THE PREPARATION AND EVALUATION OF THREE EXPERIMENTAL INACTIVATED BOVINE VIRAL DIARRHEA VACCINES

by

Leslie Glade Classick IV

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major Subject: Veterinary Microbiology

Iowa State University
Of Science and Technology
Ames, Iowa

1970

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I. INTRODUCTION

Although bovine viral diarrhea (BVD) affects primarily cattle, it has been reported in several species of animals from several countries in various parts of the world. In countries reporting this disease, serological evidence usually indicates that a large percentage of the cattle have been infected.

The disease causes considerable economic loss through a variety of clinical manifestations. BVD may be an acute disease causing severe diarrhea, dehydration, and death. The virus has also been associated with abortion, infertility, lameness, respiratory disease and decreased milk production. In some herds the infection is inapparent but causes economic losses through reduced feed conversion.

There is no satisfactory treatment for the disease, but at the present time, live attenuated vaccines are used in attempts to prevent the disease. However, there are some disadvantages in using the present vaccines. As long as "live" vaccines are used it is always possible that the virus could spread to susceptible pregnant animals and cause abortion. It is also possible that such vaccines could revert to a more virulent form by successive passages through a series of susceptible animals. The present vaccines are not presently recommended for use in pregnant animals or very young animals. The incidence of "adverse reactions" and "vaccination breaks" which may resemble the acute mucosal disease syndrome is not great enough to cause large economic losses in general, but individual livestock owners may sustain losses large enough to

discourage further use of the present vaccines. Over a period of time this could result in large numbers of nonvaccinated and susceptible herds. We have learned through experiences with other diseases, such as hog cholera, that eradication or control programs may not be successful as long as attenuated vaccines are used.

Completely inactivated vaccines have been used successfully for the prevention of a number of viral diseases such as: poliomyelitis, parainfluenza, Newcastle disease, rabies, African horse sickness, hog cholera, and equine encephalomyelitis.

Because of the disadvantages of the present BVD vaccines and because inactivated viral vaccines have been used successfully against other diseases, this study was initiated in an effort to develop and evaluate three experimental inactivated vaccines. The results of these efforts are the basis for this thesis.

II. REVIEW OF LITERATURE

A. Description of the Disease

1. Clinical manifestations

The clinical manifestations of BVD have been well described by the early workers, Olafson et al. (1) in 1946, Ramsey and Chivers (2) in 1953, Pritchard (3) in 1955, and Gillespie and Baker (4) in 1959. Infected animals usually develop varying degrees of anorexia. Diarrhea may vary from a mild form of short duration to a persistent, severe bloody type with marked dehydration; however, all infected animals do not have diarrhea. Many times respiratory symptoms may be the most obvious sign of illness. There is usually a diphasic body temperature elevation (103° - 107°) and an accompanying leukopenia (2,000 - 5,000 leukocytes per cubic millimeter). Often there is an excess nasal discharge, salivation and lacrimation. Lactating cattle may also have a marked decrease in milk production.

Erosions and ulcers of the oral mucosa, muzzle and nostrils are quite common in the field cases. Sometimes erosions and ulcers may be found on the coronet and in the interdigital spaces of the feet.

Less common clinical signs such as: encephalitis, Schipper et al. (5), cloudiness of the cornea, Ramsey and Chivers (2) and Schipper et al. (5), and according to Shope (6) hyperkeratosis may also be associated with the disease. Olafson (1) reported that abortion may occur up to 3 months after the dams become infected, and in some cases clinical signs of the disease were not observed.

Lambert (7), using a low passage NADL strain of BVD virus, experimentally infected calves and produced acute diarrhea and death in neonatal calves.

From 1953 to 1961 it was thought that mucosal disease as described by Ramsey and Chivers (2) and the virus diarrhea described by Olafson and Rickard (8) and Gillespie and Baker (4) were distinctly different diseases, but in 1961 Gillespie et al. (9) reported the antigenic relationship between various strains of viral diarrhea virus and the mucosal disease virus.

2. Necropsy findings

Ramsey (10) described in detail the gross pathology and histopathology of the disease. Lesions of the mucosa of the alimentary tract and lamina epithelia are primarily erosive, ulcerative, and cystic. Necrosis of the lymphoid tissue of the intestines, lymph nodes and spleen is common. Hemorrhages in the epicardium are often present. The mesenteric, cervical and retropharyngeal lymph nodes may be enlarged and hyperemic. The trachea and lungs usually appear normal but occasionally pneumonia may accompany the disease according to Olafson et al. (1) and Ramsey and Chivers (2).

Tyler and Ramsey (11) found that several different BVD isolates produced similar pathological changes in the tissues and cells of experimentally infected calves.

B. Virus Characterization

1. Virus isolation

Bovine virus diarrhea-mucosal disease (BVD-MD) virus can usually be isolated from a variety of tissues, secretions and excretions. Lambert (7) isolated the virus from feces, nasal mucous, leukocytes, milk, cecum, kidneys, small intestine, spleen, and mesenteric lymph nodes. Mills et al. (12) recovered the virus from the urine of infected animals and felt that urine could be another means of spreading the disease in a herd.

2. Nucleic acid

There is agreement among nearly all BVD-MD workers that the nucleic acid is ribonucleic acid (RNA), Hermodsson and Dinter (13), Ditchfield and Doane (14), Gutekunst (15), and Fernelius (16).

3. Size and morphology

Virologists do not agree on the size and morphology of the BVD-MD virus. The variations may be a result of the various methods used to determine size and morphology.

Hermodsson and Dinter (13) using Millipore filters found that C24V virus would readily pass the 100 mμ filter and reduced amounts of virus would pass the 50 mμ filter. Using the electron microscope they reported C24V virus to be spherical with a diameter of approximately 40 mμ.

Gutekunst (15) using NADL-MD and C24V viruses with Millipore filters found both viruses passed the 220 mμ and the 100 mμ filter, a small fraction of both viruses passed the 50 mμ filter, but no infective virus passed the 10 mμ filter.

Ditchfield and Doane (14) using the electron microscope to observe ultra thin sections of tissue culture cells infected with C24V or MAC-A BVD-MD viruses observed particles 150 to 250 mμ in size with projections of 10 mμ. The internal component was in the form of a helix with a diameter of 18 mμ and on this basis they suggested that the BVD-MD virus is a myxovirus.

Pritchard (17) published an electron micrograph (supplied by Kniazeff) of BVD virus-like particles 35 to 55 mμ in diameter and roughly spherical in form.

Dutta et al. (18) in 1964 using negative staining techniques reported the virus to be spherical with a diameter of 75-85 mμ. The central core was enveloped and the outer coat had surface projections 7-8 mμ in length with bulbous ends. The virus appeared to have helical symmetry thus resembling a myxovirus.

According to Fernelius (16), various strains of BVD-MD viruses vary in size. Some strains show a reduction of 3 logs in titer after passing a 220 mμ filter. The C24V virus, stored 3 1/2 years at -75 C, had a titer of 10^3 after passing a 10 mμ Millipore filter. Using the electron microscope and negative staining, he found four types of particles present in the tissue culture fluids: (1) rectangular shaped particles 15-20 mμ in size often surrounded by a membrane, which may have been part of the cellular endoplasmic reticulum; (2) 45-50 mμ particles; (3) 80 mμ particles; (4) 100-120 mμ particles. Using magnification up to 500,000 diameters, he could not detect internal structures or external morphological features that could be used for virus identification or classification, although he did find 100 mμ

particles with membrane-like structure possessing projections similar to those of a myxovirus. Fernelius also noted that the 100 mμ particle seemed to break up and release 30-40 mμ particles. The 15-20 mμ particles were thought to be soluble antigens or viral precursor units.

4. Ether sensitivity

Ether sensitivity is one of the major criteria used in viral classification and in 1961, Feldman and Wang (19) found that viruses sensitive to ether were also sensitive to 5% chloroform when incubated at room temperature for 10 minutes.

Hermodsson and Dinter (13), Gillespie et al. (20), and Gutekunst (15) reported BVD-MD viruses are ether and chloroform sensitive.

5. Thermal inactivation and cationic stability

A number of investigators have reported results of thermal inactivation under a variety of conditions.

Underdahl et al. (21) and Ditchfield and Doane (14) reported BVD-MD viruses were inactivated at 50 C, but Gratzek (22) noted a decreased titer of one log (of C24V) in 24 hours at 37 C or 27 C. At 58 C he noted a two component curve which indicated the possibility of heat resistant variants.

Dinter (23) reported no cationic stabilization of the virus, but Gutekunst (15) found 1 M solutions of either Ca^{++} or Mg^{++} actually enhanced thermal inactivation of NADL-MD virus incubated at 50 C.

6. Trypsin sensitivity

In 1958, Cheng (24) found that trypsin, chymotrypsin or papain would inactivate both the hemagglutinin and infectivity of group B arboviruses but they had no appreciable effect on the group A arboviruses. Dinter (23) found C24V BVD virus to be moderately sensitive to the enzymatic action of trypsin and thought this virus resembled the group B arboviruses and according to Gutekunst (15), both NADL-MD and Oregon C24V were moderately sensitive to trypsin.

7. Infectious RNA

Diderholm and Dinter (25) in 1966 reported cold phenol extraction of infectious RNA from BVD virus. These authors inferred that these results precluded a myxovirus classification because myxoviruses are refractory to extraction of infectious nucleic acid according to Schaffer (26).

Fernelius (16) tried to extract infectious RNA from NADL-PK_{22} strain of BVD using phenol but was unsuccessful. His findings were contrary to those of Diderholm and Dinter (25).

8. Sedimentation coefficient

The sedimentation coefficient of C24V virus, according to Hermodsson and Dinter (13), was estimated to be 80-90 S by centrifugation in a Spinco Model L centrifuge.

9. Specific gravity

Fernelius (16) using isopycnic centrifugation with sucrose, potassium tartrate, and cesium chloride gradients found that the NADL

virus had a density of 1.15 gm/ml, with cesium chloride giving the sharpest peak.

