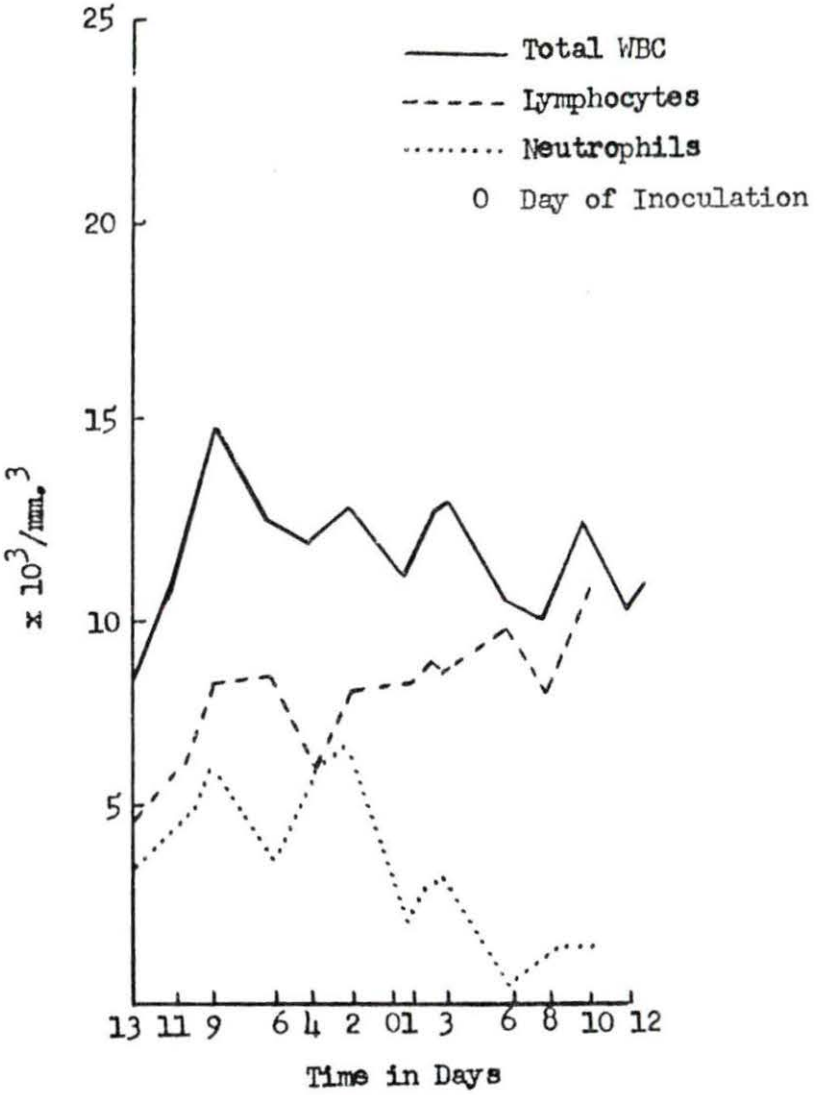
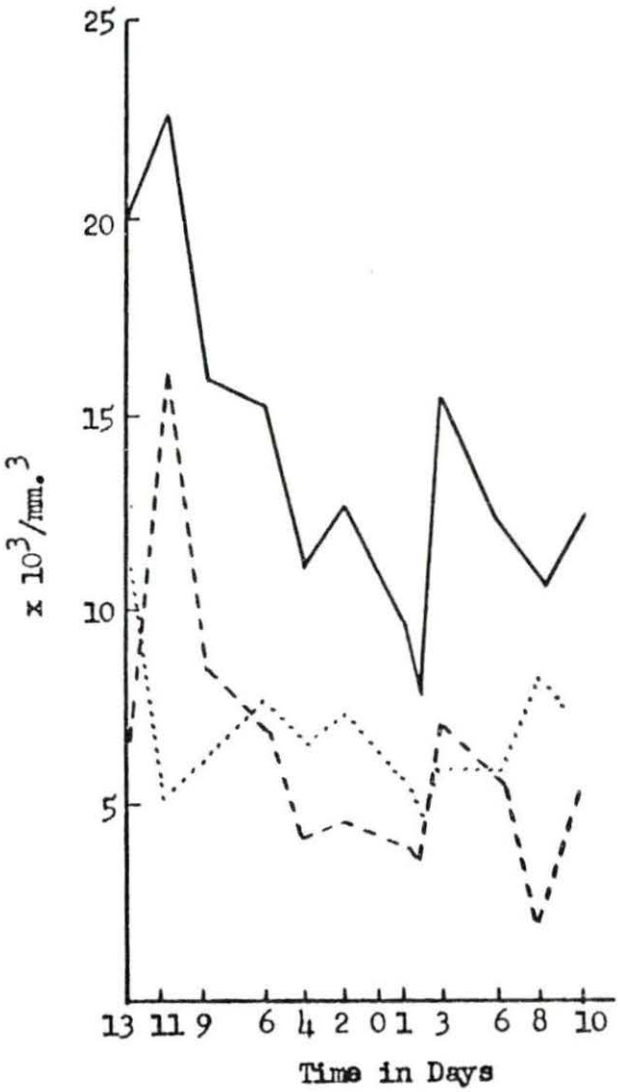
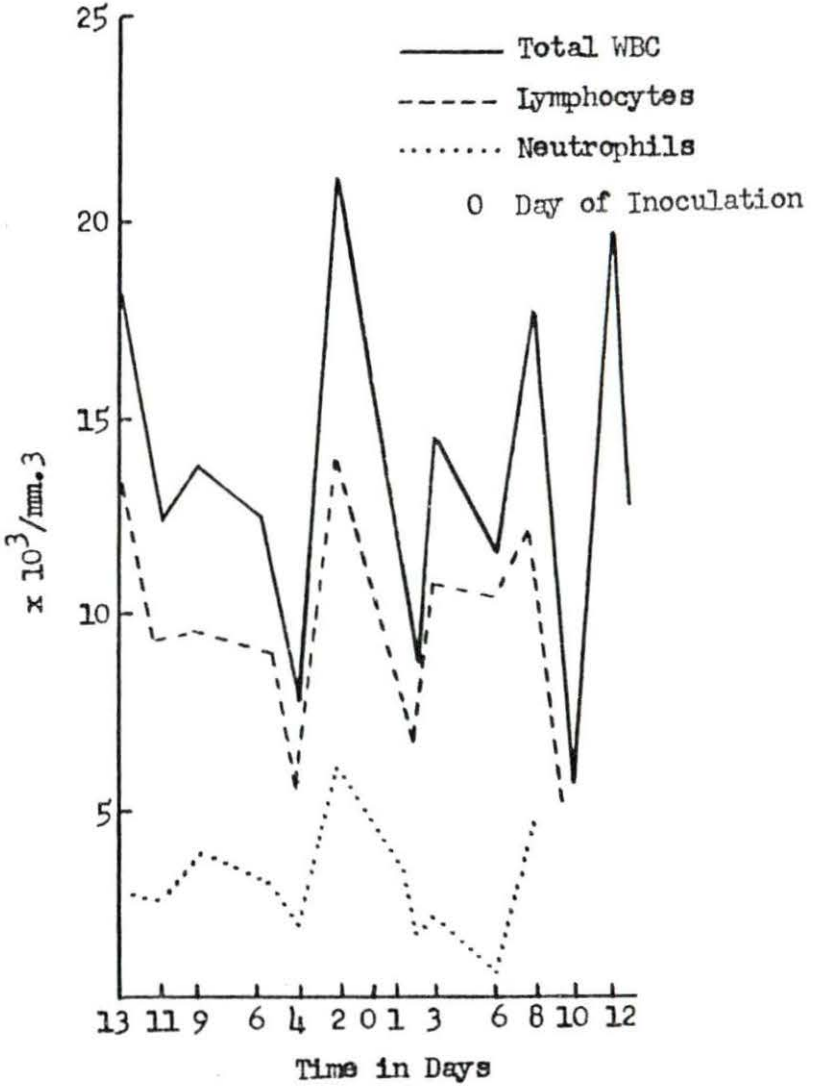

Total WBC
Lymphocytes
Neutrophils
0 Day of Inoculation
25
20
15
10
5
x 10³/mm.³
13 11 9 6 4 2 0 1 3 6 8 10 12
Time in Days

Figure 15. Hemogram of pig no. 4 which received the IBR-Fetus virus by intracerebral and intraprepputial inoculation.