10. pH Stability at 56 C

Gutekunst (15) found that at a pH of 5 the NADL-MD virus was inactivated in 45 minutes. At a lower pH it was inactivated more rapidly. Between pH of 6 and 7 the virus was not completely inactivated in 2 hours.

11. Hemagglutinins

Gutekunst (15) reported that BVD-MD viruses did not cause hemagglutination of bovine, chicken, day-old chicken, guinea pig, rabbit, mouse, sheep, swine, hamster and human type O erythrocytes.

12. Cytopathic effect

According to Gillespie and Baker (4) and Gutekunst (15) the NY-1, Indiana 46, Sander, Merrell and CG1220 strains are noncytopathogenic.

Underdahl et al. (21) isolated and propagated cytopathogenic viruses from field cases of MD. They were designated M-833 and ISC-1. At 7-10 days PI vacuolization of the EBK cells was noted. The vacuoles increased in size until the cell layer was destroyed by 10 to 14 days. Gillespie et al. (27) also isolated a virus designated Oregon C24V, which was cytopathogenic. Gutekunst (15) found that the NADL strain of BVD-MD virus was another cytopathogenic BVD-MD virus.

13. Growth cycle

The work of Gratzek (22), Gillespie et al. (20), and Gutekunst (15) indicates that absorbtion of the virus to cells is accomplished in 10-60 minutes. Cell-associated virus may be detected in 8-10 hours and virus in the extracellular fluid 4 hours later. Cytopathic effect may be noted in 18 hours but maximal CPE and virus titer may take 3-7 days.

Virus release is gradual rather than rapid as in polio or bacteriophage release.

C. Serology

Prior to 1960, the relationship between the various BVD and MD isolates was unclear, but in 1960 Gillespie et al. (27) showed the Oregon C24V virus was antigenically related to New York 1 and Indiana 46 viruses.

Kniazeff and Pritchard (28) using plaque assay procedure to conduct neutralization tests on 45 various antiserums from the United States and England against Oregon C24V virus found that there was a serologic relationship between all of the anti-BVD and anti-MD serums tested. They also showed that antiserums to bluetongue, hog cholera, IBR, bovine infectious ulcerative stomatitis, winter dysentery, malignant catarrhal fever, sporadic bovine encephalitis and bovine mycotic stomatitis did not neutralize the C24V virus. Kniazeff et al. (29) also reported C24V virus was neutralized by antiserums against BVD-MD viruses from Scotland and West Germany.

Gillespie et al. (9) showed several BVD-MD viruses were related and suggested serological tests be used before any new disease entities were postulated on the basis of clinical and pathological findings.

Using anti NADL-MD serum against 100 $TCID_{50}$ of various viruses, Gutekunst (15) showed an antigenic relationship between NADL-MD virus and Oregon C24V, New York 1, Indiana 46, Sander, Merrell, and CG-1220 BVD viruses. Gutekunst did not find any antigenic relationship between NADL-MD virus and IBR, PI-3, hog cholera, TGE, group B arbovirus, and LCM viruses when he used the serum virus neutralization tests.

Gutekunst and Malmquist (30) found that infective NADL BVD-MD virus failed to fix complement, but the soluble antigen did fix complement.

Gratzek (22) in 1962 reported a virus interference test for noncytopathogenic BVD-MD viruses using VSV for the challenge virus. This was a significant finding because noncytopathogenic BVD-MD viruses are difficult to detect and assay in the laboratory.

Gillespie et al. (31) reported a plaque inhibition assay method for detecting noncytopathogenic BVD-MD viruses based on the interference principle.

Gutekunst and Malmquist (30) reported that cells infected with noncytopathogenic BVD-MD viruses had almost 100% interference to infection with NADL-MD virus.

Inaba et al. (32) described the use of the END method to detect noncytopathogenic BVD viruses.

Darbyshire (33) reported that the agar double-diffusion technique could be used as a serological technique for diagnosis of BVD-MD.

The antigenic relationship between BVD-MD virus and hog cholera virus causes one to wonder if BVD-MD virus may have evolved from HC virus. It was almost inevitable that some workers would try to immunize swine by injecting them with BVD-MD virus.

Beckenhauer et al. (34) found that pigs inoculated with some strains of BVD-MD virus withstood challenge with virulent hog cholera virus. They concluded that this immunity was related to antibody formation rather than some interference mechanism.

Darbyshire (35) in 1962, using agar double-diffusion plates, was probably the first to report direct evidence of an antigenic relationship between BVD-MD virus and hog cholera virus.

Sheffy et al. (36) showed an antigenic relationship between hog cholera and BVD-MD virus using calves and pigs and concluded that the cross protection was a result of accelerated secondary response induced by prior exposure to the heterotypic virus.

Snowdon and French (37) reported that Australian swine may have swine fever-neutralizing antibodies as a result of previous BVD-MD infection. They exposed pigs to 4 strains of BVD-MD virus and found that 3 of the 4 strains could induce protection against subsequent SF challenge.

Tamoglia et al. (38) in 1965, tried to use New York-1 strain of BVD-MD virus to protect pigs against challenge with HC virus and found that it would not protect as well as modified live virus hog cholera

vaccine. It would not meet the licensing requirements for either a modified live virus vaccine or an inactivated hog cholera vaccine.

Mengeling et al. (39) demonstrated antigenic relationship between BVD and HC viruses using immunofluorescence and Fernelius (40) used immunofluorescence to detect and titrate various BVD-MD viruses.

Gutekunst and Malmquist (41) found that the soluble antigen formed a single line of precipitation in the agar double-diffusion plates against the homologous immune serum; however, the infective virus "pellet" failed to form a precipitin line.

Gutekunst and Malmquist (30) infected animals with BVD-MD soluble antigen and demonstrated complement-fixing antibodies to the soluble antigen and showed that the soluble antigen alone was antigenic in calves.

Fernelius and Packer (42) in 1969 reported that rabbit antisera to bovine kidney cells neutralized several strains of BVD-MD virus. Antisera to porcine kidney cells did not neutralize BVD-MD virus, even when the BVD-MD virus had been grown in porcine kidney cells. A degree of specificity was present because the anticellular serum failed to neutralize viruses of infectious bovine rhinotracheitis, vesicular stomatitis, bovine enterovirus, vaccinia, and parainfluenza-3.

D. Immunization Against BVD-MD

1. Attenuated vaccines

In 1954, Baker et al. (43) adapted BVD-MD virus to rabbits and after 75 serial passages, the virus was attenuated. Inoculation of

the seventy-fifth passage into susceptible calves produced a slight leukopenia and temperature elevation that lasted for one day and the calves were protected against challenge with a virulent virus.

Baker et al. (44) in 1958 successfully immunized 6 calves against leptospirosis, virus diarrhea and infectious bovine rhinotracheitis with a single inoculation of combined vaccine. The BVD-MD virus had been attenuated by 80 serial transfers in rabbits. The virus did not spread to contact controls.

York et al. (45) in 1960 inoculated some cattle with a rabbit-adapted modified live virus and others with a bovine kidney tissue culture modified live virus. Both vaccines were capable of stimulating a protective immune response. Vaccinated animals withstood challenge with a virulent BVD-MD virus which sickened the non-vaccinated controls. In a field test where natural challenge occurred there was a significant degree of protection.

Coggins et al. (46) made 32 serial passages of the Oregon C24V strain of BVD-MD virus in primary embryonic bovine kidney (EBK) cells. The virus, when used as a vaccine, immunized susceptible cattle and did not produce signs of clinical illness.

In 1965 Malmquist et al. (47) adapted the NADL strain of BVD-MD virus to both a primary and a subculturable cell line of swine kidney cell cultures. They also reported that hog cholera virus would interfere with the cytopathic effect and the yield of the cell-adapated NADL strain of BVD-MD virus.

In 1967, Marcus and Moll (48) adapted the NADL strain of BVD-MD virus to the Madin-Darby bovine kidney (MDBK) cell line by first

making 6 passages in bovine testicle cell cultures. The virus produced CPE in MDBK cells 3 to 5 days after inoculation.

Bittle et al. (49) reported the modification of the C24V strain of BVD-MD virus by serial passage in primary bovine kidney tissue culture. Tests at the 31st passage level indicated the virus was attenuated and did not spread to contact control animals. The vaccine strain was further developed by additional serial passages of the virus in tissue culture.

Bittle and York (50) using a commercially available bivalent vaccine composed of attenuated IBR and BVD-MD viruses demonstrated compatibility of the viruses. Animals with antibodies present against one of the viruses did not interfere with production of antibodies against the second virus. Ninety-six percent of the susceptible cattle developed antibodies against BVD-MD virus and 97% of the susceptible animals developed antibodies against IBR. Trivalent vaccines using attenuated IBR, BVD-MD and PI-3 viruses tested in the laboratory and in the field apparently reduced morbidity and did not spread to contact control animals.

Gutekunst (51) in 1968 reported that the NADL strain of BVD-MD virus adapted to porcine kidney cells by Malmquist et al. (47) was attenuated by 20 additional passages in porcine kidney cells. When inoculated into colostrum-deprived calves, it did not produce signs of illness nor infect contact control animals. The virus was immunogenic and no adverse reactions were detected.

At the present time the NADL and Oregon C24V strains of BVD-MD virus are the only ones used as seed virus for attenuated BVD-MD vaccine production.

Primary and tertiary EBK cells have been the most commonly used cell cultures for growing and attenuating the virus; however, primary EBK cells may carry noncytopathogenic BVD-MD virus and contaminate vaccines with a virulent strain of virus. To prevent such contamination, cells must be checked by fluorescent antibody techniques or interference tests. The trend is now towards the use of tertiary EBK cells, MDBK cells or porcine kidney cells in attempts to get uniform cells that are free of adventitious viruses.

BVD-MD attenuated vaccines are commercially available as monovalent, bivalent or trivalent vaccines. It is usually used in combination with IBR or IBR and PI-3 vaccines.

2. Postvaccinal reactions

The subject of postvaccinal reactions is controversial among those persons involved in BVD-MD research, vaccine production and sales, practicing veterinarians, and livestock owners.