Figure 16. Hemogram of pig no. 7 which received the IBR-Fetus virus by intracerebral and intravaginal inoculation.


25
20
15
10
5
x 10³/mm.³
13 11 9 6 4 2 0 1 3 6 8 10
Time in Days


Total WBC
Lymphocytes
Neutrophils
0 Day of Inoculation
25
20
15
10
5
x 10³/mm.³
13 11 9 6 4 2 0 1 3 6 8 10 12
Time in Days

interstitial pneumonia were observed in the right cardiac lobe of the lung. Microscopic examination of the brain revealed perivascular cuffing of one vessel in the medulla.

Congestion of the lungs was the only observable lesion in pig no. 1 which had received the IBR-Fetus virus intranasally 18 days previously. Pig no. 5 which was necropsied 31 days after the intranasal inoculation had some areas of interstitial pneumonia with perivascular and peribronchiolar localization of lesions. Some lymphocytes were evident in these areas. A small cyst was present in the right kidney.

After 18 days pig no. 6 from the pair receiving the intravenous inoculation showed congestion of the lungs. A small cyst was found in the right kidney. The other pig, no. 2 was not necropsied.

Pig no. 4 was necropsied eleven days after receiving the intracerebral inoculation. No gross lesions were observed. However, on microscopic examination a mild perivascular cuffing with lymphocytes and monocytes was noted in the medulla, pons and left cortes of the brain.

One year following intracerebral inoculation of IBR-Fetus virus pig no. 7 was necropsied. No gross lesions were observed. On microscopic examination a peribronchiolar interstitial pneumonia was observed with numerous eosinophils present. The bronchial lymph node also contained numerous eosinophils. There was a mild mononuclear infiltrate in the meninges in the ventral sulcus in the midbrain. A mild mononuclear infiltrate was observed in the area of the pons around one vessel in the ventral meninges.

Serology

The calves and pigs used in this experiment did not have IBR antibodies in the pre-inoculation serum samples. Both of the control pigs, no. 3 and no. 8 remained negative for IBR antibodies throughout the experiment.

Both calves developed significant IBR antibody titers. By the fourteenth post-inoculation day calf no. 9 developed a titer of 1:256 and calf no. 10 developed a titer of 1:1024 (Figure 17).

The intranasally inoculated pigs, no. 1 and no. 5 had a delayed antibody titer rise (Figure 18). Both pigs were negative for IBR antibodies in the serum sample obtained 7 days following inoculation. By the end of the second week pig no. 1 had a titer of 1:16 and pig no. 5 had a titer of 1:32. Pig no. 1 was necropsied after the second week. The antibody titer in pig no. 5 dropped to 1:8 by the third week and it remained at this level at 31 days post-inoculation.

The IBR antibody titer reached 1:32 in pig no. 6 seven days after the intravenous inoculation. When the second weekly sample was tested the titer was still 1:32. This pig was necropsied 17 days following inoculation. Pig no. 2 developed a titer of 1:64 by the seventh day following the intravenous administration of the virus. By the end of the second week the titer had risen to 1:128. Between the third and twelfth week the titer rose from 1:128 to 1:1024. One year following the inoculation of the virus the antibody titer was still 1:1024.

Figure 17. Antibody titers of calves no. 9 and no. 10 which were inoculated as controls for the pathogenicity of the IBR-Fetus virus.

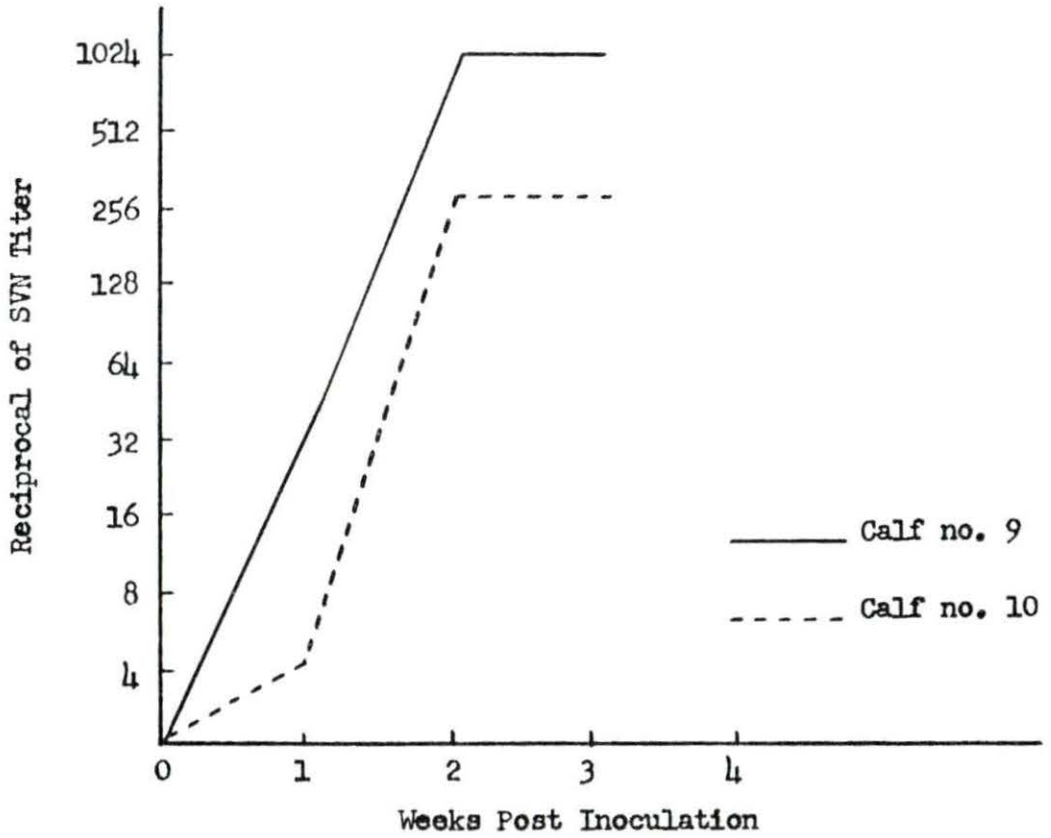
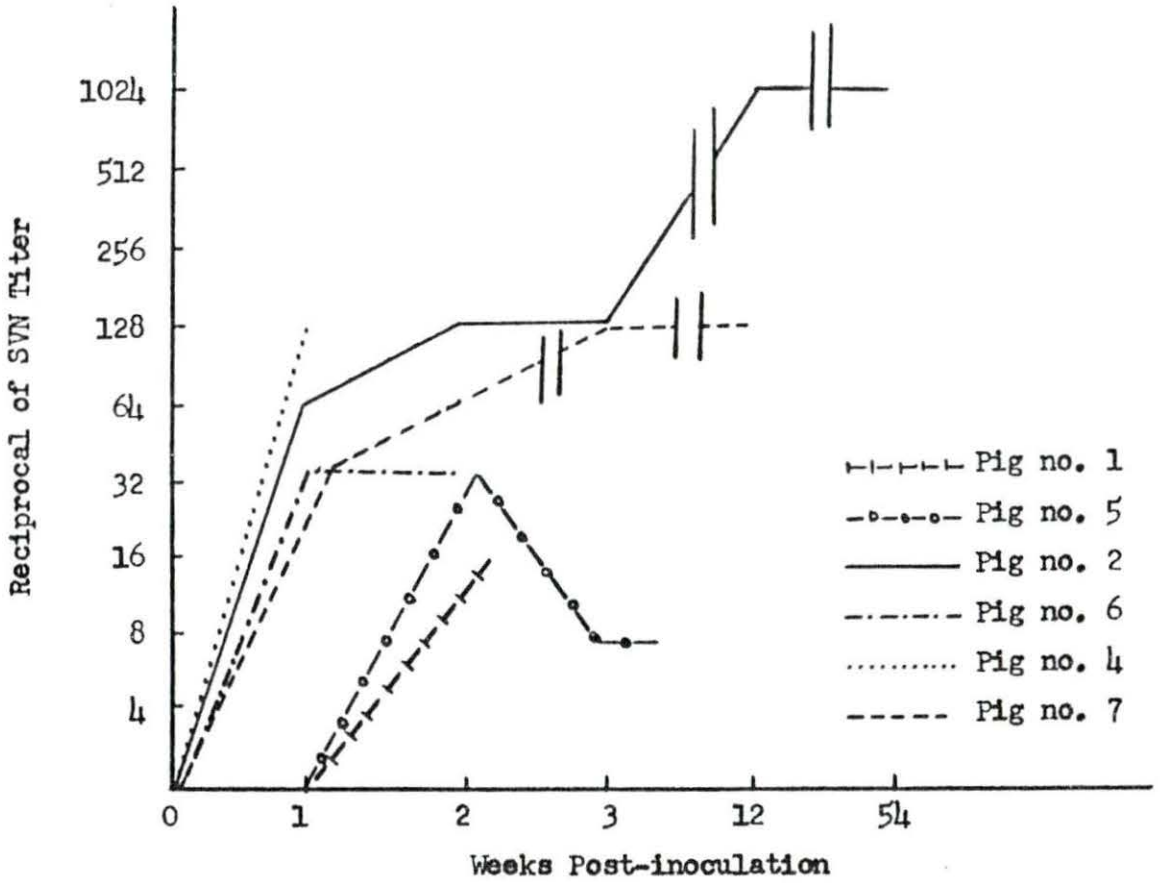

1024
512
256
128
64
32
16
8
4
Reciprocal of SVN Titer
0
1
2
3
4
Weeks Post Inoculation
Calf no. 9
Calf no. 10

Figure 18. Antibody titers of the pigs inoculated with the IBR-Fetus virus. Pigs no. 1 and no. 5 received the virus by intranasal inoculation. Pigs no. 2 and no. 6 received the virus by intravenous inoculation. Pigs no. 4 and no. 7 received the virus by intracerebral and genital inoculations.


Reciprocal of SVN Titer
1024
512
256
128
64
32
16
8
4
0
1
2
3
12
54
Weeks Post-inoculation
Pig no. 1
Pig no. 5
Pig no. 2
Pig no. 6
Pig no. 4
Pig no. 7

Pig no. 4 had an antibody titer of 1:128 on the seventh day after intracerebral and intrapreputial inoculation of virus. The pig was necropsied on the tenth day. In pig no. 7 the titer reached 1:32 after seven days, increasing to 1:64 after the second week and to 1:128 by the end of the twelfth week. The IBR titer was still 1:128 one year after the intracerebral and intravaginal inoculation.

Virus isolation

A total of 1300 samples were obtained from the eight pigs and two calves for virus isolation attempts on MDBK cell cultures.

IBR virus was regularly isolated from the nasal, conjunctival and preputial swabs from both calves from the first through the seventh post-inoculation day. After the seventh day IBR virus was isolated from the conjunctival swabs on the eighth and tenth days from calf no. 9. In calf no. 10 IBR virus was isolated from the nasal, conjunctival and preputial swabs on day no. 8 but on the ninth and tenth post-inoculation days IBR virus was recovered from only the conjunctival swabs (Table 3).

Cytopathic effect was present in the MDBK cell cultures on the first passage in all positive samples obtained from the calves except for the conjunctival sample from calf no. 9 and the conjunctival and preputial swabs fro calf no. 10 that were obtained on the eighth post-inoculation day. IBR virus was isolated from these samples on the second passage in cell cultures.

Table 3. IBR virus isolations from calves.

	Calf No. 9				Calf No. 10			
	Nasal	Conjunctival	Prepuce	Buffy Coat	Nasal	Conjunctival	Prepuce	Buffy Coat
Day								
1	+	+	+	-	+	+	+	-
2	+	+	+	-	+	+	+	-
3	+	+	+	-	+	+	+	-
4	+	+	+	-	+	+	+	-
5	+	+	+	-	+	+	+	-
6	+	+	+	-	+	+	+	-
7	+	+*	+	-	+	+*	+*	-
8	-	+	-	-	+	+	+	-
9	-	-	-	-	-	+	-	-
10	-	+	-	-	-	+	-	-
11	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-

*Isolated on second passage in cell cultures.

IBR virus was not isolated from any of the swine samples tested on MDBK cells except from the nasal swab obtained from pig no. 5 twelve hours after intranasal inoculation of the IBR virus.

Tissue specimens from the necropsied pigs were inoculated onto MDBK cell cultures. No virus isolations were made from these specimens.

Intracerebral Inoculation Experiment: Experiment No. 2

Clinical signs

Both pigs no. 13 and no. 14 developed anorexia 12 hours after the intracerebral inoculation of IBR-Fetus virus. Depression was noticed in pig no. 13 and it preferred to lie down most of the time. The pig also exhibited tremors of the muscles over the shoulders. Pig no. 14 was reluctant to move, appeared stiff, and showed some difficulty in getting up.

Both pigs were still not eating at 24 hours post-inoculation and were depressed. Incoordination was observed and they were reluctant to move. Pig no. 14 was destroyed and necropsied at 24 hours.

At 36 hours pig no. 13 showed a fair appetite. The pig was more alert but was still reluctant to move. The appetite was good and the general condition was good at 48 hours and at 72 hours following inoculation. The pig was destroyed and necropsied 72 hours after inoculation.

A rectal temperature of 107.3°F. was present in pig no. 13 at 12 hours after virus inoculation. At 24 hours the temperature was 104°F.

and by 72 hours the temperature was in the normal range. Pig no. 14 had a body temperature of 105.8°F. at 12 hours and 104.2°F. at 24 hours following inoculation (Figure 20).

Both of the pigs that received the intracerebral inoculation of the cell culture fluid were active and alert and exhibited good appetites during the period of observation. The body temperature remained within the normal range in pig no. 11 (Figure 19). Pig no. 12 had a temperature rise to 104.4°F. at 12 hours but the temperature was normal at 24 hours.