Peter et al. (52) in 1967 reported the results of their efforts to characterize BVD-MD postvaccinal conditions. The study included cattle from 23 herds which had a MD-like condition develop 10-20 days after BVD-MD vaccination. The condition was very similar to the field form of acute MD. Death usually occurred in 10 to 14 days after the first signs of illness. Morbidity rates ranged from 0.2% to 40.0%

with an average incidence of 5.2%. It was estimated that 75.0% of the cattle that developed severe clinical signs of the disease eventually died.

Calves with clinical signs of the postvaccinal condition had no significant antibody titer while calves that were apparently recovering from the postvaccinal condition usually had a titer in excess of 1:64. The BVD-MD negative calves did, however, have titers against IBR.

After discounting contaminating viruses, prior exposure to BVD-MD, simultaneous vaccination with IBR vaccine, and stress, they concluded that the probable cause was failure of the immune response against vaccine or field strains of BVD-MD virus.

These workers also detected slight differences between the lesions of MD and the postvaccinal condition. With the postvaccinal condition the skin, foot, and eye lesions are more common and the erosions in the digestive tract are more diffuse and superficial.

Kirkpatrick (53) observed no higher percentage of herds or animals with postvaccinal conditions with combination vaccines than with monovalent BVD-MD vaccines. He felt that reactions were from the lack of ability of a few animals to respond to the antigen.

Rosner (54) reported that according to his findings in 1965, the morbidity rate for mucosal disease in commercially vaccinated cattle was only 1 per 35,700 head vaccinated. In several suspected cases of postvaccinal reactions other viruses were isolated. He suggested that postvaccinal reactions could be reduced by vaccinating before

and at weaning time, before calves leave their native herd and are exposed to outside infection.

Brown and Ramsey (55), commenting on Rosner's report (54), indicated the incidence of reactions in Iowa surpassed the level of Dr. Rosner's "reported" cases. Brown's and Ramsey's figures were based on Peter's (52) work. They also mentioned that the possibility of virus spreading from vaccinates to susceptible animals must be considered.

Fuller (56) stated that the incidence of postvaccinal reactions was not high enough to warrant disuse of the vaccine, but it was high enough to warrant credence and that a presumption of IBR or BVD-MD infection prior to vaccination is not a completely satisfactory explanation in such cases.

According to Peacock (57) there is a definite indication that in some cases attenuated BVD-MD vaccine may be a predisposing factor or possibly the primary cause of severe reactions. The low morbidity and high mortality suggested to Peacock that an individual hypersusceptibility or immunologic unresponsiveness may be involved in postvaccinal reactions. Because BVD-MD virus had been found in IBR vaccines, the standard requirements for BVD-MD and IBR vaccines were amended to provide more sensitive in vitro tests for extraneous viruses in cell culture systems.

Holper (58) suggested that immune serums containing BVD-MD antibody should not be used at the time of vaccination because of probable effects of the antibody on immunogenesis of BVD-MD vaccine. He recommended delaying vaccination for 3 weeks if animals had been given antiserum.

Clark (59) reported seeing 40 cases of postvaccinal reactions in 1000 vaccinates over a 3-month period. Cytopathogenic BVD-MD viruses were isolated at necropsy from some of these cattle.

Clark (60) reported the results of his study of the occurrence of postvaccinal reactions in 92,621 vaccinated animals. One hundred and seventy-eight were diagnosed clinically as having MD and 162 died. Ten percent of the cattle were checked serologically and only 2 of 18 had BVD-MD antibodies and these had a titer of only 1:4. There was no significant difference in the incidence of reactions when BVD-MD vaccines were used alone or when used in combination with IBR vaccine. Clark's studies confirmed the findings of others that the postvaccinal reactions occur in those few animals that fail to develop antibodies against BVD-MD virus. There appeared to be no difference between Hereford and Angus breeds in susceptibility to reactions although steers seemed to be slightly more susceptible than heifers.

Some investigators advocate the use of bovine antiserum alone or with vaccine in the BVD-MD immunizing procedures in attempts to provide immediate protection and avoid possible postvaccinal reactions.

Simonyi et al. (61) used antiserum prepared against the Oregon C24V strain of BVD-MD virus to prevent and treat BVD-MD. Calves injected I.V. or subcutaneously with 50-100 ml of 1:512 titer serum were protected against challenge while those injected with lower titered serum were not protected. Sick animals treated with antiserum had a recovery rate of 72% and mortality rate of 6.2% while 40.5% of the untreated controls recovered and 25.2% died.

Simonyi et al. (62) attenuated the C24V BVD-MD virus by serial passage in EBK cells before using the virus as a vaccine. They reported that occasional vaccinates evidenced mild signs of the disease, some vaccinated animals excreted virus, and contact controls developed a low antibody titer against BVD-MD virus. It was further observed that 18 weeks after the second vaccination some animals were no longer protected against challenge. They reported that the monthly weight gains of vaccinated animals were higher than that of the controls. They recommended immunizing newborn or diseased calves with immune serum alone or immune serum plus vaccine followed by a second dose of vaccine 4 weeks later.

Lambert et al. (63) reported that a prophylactic dose of concentrated homologous bovine antiserum administered simultaneously with IBR-BVD vaccine did not interfere with active immunization. They felt that cattle vaccinated with serum and vaccine could be protected by the serum until they could develop an active immunity.

Lambert et al (64) using concentrated bovine antiserum from cattle given repeated injections of Pasteurella multocida, Pasteurella homolytica, Corynebacterium pyogenes, IBR, BVD and PI-3 found that 20 cc of antiserum inoculated subcutaneously into neonatal calves reduced the morbidity and mortality rates of pneumonia-enteritis in newborn calves.

E. Inactivated Vaccines

Attenuated vaccines will always be popular because they are relatively inexpensive to make and have been effective against a number of

diseases. Inactivated vaccines have also been used successfully to control a number of viral diseases such as: poliomyelitis, rabies, Newcastle disease, parainfluenza, African horse sickness, hog cholera, and equine encephalomyelitis.

A number of physical and chemical agents are currently used to inactivate virus: heat, ultraviolet irradiation, phenol, formalin, beta-propiolactone (BPL), and acetylethyleneimine (AEI) being some of the more common.

1. Beta-propiolactone inactivated (BPL) vaccines

In 1955, LoGrippo and Hartman (65) reported the results of screening over 600 physical and chemical agents in their search for a more satisfactory agent for preparation of inactivated virus vaccines. They found that BPL was capable of inactivating viruses in 10-15 minutes at 37 C, whereas formalin and phenol required days for inactivation of virus. Satisfactory inactivated vaccines were prepared with viruses of eastern equine encephalomyelitis, rabies and the MM strain of murine encephalomyelitis. BPL had not been used previously in the preparation of vaccines. The antigenicity of the BPL vaccines was better than the formalin or phenol-inactivated vaccines. They also found that 2 to 4 times the minimal effective viricidal concentration of BPL can be used without critical loss of antigenicity.

LoGrippo (66) reported that BPL-inactivated biologicals are non-toxic because BPL is completely degraded and the products of hydrolysis are non-toxic. He also found that a combination of BPL and ultraviolet (U.V.) light is better than BPL alone because less BPL is

required. There is less alteration of the protein and less tailing effect of the inactivation curve.

One of the more widely used BPL-inactivated vaccines has been NDV vaccines. Mack and Chotisen (67) in 1955 demonstrated the immunogenicity of the vaccine and this report stimulated much interest in the development of BPL-inactivated Newcastle disease virus (NDV) vaccines.

Gill et al. (68) studied the effects of aluminum hydroxide, ethylene glycol and phosphorylated hesperiden as adjuvants with BPL-inactivated NDV vaccine and found that all 3 gave similar results.

Hofstad (69) evaluated 6 commercial inactivated NDV vaccines. The chickens were injected at 6 weeks of age and challenged with virulent virus 6 weeks later. Two of the vaccines were considered inferior.

Hofstad (70) used the B_1, GB, and Manhattan strains of NDV for his vaccine production. In his first experiment the viruses were inactivated with formalin and the B_1 vaccine provided the best immunity. In the second experiment BPL was used to inactivate the viruses and there was no significant difference in the immunogenicity of the 3 strains. The BPL-inactivated vaccines seemed to produce better immunity than formalin-inactivated vaccine. Hofstad concluded that the inactivating substance, the adjuvant, and the type of virus - bearing material are more important than the particular strain of virus used or the route of inoculation.

Chang et al. (71) compared six strains of BPL-inactivated Newcastle disease virus in chickens. The immune response was measured

by the level of hemagglutination inhibition antibody. Virulence appeared to be unrelated to antigenicity as the Texas GB, Kansas-Manhattan, and Bl-Hitchner strains induced significantly higher HI antibody levels than NJ-LA Sota, Mass-MK-107, and California 11914 strains. All chickens gave an anamnestic response following the second inoculation.

Stone and Boney (72) used NDV antigen-antibody complexes to vaccinate 2-day-old chicks with different levels of passive immunity. They concluded that immunological refractoriness of congenitally immune chicks could be overcome by vaccination with antigen-antibody complex vaccine.

Stone et al. (73) immunized young chickens against NDV with BPL-killed-NDV administered in the drinking water. Although this method of vaccination saves time and labor it requires a large amount of antigen.

Winterfield (74), using a BPL-inactivated infectious bronchitis virus (IBV) that is used successfully in Great Britain, could not detect formation of significant neutralizing antibodies against Massachusetts 41 and Beaudette IBV strains. When the chickens were challenged they all developed severe respiratory signs. No difference could be noted between vaccinated chickens and the unvaccinated controls.

Price and Thind (75) found that BPL-inactivated Langat E5 virus injected into mice induced production of protective levels of neutralizing antibodies but did not induce production of serum protective-factor (SPF). Live virus induced the production of both SPF and

neutralizing antibodies. Mice with no detectable neutralizing antibodies were protected against challenge with virulent virus if they had the SPF.

Pilchard (76) evaluated 10 experimental inactivated hog cholera vaccines and found that a Bordetella-azo-BPL-inactivated-virus hog cholera vaccine and an endotoxin-BPL-inactivated-virus hog cholera vaccine induced immunity in 7 days. Other inactivated hog cholera vaccines require 2 inoculations and the induction of immunity takes 2 to 3 weeks.

Mirchamsy and Taslimi (77) evaluated both formalin- and BPL-inactivated African horse sickness (AHS) virus vaccines and found that these vaccines protected horses for at least 6 months. The virus was grown on monkey kidney cells. The formalin-inactivated product induced slightly higher neutralizing antibody titers.

Fellowes (78) found that 0.05% BPL did not completely inactivate foot-and-mouth disease virus; however, 10 minutes exposure to U.V. light prior to BPL treatment did completely inactivate the virus. The immunogenicity of either the AEI or the U.V.-BPL-inactivated products was approximately the same.

Chloroform has not been used widely as an inactivating agent. However, Walker et al. (79) in 1946 reported on the development of a satisfactory chloroform-inactivated rinderpest virus vaccine. The source of the virus was lymph nodes, spleen and lungs from infected calves that had been febrile for 2 or 3 days. Chloroform appeared to be superior to formalin as an inactivating agent. They recommended a

single 20 cc dose administered subcutaneously for use under field conditions.

Rouhandeh et al. (80) found that ether-inactivated IBR virus could be reactivated when resuspended in water containing insoluble inorganic compounds which adsorbed the virus and adhered to cells, thus providing a cell entry mechanism for the damaged virus. This finding of reactivation may cast doubt on the rationale of ether or chloroform inactivation which in theory may be based on blocking the cell attachment or cell entry of the virus.

2. Viral-component vaccines

Recent work with some of the viruses infecting man has shown that viral protein subunits can stimulate production of neutralizing antibodies and may be used as vaccines. Such products might avoid side effects caused from certain other components of the virus.

Crick and Brown (81) treated rabies virus with "Tween"-ether and then inactivated the residual infectivity with AEI. Subunits which were separated by sucrose density gradient centrifugation and inoculated into mice induced protective levels of neutralizing antibodies. This indicated that viral subunits may be useful in rabies vaccination.

Brown et al. (82) separated 3 fractions of VSV by sucrose density gradient centrifugation. All 3 fractions were then inactivated with AEI. The inactivated fractions were immunogenic in guinea pigs.

Davenport (83) reported that the antigenicity of formalin-inactivated influenza HA-vaccines was almost equal to that of whole virus vaccines if $AlPO_4$ was used as an adjuvant with the inactivated vaccine.

In 1964, Kasel et al. (84) vaccinated human volunteers with non-infectious soluble antigens of adenovirus type 1. The vaccine induced production of neutralizing antibodies and following challenge, the immunized volunteers had a significant reduction in virus shedding and almost complete protection against illness.

Norrby et al. (85) compared a measles HA vaccine with a formalin-killed vaccine. Both vaccines produced 100% serological conversions and HI antibodies were detectable 1 year after vaccination. Two mild cases of measles occurred among children given the formalin-killed vaccine but none occurred in those given HA vaccine.

III. MATERIALS AND METHODS

A. Preparation of Vaccines

1. Virus

The bovine viral diarrhea virus used in this study was the NADL-MD strain which has been previously described by Gutekunst (15). This virus was isolated from several tissues of a naturally-occurring fatal case in a group of yearling Holstein-Friesian heifers at the National Animal Disease Laboratory in 1962. The seed virus used in this work was the second passage in primary embryonic bovine kidney (EBK) cells.

2. Tissue culture system

Primary EBK cell cultures were used throughout the entire experiment. Cell cultures were prepared by the general method of Younger (86) as modified by Gutekunst (15). Kidneys were collected aseptically from selected bovine fetuses in the sixth to ninth month of development and placed in a sterile container of Hank's balanced salt solution (HBSS). The capsule was removed from the kidney and the cortical region separated from the medulla. The cortical tissue was minced until pieces were approximately one cubic millimeter in size. The minced cortical tissue was washed three times with cold GKN[1] solution, with the washing fluids being discarded. The tissues were placed in a sterile trypsinization flask[2] containing a sterile Teflon covered

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[1] See Appendix for formula.

[2] Bellco Glass Company, Vineland, New Jersey.

magnetic stirring bar. Sterile trypsin solution (0.25 percent) chilled to 4 C was added to the trypsinization flask and incubated at 4 C for 4 to 6 hours. After incubation the supernatant fluid which contained toxic debris was discarded and fresh cold trypsin was added. Trypsinization was continued for an additional 18 hours at 4 C. Following trypsinization, fibrous tissue was separated from the trypsinized cells by filtration through sterile cheesecloth. The cells were sedimented by centrifugation at 1,000 RPM in a refrigerated centrifuge and the supernatant fluid was discarded. The cells were then washed three times with cold GKN and diluted in HBSS containing 10% specific-pathogen-free (SPF) calf serum to give a final concentration of approximately 1 X 10^6 cells/ml. The EBK cells were then propagated in sterile tissue culture tubes. The cells were planted in medium containing 89.5% HBSS, 0.5% lactalbumin hydrolysate, 10.0% SPF calf serum, and penicillin and streptomycin at a concentration of 100 units and 100 µg/ml, respectively. After three days' incubation at 37 C, the medium was replaced with 89.5% Earle's balanced salt solution (EBSS), 0.5% lactalbumin hydrolysate, 10% SPF calf serum, and antibiotics.

After complete monolayers had formed (approximately 5 days), the medium was removed and EBSS medium with 5% SPF calf serum was added for maintenance.

3. Preparation of virus suspension for vaccines

Twenty-four 500 ml Blake bottles of primary EBK cells were treated with 10 ml of 0.2% EDTA for 30 minutes at 37 C to facilitate removal of the cells.

The cells were washed with Earle's medium, centrifuged at 800 RPM, supernatant fluid removed and resuspended in 100 ml of HBSS with 10% SPF calf serum. Cell suspensions were then transferred into 24 two-liter roller bottles and rotated at 12 RPH at 37 C.

After 48 hours the medium was replaced with EBSS containing 0.05% lactalbumin hydrolysate (LAH) and 10% SPF calf serum.

Seventy-two hours later the medium was removed and the roller bottles with EBK monolayers were inoculated with 10 ml of a second passage NADL BVD-MD virus in Earle's medium. The roller bottles were allowed to rotate for 1 hour at 37 C, then 90 ml of Earle's with 0.5% LAH plus 5% SPF calf serum was added to the roller bottles. In 96 hours the cultures were harvested and clarified by centrifugation at 19,000 RPM (53,700 x G) in a Beckman Model L-2 ultracentrifuge.

4. Virus titration

The NADL strain of BVD-MD virus is cytopathogenic so its presence could be detected by CPE in EBK cells. The virus suspension was quantitated by titrations of infectivity in susceptible cells by 10-fold serial dilutions in EBSS, then 0.2 ml. of each dilution was inoculated into each of 4 roller tubes containing monolayers of EBK cells. The cultures were incubated at 37 C and observed for appearance of CPE after 7 days. The 50% end points were calculated by the method of Reed and Muench (87).

5. Beta-propiolactone inactivation

The inactivation by BPL was carried out with some modifications of the methods described by LoGrippo and Hartman (65). The solution

of BPL[1] (97%) stored at 4 C was slowly mixed with virus suspension to a final concentration of 0.2%. The pH of the virus suspension was adjusted to 8.0 with 1M potassium dihydrogen phosphate before the addition of BPL. The pH of the mixture was periodically adjusted to 7.4 during the 20-minute incubation period at 25 C. Samples were taken for in vitro tests for residual virus.

Ten ml Rehsorptar[2] (aluminum hydroxide gel) was added to 90 ml of the BPL-inactivated vaccine to give a final concentration of 10%. Each lot of vaccine was shaken vigorously for 2 hours on a mechanical shaker and distributed into 50 ml vials, sealed, labeled and stored at 4 C till used.

6. Chloroform inactivation

One hundred ml of chloroform was added to 1900 ml of the virus suspension. The mixture was incubated for 96 hours at 4 C in a sterile flask and stirred continuously with a Teflon covered magnetic stirring bar. Following incubation the chloroform was sedimented by centrifugation at 1500 RPM for 20 minutes in a refrigerated centrifuge. The supernatant fluid was saved. This sedimentation procedure was repeated 3 times.

The residual chloroform was removed by bubbling filtered air through the inactivated virus suspension for 12 hours.

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[1]Wilmot Castle Company, Rochester, New York.

[2]Reheis Chemical Company, Chicago, Illinois.

At this time samples were taken for *in vitro* tests for any remaining infectivity. Ten ml of Rehsorptar was added to each 90 ml of chloroform-inactivated vaccine to give a final concentration of 10%. After vigorous shaking each lot of vaccine was distributed into 50 ml vials, sealed, labeled, and stored at 4 C until used.

Approximately 1500 ml of the chloroform-inactivated virus, without adjuvant, was saved for the preparation of the soluble antigen vaccine.

7. Soluble antigen vaccine

Chloroform-inactivated virus suspension was first concentrated approximately 10-fold by dialysis against polyethylene glycol (Carbowax 20-M).[1] Carbowax was then removed by dialysis against distilled water for 16 hours at 4 C.

The concentrated inactivated virus suspension was then centrifuged in a Spinco model L-2 HV ultracentrifuge at 220,000 g for 16 hours in a cesium chloride (C_sCl) density gradient system. Five ml of concentrated inactivated virus suspension was layered on each of the CsCl density gradient tubes which were prepared by layering 5 ml of a 20% CsCl solution on 2 ml of 35% CsCl solution. After centrifugation the gradient ranged from approximately 1.02 to 1.34 g/ml as determined by an Abbe 3L refractometer.

Preliminary experiments using infectious virus indicated that the infectious portions of the suspension are in those fractions of the

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[1]Union Carbide Corporation, New York, New York.

density gradient between 1.02 and 1.20 g/ml. The soluble antigens, as determined by agar gel double-diffusion methods, were in the gradient between 1.20 and 1.34 g/ml.