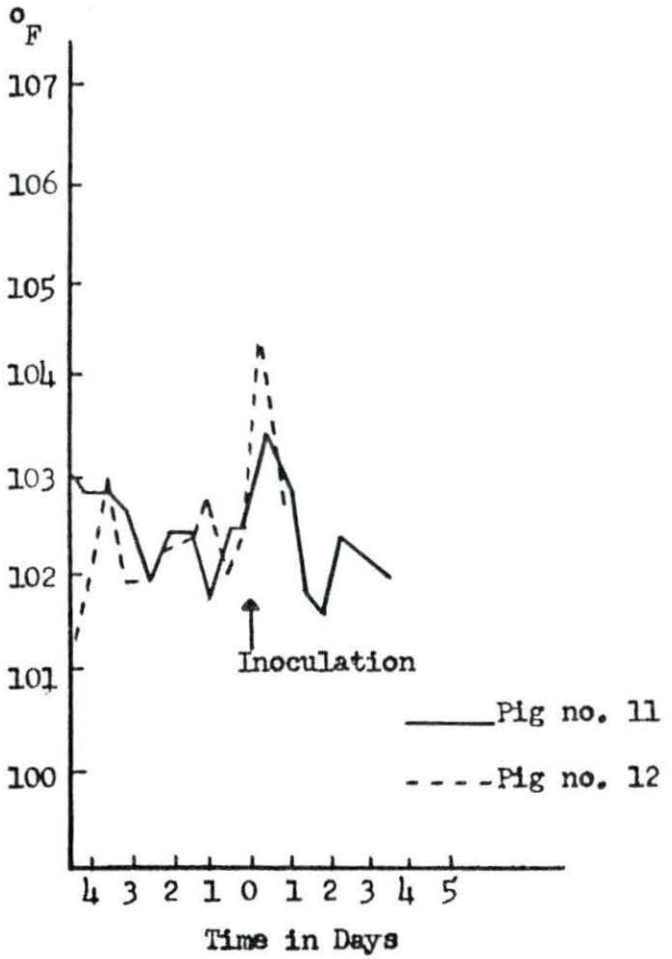
Hematology

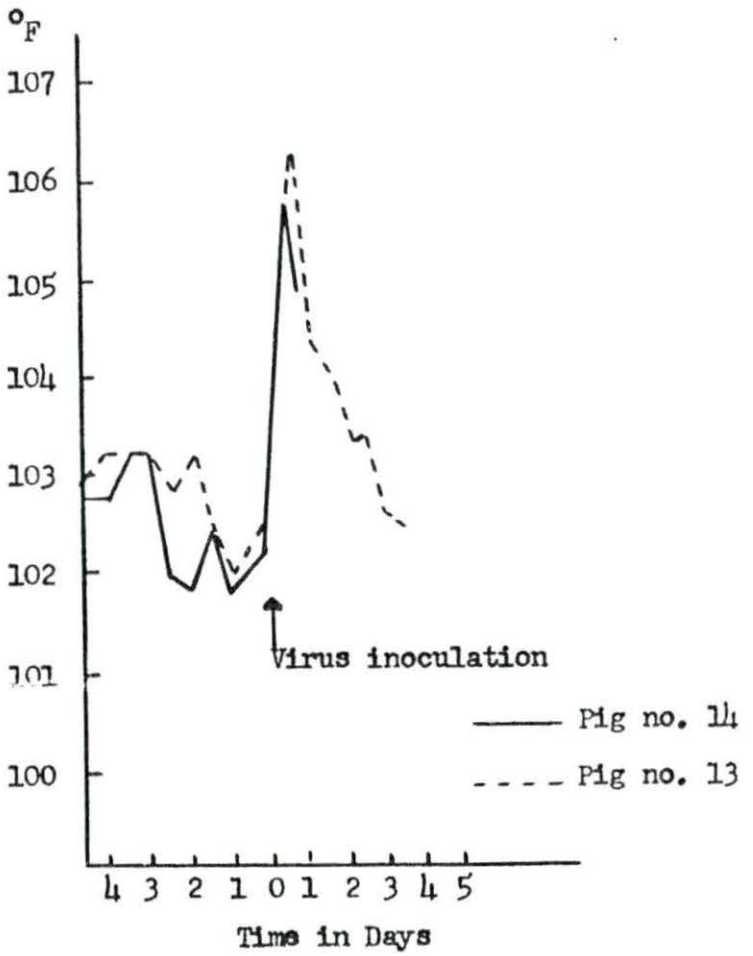
The pre-inoculation leucocyte count of pig no. 11 was 30,000. After receiving the intracerebral inoculation of the cell culture fluid there was little change in the leucocyte count or in the lymphocyte count during the 72 hour observation period. The other control pig, no. 12 had a definite leucocytosis in which the total leucocyte numbers increased from 19,600 to 33,700. The increase was primarily in the lymphocytes with an increase of 12,500 cells in 24 hours while the neutrophils increased only 2,400 cells during the same period.

The virus-infected pig no. 14 when necropsied after 24 hours, showed a decrease in the total leucocyte count with a reduction in both lymphocyte and neutrophil count. In pig no. 13 there was a leucocytosis with an initial total leucocyte count of 12,900 and a count of 17,550 after 72 hours. There was a slight decrease in the neutrophils at this time. The increase in the total leucocyte count was due primarily to a lympho-

Figure 19. Morning and evening body temperatures of pigs no. 11 and no. 12 which received intracerebral inoculations of cell culture fluid.

Figure 20. Morning and evening body temperatures of pigs no. 13 and no. 14 which received intracerebral inoculations of IBR-Fetus virus.


°F
107
106
105
104
103
102
101
100
Inoculation
Pig no. 11
Pig no. 12
4 3 2 1 0 1 2 3 4 5
Time in Days


°F
107
106
105
104
103
102
101
100
Virus inoculation
Pig no. 14
Pig no. 13
4 3 2 1 0 1 2 3 4 5
Time in Days

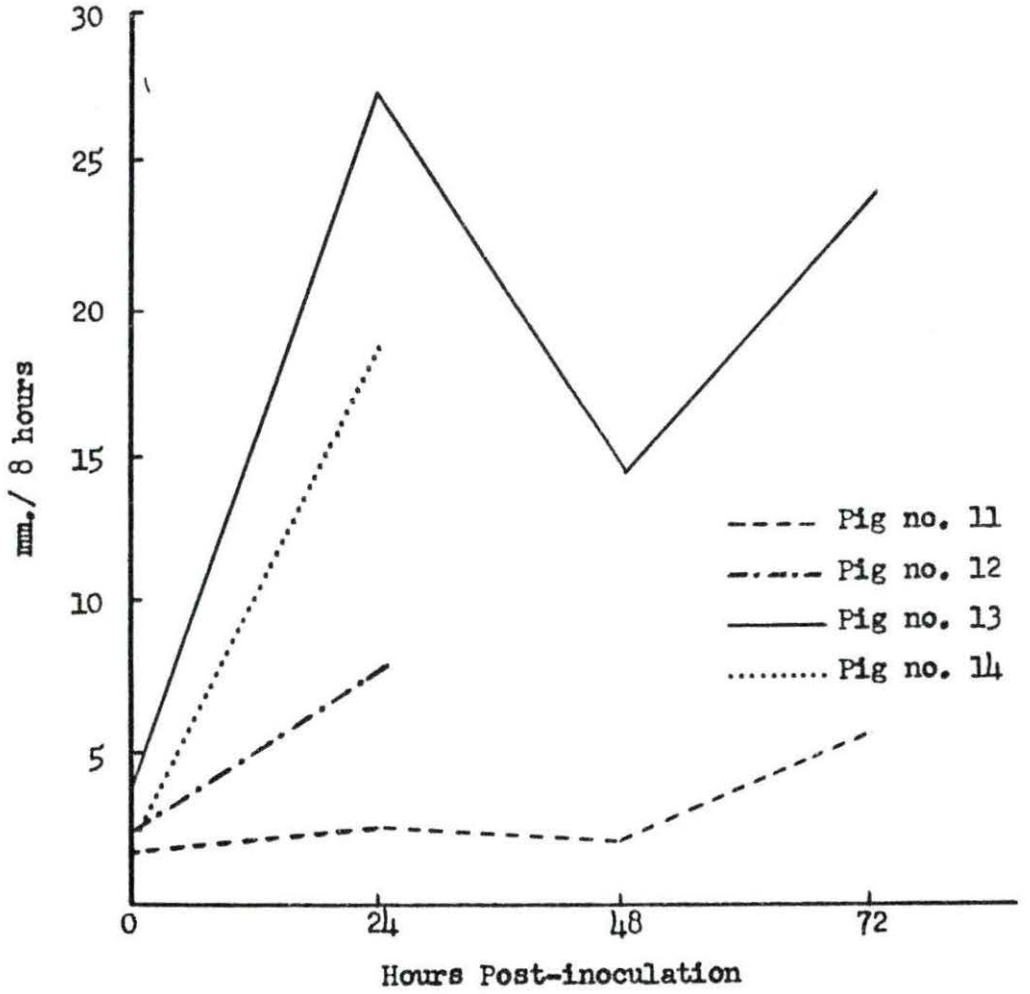
cytosis with a 50% increase in the lymphocyte count over the three day period.

It was noticed in Experiment No. 1 that the cellular elements of the blood tended to settle very rapidly in the blood samples from the pigs receiving the intracerebral inoculation of virus. The erythrocyte sedimentation rate (ESR) during an eight hour period was determined on the daily blood samples from both the virus-infected pigs and the cell-culture-inoculated pigs. A marked difference in the ESR was observed between the two groups. Pigs no. 11 and 12, the cell culture inoculated pigs, had very low sedimentation rates with a high reading of 8 mm. per 8 hours in pig no. 12 at 24 hours post-inoculation. Twenty-four hours after inoculation the virus-infected pigs had accelerated sedimentation rates with no. 13 showing 27 mm. per 8 hours and no. 14 being 19 mm. per 8 hours. Pig no. 13 had an ESR of 25 mm. per 8 hours on the third day after inoculation (Figure 21).

Necropsy results

With the exception of a reddish coloration of the cerebrospinal fluid there were no other gross pathological lesions observed in control pig no. 12 when examined at necropsy 24 hours after receiving the intracerebral injection of the cell culture inoculum. Microscopic examination of the brain revealed a mild meningitis with perivascular cuffing of a few blood vessels. At the site of the needle track there was focal malacia with erythrocytes and neutrophils in the area.

Figure 21. Erythrocyte sedimentation rates of the pigs in the Intracerebral Inoculation Experiment (Experiment no. 2). Pigs no. 11 and 12 were control pigs receiving intracerebral inoculations of cell culture fluid. Pigs nos. 13 and 14 received intracerebral inoculations of IBR-Fetus virus.