The soluble antigen-containing fractions from 12 tubes were pooled and dialyzed against distilled water for 8 hours followed by dialysis against 0.975% phosphate buffered saline (PBS) for 16 hours.

A sample of the vaccine was saved for agar gel double-diffusion tests and the remainder was divided into 9.0 ml aliquots, placed in 20 ml vaccine bottles containing 1 ml of Rehsorptar, and stored at 4 C until used for animal experiments.

8. In vitro test for residual infectivity

Before the 3 experimental vaccines were inoculated into calves, 20 ml from each 100 ml lot of vaccine was checked for infectious virus by inoculating 0.2 ml of vaccine into each of 100 roller tubes containing monolayers of EBK cells. Prior to inoculation, each roller tube of cells was examined to make sure all cells appeared normal. Seven days after inoculation cells were examined for evidence of CPE and at that time 0.2 ml of fluid from each tube was transferred to another tube of fresh EBK cells. If, after 4 such passages, all 100 tubes were negative for CPE the vaccine was considered free of infectious virus.

After 4 passages there was no CPE in any of the 300 tubes of EBK cells and all 3 vaccines were considered free of residual infective NADL strain of BVD-MD virus.

B. Animal Experimentation

In order to obtain enough calves and to be reasonably sure that experimental calves were free of BVD-MD virus and antibodies, it was necessary to obtain calves by 2 methods. Twelve calves were obtained from the NADL BVD-negative herd and 11 calves were collected from a BVD-positive herd by using a modified SPF technique and depriving the calves of colostrum. Eight of the 11 calves were from BVD-positive dams.

1. SPF calves

The group of 11 Holstein-Friesian calves was obtained by means of the following modified SPF technique. At the time of parturition the placenta was incised and manipulated to minimize or eliminate contact between the calf and the birth canal of the dam. Calves were taken from the dam and put into sterile galvanized steel cans, then delivered immediately to an isolated barn at the research laboratory. The calves did not receive colostrum, but were fed synthetic milk without antibiotics until two months old. After two months the calves were weaned by gradually changing their ration from synthetic milk and calf concentrate to a complete built-in roughage (BIR) ration. At approximately 3 months of age the calves were transferred to another isolation barn for the experiment. At that time they were divided into three groups of 3 calves each and one group of 2 calves. Each group of calves was kept in a separate room for the duration of the experiment. During the experiment all personnel showered and changed clothes before entering the barn in order to avoid the possibility of carrying infectious agents from outside sources to the

experimental animals. These calves were used in experiments 1, 2, 3, and 7 as outlined in Figure 1 and tested BVD-negative prior to vaccination.

2. Naturally born (NB) calves

The group of 12 Holstein-Friesian calves from serologically BVD-negative dams was assembled from the NADL BVD-negative herd. These calves were born naturally and allowed to suckle in order to obtain the benefits of colostrum. These calves were later fed commercial milk replacer and concentrate according to manufacturer's directions. At 2 months of age they were weaned and put on BIR rations in the same manner as the SPF calves. At approximately 3 months of age they were moved to the isolation barn, tested, divided into 4 groups with 3 animals in each group, and handled in the same manner as described for the SPF calves.

3. Plan of animal experimentation

The immunogenicity of the soluble antigen vaccine (SA), the chloroform-inactivated vaccine (Chl), and the BPL-inactivated vaccine (BPL) was evaluated in SPF and in NB calves. In each group, 2 calves served as vaccinates and 1 calf served as a contact control. All vaccinates were given 2,5-ml intramuscular injections of vaccine. The first inoculation was given when the calves were approximately 4 months of age and the second inoculation was given 4 weeks later. Contact control calves were not vaccinated.

One hundred eighty-two days after the second inoculation all vaccinates and contact controls were challenged with an intravenous

inoculation of 10 ml of tissue culture fluid containing approximately 10^7 $CCID_{50}$ per ml of the third passage NADL strain of BVD-MD virus. The barn control animals, 6120 and 6104 in experiment 7, were challenged by an intraocular instillation of virus into each eye. Animals 6127 and 6128, in experiment 8, were challenged by spraying approximately 1 ml of virus suspension into each nostril and calf 6114 was given 10 ml of the challenge virus orally. The postvaccination and postchallenge responses of the calves were studied from clinical, serologic, immunologic and pathologic aspects. The methods of measuring these responses will be described later.

Two SPF calves and 3 NB calves served as barn controls. These calves were used to detect any possible BVD-MD contamination during the experiment. These animals were not vaccinated but were challenged at the same time as the vaccinates and contact controls. Their response to challenge virus was to serve as a basis for comparison with that of the vaccinated calves.

C. Procedures for Evaluating Post-Vaccination and Post-Challenge Responses

1. Clinical

Pre-vaccination and pre-challenge temperatures were recorded daily for 14 days before the calves were vaccinated and challenged, and post-vaccination and post-challenge body temperatures were also recorded daily for at least 14 days.

All experimental calves were observed daily for evidence of clinical signs of illness such as anorexia, nasal discharge, excessive lachrymation

Experiment number	Type of birth	Colostrum	Calf number	Vaccine used
1	SPF	Deprived	6119, 6123, 6121	SA None (contact control)
2	Natural	Permitted	6086, 6116, 6071	SA None (contact control)
3	SPF	Deprived	6098, 6099, 6066	Chl None (contact control)
4	Natural	Permitted	6105, 6102 6087	Chl None (contact control)
5	SPF	Deprived	6060, 6080, 6088	BPL None (contact control)
6	Natural	Permitted	6097, 6100, 6106	BPL None (contact control)
7	SPF	Deprived	6120, 6104	None (barn control)
8	Natural	Permitted	6114, 6127, 6128	None (barn control)

Figure 1. Precis of animal experiments

and salivation, listlessness, diarrhea, constipation, lameness and for evidence of lesions on the tongue, gums, dental pad, muzzle, lips, and hard palate.

2. Laboratory

a. Hematology Blood samples were collected from each calf for leukocyte counts. Several pre-vaccination and pre-challenge samples were obtained and the post-vaccination and post-challenge bleeding schedule was as follows: daily samples for 14 days, followed by weekly samples until challenge. Samples of blood were collected in 10% ammonium and potassium oxalate in sterile 10 ml tubes. The anticoagulant and the blood were mixed thoroughly and taken immediately to the laboratory for leukocyte counts. After the leukocyte count was made the remainder of the sample was used for virus isolation.

b. Virology Pre-vaccination, post-vaccination, pre-challenge and post-challenge samples of blood for virus isolation were collected at various times in 10 ml tubes containing 10% ammonium and potassium oxalate solution. Samples were immediately placed in a PR-2 refrigerated centrifuge[1] and spun at 2000 RPM for 50 minutes. After the plasma had been pipetted off, the leukocytes were carfully removed with a Pasteur pipette. Approximately 0.2 ml of leukocytes was inoculated into each of 4 tubes of primary EBK cell cultures. The inoculated cultures were incubated at 37 C and were observed daily for evidence of cytopathic effect (CPE). When CPE was not apparent,

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[1]International Equipment Company, Boston, Massachusetts.

the fluids were subcultured into 4 additional tubes of primary EBK cell cultures and observed daily for 7 days for evidence of CPE. Virus isolations were considered negative when at least 3 passages were made on EBK cells and there was no CPE.

At various times before and after vaccination and challenge nasal secretions were collected with cotton swabs and examined for the presence of BVD virus in the secretions. As these samples were obtained they were placed immediately into EBSS and stored at -20 F until examined. Approximately 0.2 ml of this solution was inoculated into each of 4 tubes of primary EBK cell cultures. These were likewise incubated at 37 C and observed daily for evidence of CPE and considered negative when at least 3 passages were made on EBK cells and there was no CPE.

Rectal swabs were also taken from each experimental animal at the identical intervals as for collecting nasal secretions. Samples were placed in EBSS containing antibiotics and stored at -20 F until inoculated into primary EBK cell cultures. Maximum storage time before examination was 6 days. Inoculation and incubation of primary EBK cell cultures was similar to the procedures described for nasal swabs.

c. Serology Blood samples for serologic studies were collected at identical times to the blood collection schedule described previously.

The serum neutralization test was used to study the antibody responses of the cattle. The beta method of constant virus and varying

serum dilutions was used. Test sera were heat-inactivated in a 56 C water bath for 30 minutes. Serial 4-fold dilutions of each serum to be tested were prepared in 1.0 ml of EBSS. An equal amount of EBSS containing approximately 100 $CCID_{50}$ per ml of NADL-MD virus was added to the serum dilutions. The mixtures were incubated at 37 C for one hour and then 0.2 ml of each serum-virus mixture was inoculated into each of 4 tubes containing primary EBK cell cultures. The cultures were incubated at 37 C and observed daily for evidence of cytopathic effect for 7 days. The antibody titer was expressed as the reciprocal of the dilution giving CPE in 50% of the tubes.

IV. RESULTS

A table for each animal, listing the recorded body temperature, leukocyte counts, antibody titers, signs of illness, and results of attempted virus isolations may be found in the appendix. Graphs showing the serologic response, temperature, and leukocyte count for each animal appear in Figures 2 through 24.

None of the control animals developed detectable neutralizing antibodies against BVD-MD virus before being challenged with virulent virus; however, all of these animals developed relatively high titers after challenge.

Following the first inoculation of vaccine, 9 of the 12 calves developed a low level of BVD antibodies but 3 calves failed to develop detectable levels of antibody. After the second inoculation, a strong secondary serologic response was detected in all vaccinates.

After challenge, neither the vaccinates nor the control animals developed ulcers in the oral cavity that are usually associated with the disease. Virus isolation tests were negative for all vaccinates, but BVD-MD virus was isolated from all the controls after challenge.

A. Soluble Antigen Vaccine

1. Experiment 1 (SPF calves)

Calf 6123 (Figure 2) and calf 6119 (Figure 3) developed low antibody titers 7 days after the first vaccination and peak titers of 16,384 following the second inoculation. After calf 6123 was challenged it had a slight temperature elevation and leukopenia but no clinical

signs of disease. Calf 6119 developed a temperature elevation, leukopenia and acute signs of illness such as depression, anorexia, and respiratory involvement.