30
25
20
15
10
5
mm./ 8 hours
0
24
48
72
Hours Post-inoculation
Pig no. 11
Pig no. 12
Pig no. 13
Pig no. 14

In control pig no. 11 which was necropsied 72 hours after receiving the cell culture fluid there were no gross or microscopic lesions.

Post mortem examination of pig no. 14, twenty-four hours following virus inoculation, revealed a moderate non-supperative meningitis over the cerebral cortex area. Microscopic examination of the brain revealed vasculitis and perivascular cuffing in the cortex, midbrain, medulla and brainstem. The inflammatory cells were primarily of the mononuclear series. There were some neutrophils and some hemorrhage was also present.

Similar lesions were observed in the other virus infected pig, no. 13, except that the lesions appeared to be more severe. A severe non-suppurative meningitis was apparent over the cerebral cortex and midbrain areas. Vasculitis and perivascular cuffing were apparent throughout the brain and brainstem on microscopic examination. The inflammatory cells were of the mononuclear series.

Serology

Examination of pre-inoculation serum samples from each pig revealed no IBR antibodies. Post-inoculation samples were not tested.

Virus isolation

Virus isolation attempts on the tissue specimens obtained on post-mortem examination were made on MDBK cell cultures. No viruses were isolated.

Bacteriological examination

Bacteriological examination of swabs obtained from the meningeal surface and the subarachnoid space revealed no bacterial organisms in pigs 11, 13 and 14, but a Streptococcus species was isolated from the meningeal surface swab from pig no. 12.

Pregnant Sow Pathogenicity Experiment: Experiment No. 3

Clinical signs

Eight days following intravenous inoculation with IBR-Fetus virus sow no. 16 developed a bilateral serous ocular discharge which persisted for eight days. On the eleventh and twelfth days there was a bilateral nasal discharge. Also on these days there was a noticeable amount of blood present on the nasal swab.

On the sixteenth post-inoculation day sow no. 15 was irritable and reluctant to move. Breathing was heavy and anorexia was evident. On the following day the appetite had improved but the sow was reluctant to move and refused to get up. On day no. 18 the sow appeared normal.

No further signs were observed in either sow until farrowing. Sow no. 15 farrowed 59 days after receiving the intravenous inoculation of virus. Ten living pigs were born. On the following day the sow developed a diarrhea. The mammary glands were congested and firm, and the vulva was swollen. The appetite was good and the rectal temperature was 103.8°F. On the second post-parturient day the sow's appetite was poor but the sow

was more alert. The mammary glands had improved, there was no evidence of mastitis, and the sow was milking fairly well. The vulva was still swollen and there was a sero-hemorrhagic vaginal discharge. The consistency of the feces was improved but the sow's body temperature was 105.8°F. The pigs were active and all were nursing well.

On the third day following parturition there was a slight purulent vaginal discharge in sow no. 15. Her appetite was good, the feces were of normal consistency and the rectal temperature was down to 103.6°F.

Bacterial examination of vaginal swabs obtained on the second and third post-parturient days did not result in the isolation of any pathogenic organisms.

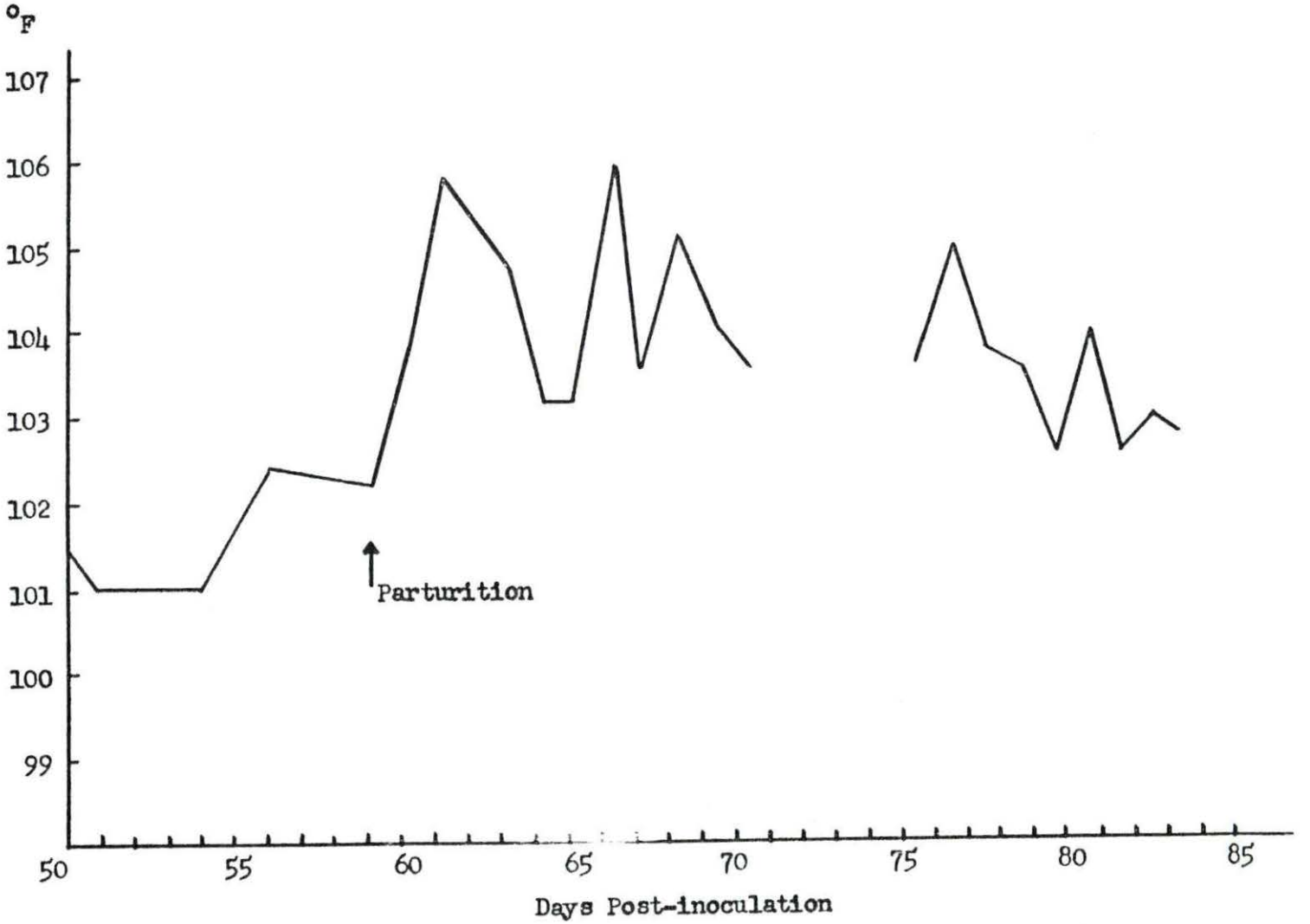
There was no vaginal discharge on the fourth post-parturient day. The sow was alert, her appetite was good and the bowels were normal. The sow's temperature was 104.8°F.

Sow no. 15 remained alert and active throughout the remainder of the experiment. Her appetite was good and her feces were normal. The piglets were healthy and grew well. However, during the 20 days following parturition the sow's temperature was elevated on several occasions (Figure 22).

Post partum day	no. 7	106°F.
	no. 9	105.2°F.
	no. 10	104°F.
	no. 12	105°F.
	no. 16	104°F.

The temperatures of two other sows housed in an adjacent pen remained normal throughout this period.

Figure 22. Daily body temperatures of sow no. 15 following parturition. IBR-Fetus virus was administered by intravenous inoculation 59 days prior to parturition.


°F
107
106
105
104
103
102
101
100
99
Parturition
50
55
60
65
70
75
80
85
Days Post-inoculation

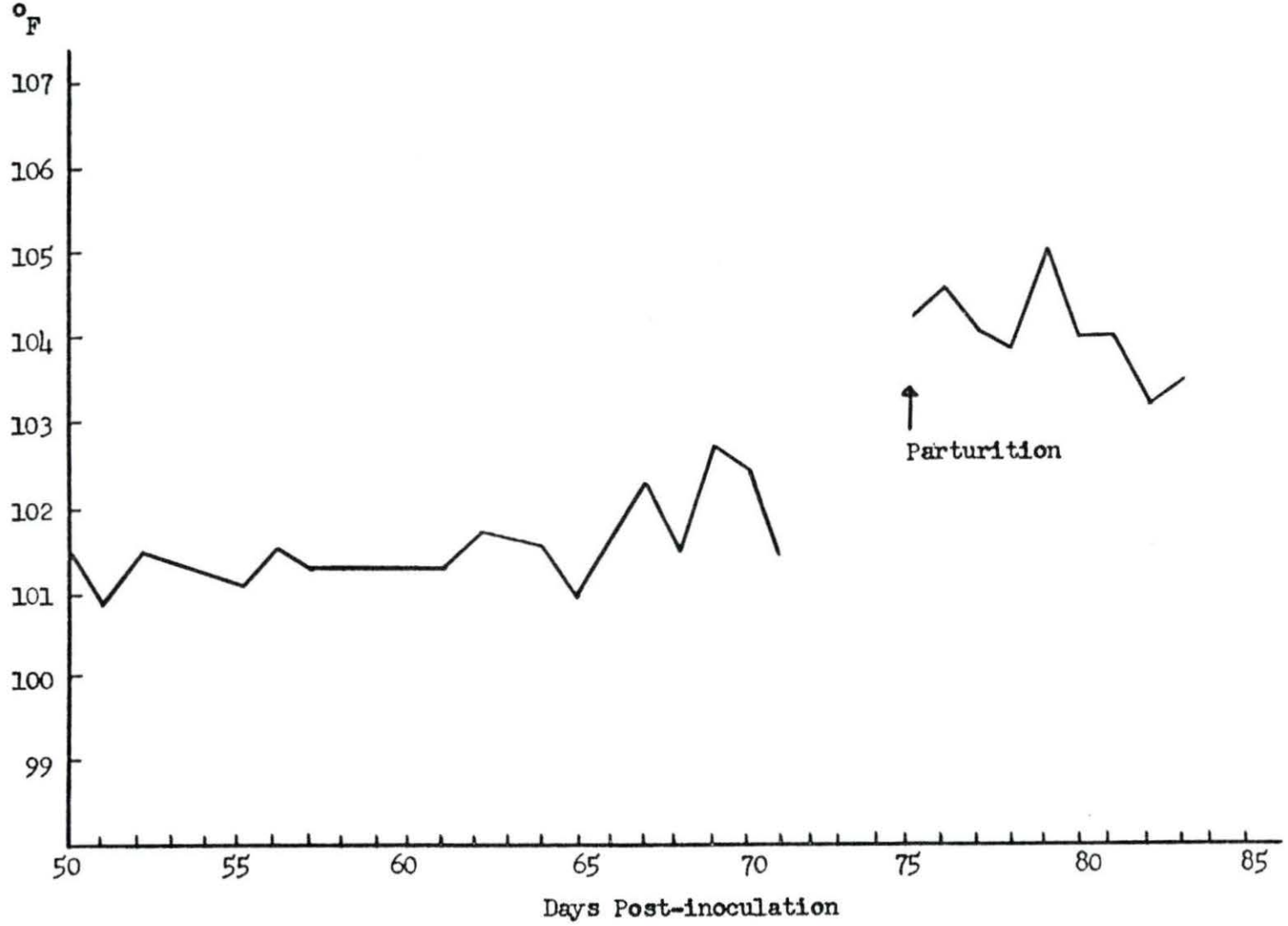
Sow no. 16 farrowed 76 days following the inoculation of the IBR-Fetus virus. Nine living pigs were born and three pigs (no. 16I, no. 16J, no. 16K) were dead but were full-term. Another dead pig was born on the following day. One of the live pigs (no. 16L) died on the day following parturition after being crushed by the sow. However, this pig had been lethargic since shortly after birth.

Forty-eight hours after parturition sow no. 16 had a slight purulent vaginal discharge. There was a laceration at the dorsal commissure of the vulva. Bacterial examination of a vaginal swab did not result in the isolation of any pathogenic organisms. The sow's appetite was good and the consistency of her feces was normal.

The sow's temperature was 104.2°F. on the day of parturition and remained above 104°F. for seven days except on the fourth post-parturient day the temperature was 103.8°F. On the fifth day the temperature was 105°F. (Figure 23). The sow was alert and active and had a good appetite throughout the observed post-parturient period.

On the sixth day following parturition rectal temperatures of the piglets were taken. Pig no. 16A had a temperature of 104°F. and pig no. 16F had 104.4°F. On the seventh post-parturient day these two pigs were lethargic and depressed. The bowel movements were normal and both pigs nursed well. The temperature of 16A was 105.8°F. and of 16F was 104°F. On the following day the pigs appeared normal and the temperatures were within the normal range. There were moist scabby areas below the eyes of five piglets (16A, 16B, 16C, 16G, 16H).

Figure 23. Daily body temperatures of sow no. 16 following parturition. IBR-Fetus virus was administered by intravenous inoculation 75 days prior to parturition.


°F
107
106
105
104
103
102
101
100
99
50
55
60
65
70
75
80
85
Days Post-inoculation
Parturition

Necropsy results

Sow no. 16 was submitted to post mortem examination 86 days following inoculation of the IBR-Fetus virus and ten days following parturition. There were no gross lesions. Microscopic examination of the tissues revealed the presence of necrotic debris and neutrophils in one follicle of the left ovary. There was a mild peribronchiolar thickening of the interstitial areas of the lungs.

Post mortem examinations were conducted on two of the pigs from sow no. 15 approximately six hours after parturition. No pathological changes were noted either on gross examination or on microscopic examination.

The three stillborn pigs from sow no. 16 were submitted to a necropsy examination. The lungs were collapsed in all three pigs. In one pig, no. 16I, there was a sero-hemorrhagic transudate in the peritoneal cavity. The liver was soft and friable. In another of the pigs, no. 16J the lymph nodes in the cervical area were dark and hemorrhagic. The other pig, no. 16K had no observable lesions.

Post mortem examination of the pig crushed by the sow, no. 16L was conducted. A ruptured diaphragm was observed with a large amount of hemorrhagic fluid in the thoracic cavity. The liver was very yellow and the spleen was dark in color. The peritoneal cavity contained fibrin as well as a dark hemorrhagic fluid. The mesenteric blood vessels were injected and the mesenteric lymph nodes were dark and hemorrhagic.

An intestinal strangulation through a tear in the mesentery was found. A portion of the strangulated intestine was dark, hemorrhagic, and filled with gas.

Bacteriological examination of the kidney, liver and the peritoneal fluid of pig no. 16L resulted in the isolation of a pure culture of a non-hemolytic Escherichia coli from all three specimens.

Pigs no. 16A and 16 F were examined on post mortem on the eighth day following parturition. No lesions were observed.