The contact control, calf 6121 (Figure 4), had a mild depression, anorexia, and a dry non-productive cough following challenge. One may also note that there was a temperature spike of 105 F and a leukopenia.

2. Experiment 2 (NB calves)

Both vaccinates, 6086 and 6116 (Figures 5 and 6), developed an antibody titer of 8 after the first inoculation and peak titers of 16,384 following the second inoculation of vaccine. Calf 6116 had a marked rise in titer following challenge but calf 6086 had only a 4-fold increase in titer. The body temperature of both calves was normal during the entire time of the experiment but calf 6086 developed a slight leukocytosis on the ninth day postchallenge while calf 6116 had a slight leukopenia on the fifth day postchallenge. Neither of the vaccinates had clinical signs of illness following challenge.

Calf 6071, the contact control, did have a slight temperature elevation and leukopenia the third day postchallenge and was anorectic and depressed from the third to the fifth day after receiving the challenge virus.

B. Chloroform Inactivated Vaccine

1. Experiment 3 (SPF calves)

It is interesting to note that neither of the twin calves, 6098 and 6099, (Figures 8 and 9) developed detectable levels of antibodies

after the first inoculation of vaccine but did have strong and nearly identical secondary responses. Following challenge neither of the vaccinates appeared ill although both had a slight leukopenia and calf 6098 had a slight temperature elevation on the eighth day postchallenge.

The control animal, 6066 (Figure 10), had 2 temperature spikes and a diphasic leukopenia; however, the only signs of illness were a loss of appetite on the seventh day and a depressed appearance on the seventh and tenth day postchallenge.

2. Experiment 4 (NB calves)

Calves 6105 and 6102 (Figures 11 and 12) both had detectable but low levels of antibodies 7 days after the first inoculation and both showed a good secondary response to the vaccine. Calf 6105 had a leukopenia following challenge but was not clinically ill. Calf 6102 had a temperature of 107 F and was slightly depressed on the ninth day postchallenge.

The control calf, 6087 (Figure 13), was clinically ill from the third through the eighth day postchallenge as evidenced by marked depression and loss of appetite. This animal also had a temperature elevation on the seventh and eighth day after challenge and a leukopenia on the third and seventh day postchallenge.

C. Beta-Propiolactone Inactivated Vaccine

1. Experiment 5 (SPF calves)

Calf 6080 (Figure 14) had a typical primary response with a low antibody titer and a strong secondary response to the vaccine with

a peak titer of 4096. The other vaccinate, calf 6060 (Figure 15), was the third example of priming as a result of the first inoculation followed by a strong secondary response with a peak titer of 2048. Both vaccinated calves remained free of clinical signs of disease after challenge and the body temperature and leukocyte count remained normal for calf 6080; however, calf 6060 did have a slight leukopenia for 2 days and a temperature of 105 F for 1 day.

Calf 6088 (Figure 16), the contact control, was ill from the second to the seventh day postchallenge as evidenced by depression, anorexia, respiratory involvement, laminitis, leukopenia and temperature elevation.

2. Experiment 6 (NB calves)

The primary response of calf 6100 (Figure 17) was not detectable until 21 days after the first inoculation but the secondary response was prompt and reached a peak titer of 8,182. In spite of a high titer of neutralizing antibodies at the time of challenge, the calf evidenced signs of depression on the seventh and eighth days postchallenge and a temperature spike of 104 F occurred on the seventh day. A slight diphasic leukopenia was detected on the seventh and ninth days after challenge.

Calf 6097 (Figure 18) had an antibody titer of only 2 at the time of the second inoculation of vaccine, but the titer increased to 4096 after 3 weeks. On the seventh day after challenge, the calf had a mild diarrhea, a temperature of 107 F and a leukopenia.

The contact control, 6106 (Figure 19), appeared depressed from the third to the seventh day postchallenge, also had diarrhea on the sixth and seventh day. Although a rather marked leukopenia did occur on the fourth and sixth days postchallenge a body temperature elevation was not detected.

D. Challenge of Barn Control Calves

1. Experiment 7 (SPF calves)

Calves 6104 (Figure 20) and 6120 (Figure 21) were challenged by an intraocular instillation of approximately 1 ml of the challenge virus. Both calves were infected as evidenced by the immune response to the challenge virus and the appearance of a very mild depression following challenge. The clinical signs of illness were almost inapparent. Although calf 6104 had an elevated temperature and leukopenia, calf 6120 did not.

2. Experiment 8 (NB calves)

Calf 6127 (Figure 22) and calf 6128 (Figure 23) were challenged with 1 ml of virus sprayed into each nostril and calf 6114 (Figure 24) was given 10 ml of the virus orally. All three calves developed peak BVD antibody titers of 4096 in 5 to 6 weeks.

Calves 6127 and 6128 became clinically ill after challenge as evidenced by depression, anorexia and a transient respiratory involvement. Calf 6127 had a temperature elevation and leukopenia and 6128 had a leukopenia but no temperature spike was detected. Calf 6128 did have diarrhea on the second and third day after challenge.

Of the 23 calves used in the entire experiment, calf 6114 developed the most acute signs of illness. It had a severe diarrhea with flecks of blood, dehydration, loss of appetite and was depressed. It also had a diphasic leukopenia with concurrent temperature spikes.

Figure 2. Calf 6123, an SPF calf, vaccinated IM with SA vaccine and challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

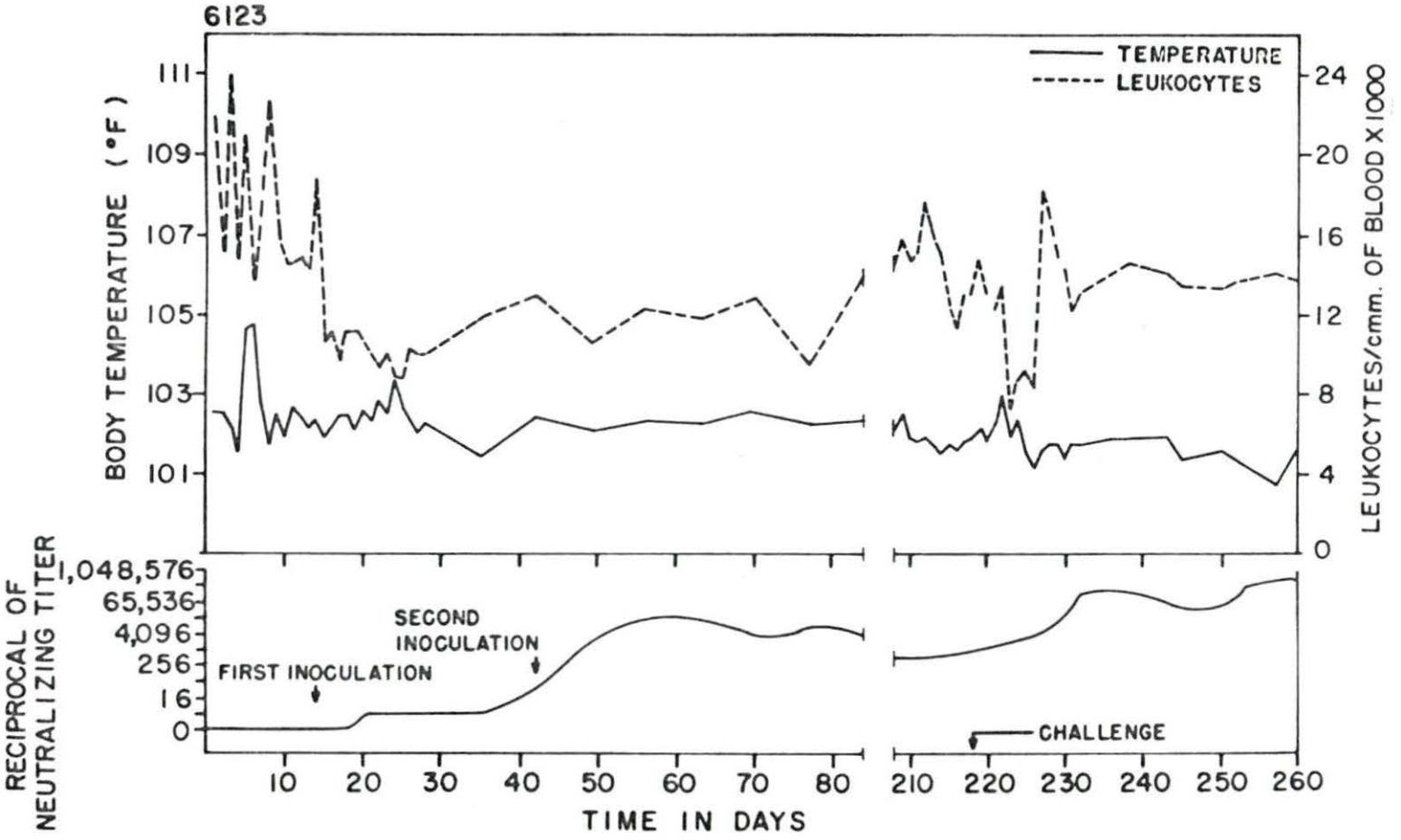

6123
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
LEUKOCYTES/cmm. OF BLOOD X 1000
24
20
16
12
8
4
0
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10 20 30 40 50 60 70 80
210 220 230 240 250 260
TIME IN DAYS

Figure 3. Calf 6119, an SPF calf, vaccinated IM with SA vaccine and challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