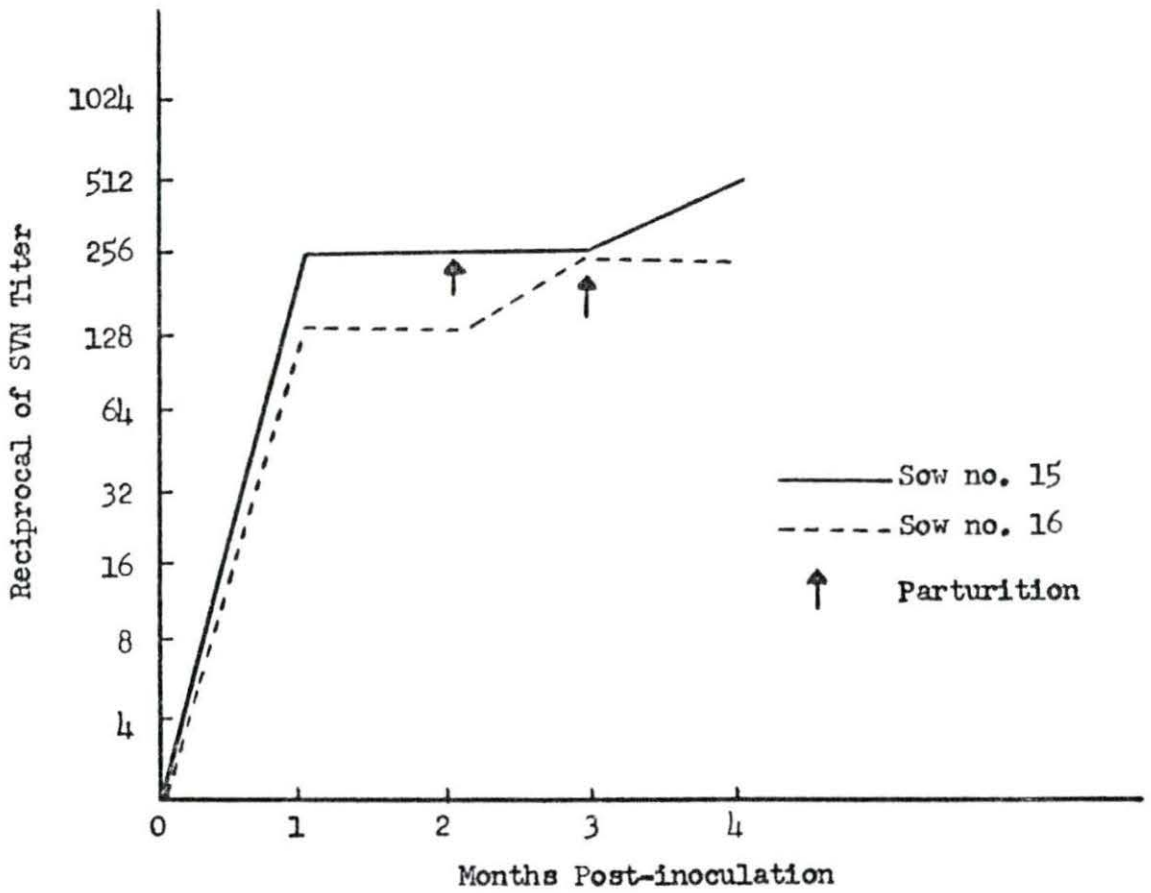
Serological results

Serum samples were obtained at monthly intervals from both sows. Sow no. 15 had a 1:256 titer by the end of the first month following virus inoculation. The antibody titer persisted at this level through the next two samplings. A sample was obtained 97 days following inoculation and the titer had risen to 1:512 (Figure 24).

Sow no. 16 had a titer rise to 1:128 at the end of the first month. By 76 days the titer had risen to 1:256 and was still present at this level at 86 days.

Serum samples were obtained from the pigs from sow no. 16. Samples were taken at necropsy from 16A and 16F. Pig 16A was negative and 16F

Figure 24. Antibody titers of sows no. 15 and no. 16 which received IBR-Fetus virus by intravenous inoculation during pregnancy.


1024
512
256
128
64
32
16
8
4
Reciprocal of SVN Titer
0
1
2
3
4
Months Post-inoculation
Sow no. 15
Sow no. 16
Parturition

had an IBR titer of 1:8. The other pigs were sampled at eleven days of age and the following IBR titers were present:

16 B	1:8
16 C	1:8
16 D	1:4
16 E	1:4
16 G	1:4
16 H	1:4

Virus Isolation

Virus isolation attempts were made on the nasal, conjunctival and vaginal swabs collected daily from the two sows. A total of 465 samples were inoculated onto MDBK cell cultures and passaged four times. No virus isolations were made.

Several weeks after the completion of this experiment a swine testicle cell line was made available. One hundred and fifteen of the original samples were inoculated onto the ST cell cultures and passaged twice. No viruses were isolated.

Tissue specimens from sow no. 16 and from pigs no. 16A and 16 F were processed and inoculated onto MDBK cell cultures. No virus isolations were made. Later these same samples were inoculated onto ST cell cultures with negative results.

DISCUSSION

Serological survey

The results of the serological survey of 108 swine herds in Iowa indicate that Iowa swine have had exposure to infectious bovine rhinotracheitis (IBR) virus. Of the 1220 animals sampled, 139 or 11.38% possessed antibodies to IBR virus. Of the 108 herds sampled, 32 or 29.6% had one or more pigs with antibodies to IBR virus. The only reported survey of swine sera for IBR antibodies was by Spradbow (96). One hundred porcine sera collected from abattoirs in Queensland, Australia, were all found to be negative for IBR antibodies. Similar surveys in cattle in Canada by Niilo, et al. (73) and by Greig (30) indicated an incidence of 37% in Alberta and 8.13% in Ontario respectively. Clinical IBR had not been previously reported in Ontario, however IPV had been observed and the virus isolated. Newberne, et al. (72) in 1961 reported an IBR antibody incidence of 35% in the United States and a 46% incidence in Iowa. Though some time has elapsed since this survey, it is very likely that the present incidence of IBR in cattle is sufficient to provide adequate exposure of Iowa swine to the IBR virus. The 11.38% incidence of IBR antibodies reported in this study when compared to the other surveys appears to represent a significant incidence of this virus in swine.

Whether clinical disease was involved with the development of the antibody titers is not known. It must be assumed that the animals in this survey represent primarily breeding stock since samples were

originally submitted for brucellosis and leptospirosis testing. It is probable that most of the animals were tested as a prerequisite for sale. However some of the animals were undoubtedly tested because of breeding problems or infectious disease problems. Usually there was no pertinent history accompanying the samples and correlation of the antibody titers with a disease process was impossible.

If Iowa swine are exposed to IBR virus what is the source of the virus? Is the virus strain being maintained in the swine population or is the virus being transmitted from infected cattle to the swine? Infectious bovine rhinotracheitis is well distributed in the cattle population of Iowa. Newberne, et al. (72) reported that 46% of 318 serum samples from Iowa cattle were positive for IBR antibodies. However since the swine in the sample population are primarily breeding animals one would suspect that the swine-cattle contact would be minimal. One could then speculate that the IBR virus is maintained in the swine population by a pig to pig transfer rather than a cattle to swine transmission.

A firm producing hybrid swine has several herds scattered throughout Iowa which were included in this survey. Herd no. 79 in the north-central area and herd no. 54 in the northwest area were two herds with IBR positive animals that were owned by this firm. Herd no. 8 in close proximity to herd no. 79 also had the same breed of hybrid hogs. In northwest Iowa herds no. 51, 67 and 108 also have these hybrid hogs. On might speculate that there would be traffic of hogs between some of these farms.

Seven of the herds had two or more submissions of samples. Four of these herds had positive samples on more than one submission.

A possibility to be considered is cross-reactivity with other Herpesviruses, both known and unknown. Plummer (79) reported that there was no cross reactivity between Herpes simplex, B-virus, pseudorabies, IBR and equine Herpesvirus types 1 and 2 except cross neutralization of Herpes simplex by Herpes B virus antiserum. Cross complement fixation between IBR virus and equine Herpesvirus type 1 was reported by Carmichael and Barnes (12) and Plummer (79). Maré[a] tested a number of the same sera used in this study for pseudorabies antibodies and there was no correlation between positive IBR samples and positive pseudorabies samples.

The evidence indicates that a porcine strain of IBR virus is present in Iowa swine. Whether clinical disease is present or whether the infection is subclinical is not known.

Swine inoculation studies

It would be difficult to attribute the symptoms and lesions that were observed in the young pigs inoculated intravenously to the effects of the IBR virus. Both pigs showed a high temperature and anorexia on the 14th day and one had a nasal discharge on days no. 13 and 14. However significant antibody titers had developed in both pigs by the seventh day. Symptoms would not appear so late after inoculation unless

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[a]Maré, C. J., Ames, Iowa. Serological survey for pseudorabies antibodies in Iowa swine. Private communication. 1969.

there was a delay in antibody formation. Also one of the pigs receiving the intranasal inoculation showed a temperature rise and the other a conjunctivitis on day no. 14. It would be highly improbable that clinical signs would develop on the same day when the virus was administered by two different routes. The serological response of the pigs to the IBR virus was much more rapid in the intravenously inoculated pigs than in the intranasally inoculated pigs.