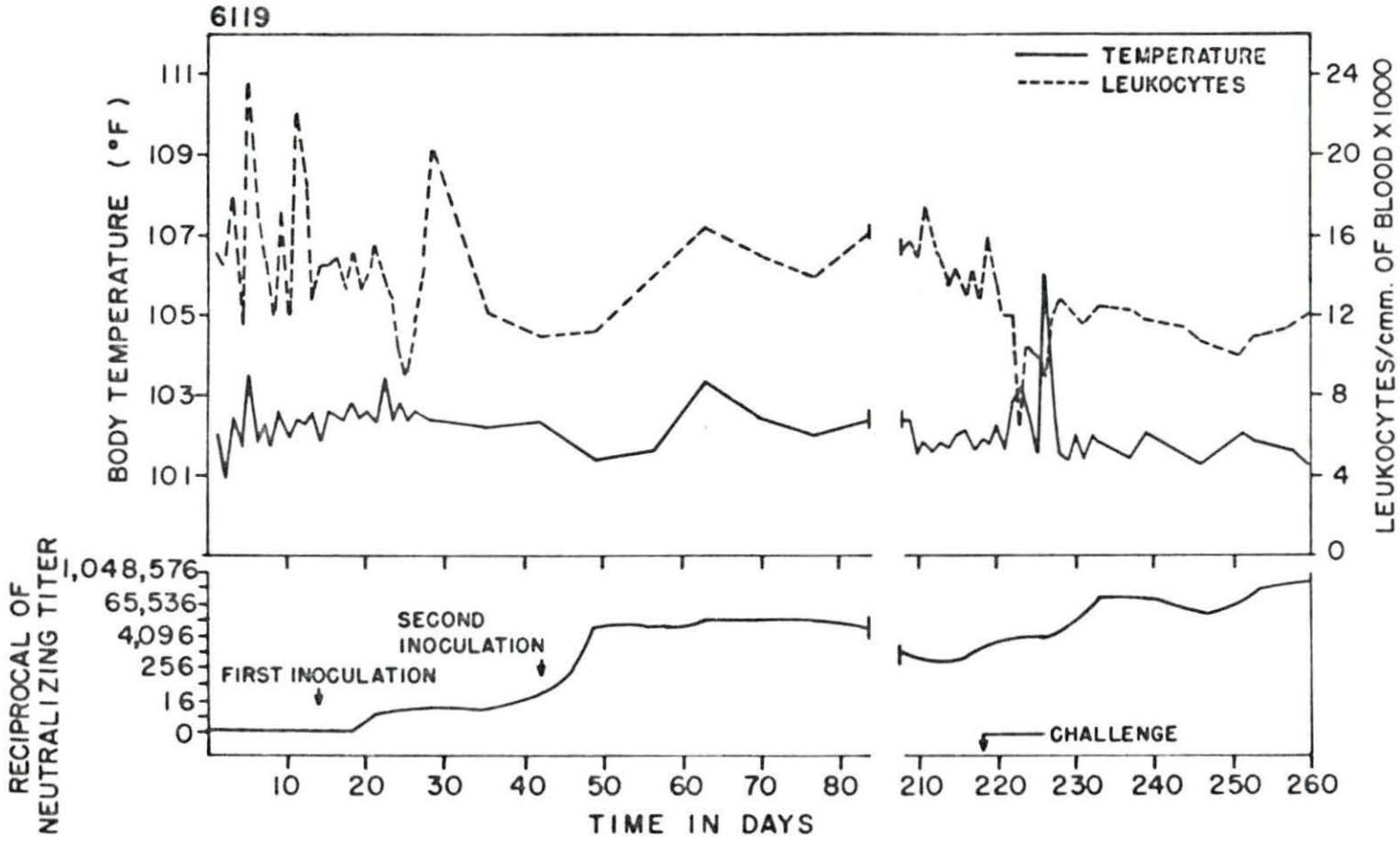

6119
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
LEUKOCYTES/cmm. OF BLOOD X1000
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
TIME IN DAYS

Figure 4. Calf 6121, an SPF calf, was the contact control with calves 6123 and 6119. It did not receive vaccine but was challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

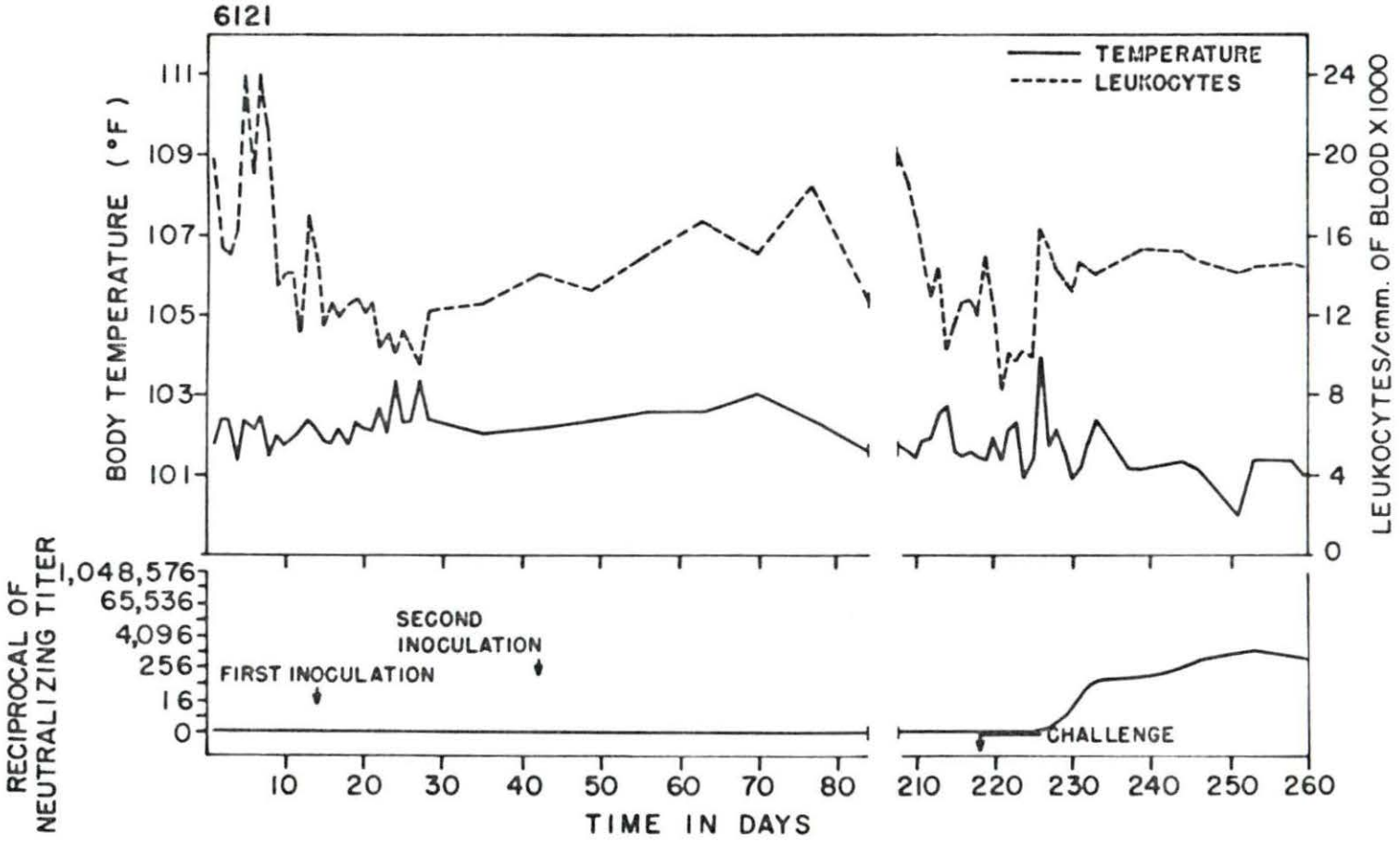

6121
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
LEUKOCYTES/cmm. OF BLOOD X 1000
24
20
16
12
8
4
0
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10
20
30
40
50
60
70
80
210
220
230
240
250
260
TIME IN DAYS

Figure 5. Calf 6086, an NB calf, was vaccinated IM with SA vaccine and challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