Clinical disease resulted from the intracerebral inoculation of the IBR virus. All four pigs developed high temperatures, disturbances of motor function, hyperesthesia and anorexia. Two of the pigs recovered after several days and no permanent alterations were observed. The infection was similar to that observed by Snowdon (94) in calves inoculated intracerebrally with a vaginal strain of IBR virus. In the calves the temperature rose to 106°F. on the day following inoculation and signs of motor disturbance and hyperesthesia occurred. A rhinitis and conjunctivitis was also present. Straub and Böhm (99) inoculated cattle intracerebrally with a strain of IBR virus isolated from the brain and cerebrospinal fluid of calves having a generalized IBR infection without evidence of nervous symptoms or lesions. The severity of the infection varied with the volume of virus used in inoculation. Calves receiving ten milliliters of virus died 2-4 days following inoculation. The other calves recovered after having high temperatures, disturbances of motor function and anorexia. Johnston, et al. (38, 39), French (23, 24), Gardiner and Nairn (25) and Hall,

et al. (34) in studies of their neurotropic IBR strains produced severe meningo-encephalitis and death by intracerebral inoculation of calves. Intranasal inoculation of these strains produced an equally severe infection indicating that the virus had the capacity to invade the central nervous system from the nasal passages.

Intracerebral inoculation of the cell-culture fluid into the control pigs in Experiment no. 2 did not produce symptoms or lesions except in pig no. 12 which had a temperature rise after inoculation. At necropsy a Streptococcus sp. which could have caused the temperature response was isolated from the surface of the brain of this pig. Straub and Böhm (99) inoculated an IBR-immune calf intracerebrally with IBR virus as a control to determine the effects of the injection and the cell culture fluids on the brain. No symptoms or lesions were noted. It would appear that the symptoms and lesions observed in the pigs inoculated intracerebrally with IBR virus are the result of the viral infection rather than irritation from the inoculation fluids.

The necropsy results of the pigs inoculated by the intracerebral route indicate that the IBR virus is capable of replication and of producing an infection of the nervous tissue. Meningitis, perivascular cuffing and gliosis were present. The lesions were not severe but were sufficient to indicate viral infections.

Antibody formation from the intracerebral and intravenous administration of the virus developed early and rapidly increased to substantial

levels indicating that viral multiplication did occur. The rapidity of the antibody response was similar to that observed by McKercher (64) in cattle in which the average antibody titer after twelve days was near the maximum observed over a ten-month period. Two of these pigs maintained high antibody levels for a year after infection.

Antibody formation in the intranasally-inoculated pigs was delayed and increased only to low levels. Undoubtedly the rate of virus survival and the ability to multiply in the nasal passages was limited. The IBR virus isolated in the nasal passages of pig no. 5 was probably residual virus from the original inoculum.

Slight nasal and ocular discharges were observed in one sow shortly after inoculation and both sows showed a slight vaginal discharge briefly during the early post-parturient period. Abortions were not produced in the sows by intravenous inoculation of the IBR virus. Four of the pigs born to sow no. 16 were dead at birth. No microscopic lesions were found in these pigs and attempts at virus isolation were not successful. Antibody production is an indication that viral replication occurred in the sows. It is possible that the virus may have crossed from the maternal circulation and became established in the fetuses. These symptoms may have been due to the effects of the virus, but since no virus could be isolated there is no evidence to support this assumption. It is interesting that both sows developed high temperatures for a number of days following parturition while appearing clinically normal otherwise. Could this be a recrudescence of the virus infection initiated by parturition as observed in cows by Snowdon (94)?

The antibody response in the sows was rapid and high titers developed indicating viral multiplication had occurred. The antibodies were apparently sufficient in the colostrum to produce low levels of antibodies in the baby pigs.

Failure to re-isolate IBR virus from the experimentally-inoculated animals could be attributed to one of several factors. (1) The storage conditions for the samples obtained from the animals and for the cell culture passaged material were not ideal for long term storage. Virus-isolation attempts on 1300 samples and the subsequent re-passaging of the samples necessitated storage for several weeks. Storage at -20°C. for this period of time may have affected the re-isolation of virus. (2) Each sample was carried through a total of four passages on cell cultures. If the titer of the excreted virus was low it is possible that further passages in cell culture would have been advantageous. Saxegaard (90) examined a group of clinically normal bulls as well as two bulls with a purulent balano-posthitis in an artificial-breeding stud. He was able to isolate IBR virus from all of the 24 bulls but extensive passages in cell culture were required. Three animals including the two bulls showing symptoms excreted virus which was isolated on the initial passage in cell culture. Two bulls were positive for IBR virus on the 3rd passage, two on the 4th passage, twelve on the 6th passage, four on the 7th passage and one on the 9th passage. Saxegaard suggested that a minimum of ten passages be accomplished before a sample be considered negative. In this study such an extensive number of passages was not

possible due to the large number of specimens tested. (3) The cell culture system utilized in this study for virus isolation attempts was possibly no longer as susceptible to the IBR virus after passage through the experimental pigs. The stock virus was propagated on MDBK cells and high titers of virus were consistently produced. However passage of the virus through the pigs may have reduced or altered the virulence of the virus for the MDBK cells.

Snowdon (95) reported that the isolation of IBR virus from vaginal swabs, preputial swabs, and nasal swabs was possible using several different cell types. However, bovine adrenal (BAD) and bovine thymus (BY) cell cultures were more susceptible since the CPE appeared earlier and progressed more rapidly than in bovine thyroid (BTY), bovine pancreas (BP), bovine kidney (BK) and porcine kidney (PK) cell cultures. In the case of a nasal strain the CPE was absent or minimal in the BK and BY cell cultures by the ninth day while the BAD and BY cell cultures were completely destroyed. Madin (50) reported in 1956 that HeLa cells were not susceptible to IBR virus. However Cabasso, et al. (11) were able to adapt the IBR virus to HeLa cells by an alternating passage between HeLa and bovine embryonic kidney cells (BEK). Attempts were made to utilize another cell type of swine origin, the swine kidney cell line (SK). However, the samples had been stored for a period of time and if one assumes the virus titer was low, the negative results are not surprising. If there was an alteration in the virulence of the virus for MDBK cells, and if the titer of excreted

virus was low it would in retrospect indicate that extensive passages would be required to isolate the virus, possibly as many as Saxegaard (90) recommends.

It is evident that despite the failure to isolate IBR virus from the pigs, the virus is capable of multiplying and under some circumstances producing clinical evidence of infection. The serological survey indicates that there is a porcine strain of IBR virus in Iowa.

For seventy years or more the IBR virus caused only a localized venereal infection in cattle. With the drastic changes in cattle husbandry circumstances developed which favored some subtle alteration in the IBR virus which increased its invasiveness and changed its tropism. Buening and Gratzek (9) have shown that there are detectable differences between IBR strains. Since 1954 new strains have developed or at least we have discovered strains which have different infective properties and a broad spectrum of disease syndromes has subsequently been described. What bearing does this have on swine? The circumstances may develop or may already be present in which a porcine strain becomes established and clinical disease may occur. The IBR virus has been shown to be very adaptable and if a porcine strain is established, a repetition of its history in cattle may occur.

SUMMARY

A serological survey for infectious bovine rhinotracheitis antibodies was conducted on swine serum samples from 108 Iowa herds. Of the 1220 animals sampled, 139 or 11.38% possessed antibody titers of 1:4 or higher. It is probable that the sampling represents primarily breeding animals that have limited contact with cattle. A porcine strain of IBR virus is present in Iowa swine and is probably being maintained in the swine population by a pig to pig transfer rather than by cattle to swine transmission.

Inoculation of IBR virus into swine by intravenous and intranasal routes produced asymptomatic infections. Significant antibody titers developed although no virus was isolated. Inoculation of IBR virus into swine by the intracerebral route produced symptoms of incoordination, hyperesthesia, high temperatures and anorexia. Significant antibody titers also developed although no virus was isolated. In two of the pigs receiving the virus by the genital route as well as the intracerebral route, a purulent vaginal and preputial discharge was observed.

Two pregnant sows receiving IBR virus intravenously failed to abort. A slight nasal discharge and ocular discharge were observed in one sow shortly after inoculation. Both sows had a slight vaginal discharge shortly after parturition followed by a period of days in which high temperatures were observed while they remained clinically normal otherwise. Antibody titers reached significant levels in both sows. No virus was isolated.

Swine are capable of being infected with IBR virus. The failure to isolate virus may be due to the extremely low titer of the excreted virus and the alteration of the virus envelope after passage through the pig which necessitates extensive passages in tissue culture for isolation.

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