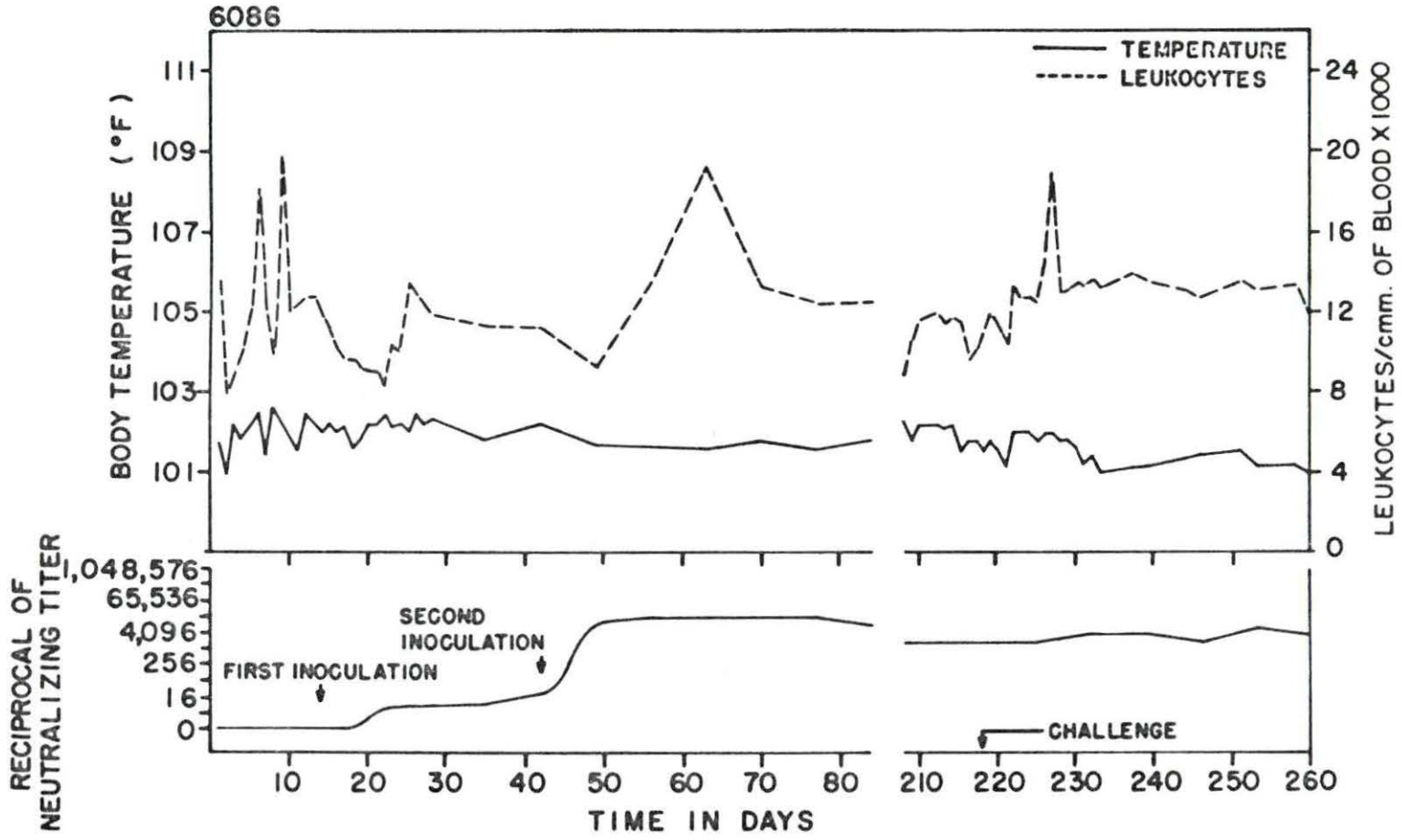

6086
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
24
20
16
12
8
4
0
LEUKOCYTES/cmm. OF BLOOD X 1000
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10 20 30 40 50 60 70 80
210 220 230 240 250 260
TIME IN DAYS

Figure 6. Calf 6116, an NB calf, was vaccinated IM with SA vaccine and challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

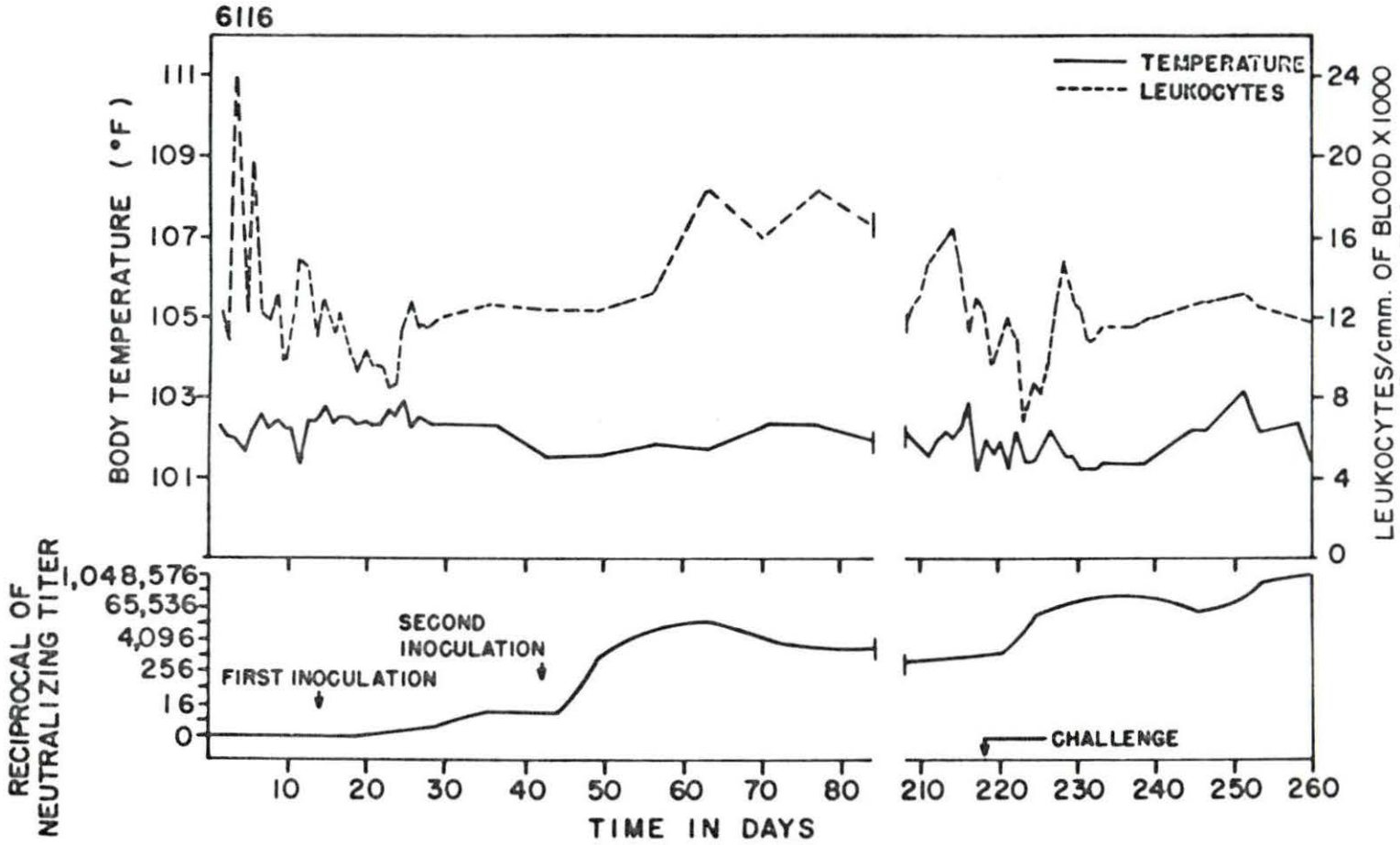

6116
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
LEUKOCYTES/cmm. OF BLOOD X 1000
24
20
16
12
8
4
0
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10 20 30 40 50 60 70 80
210 220 230 240 250 260
TIME IN DAYS

Figure 7. Calf 6071, an NB calf, was the contact control with calves 6086 and 6116. It did not receive vaccine but was challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

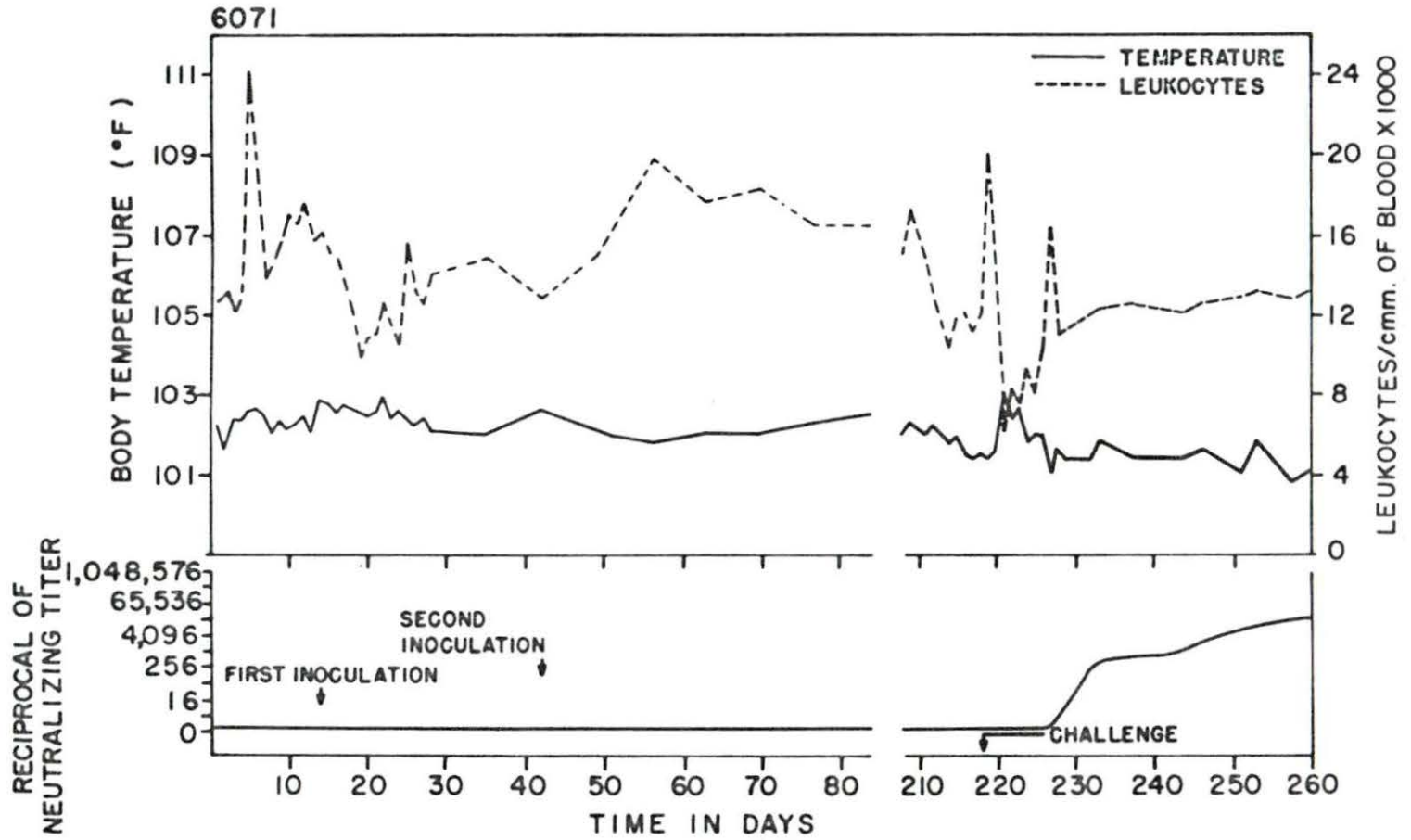

6071
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
LEUKOCYTES/cmm. OF BLOOD X 1000
24
20
16
12
8
4
0
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10 20 30 40 50 60 70 80
210 220 230 240 250 260
TIME IN DAYS

Figure 8. Calf 6098, an SPF calf, was vaccinated IM with Chl vaccine and challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

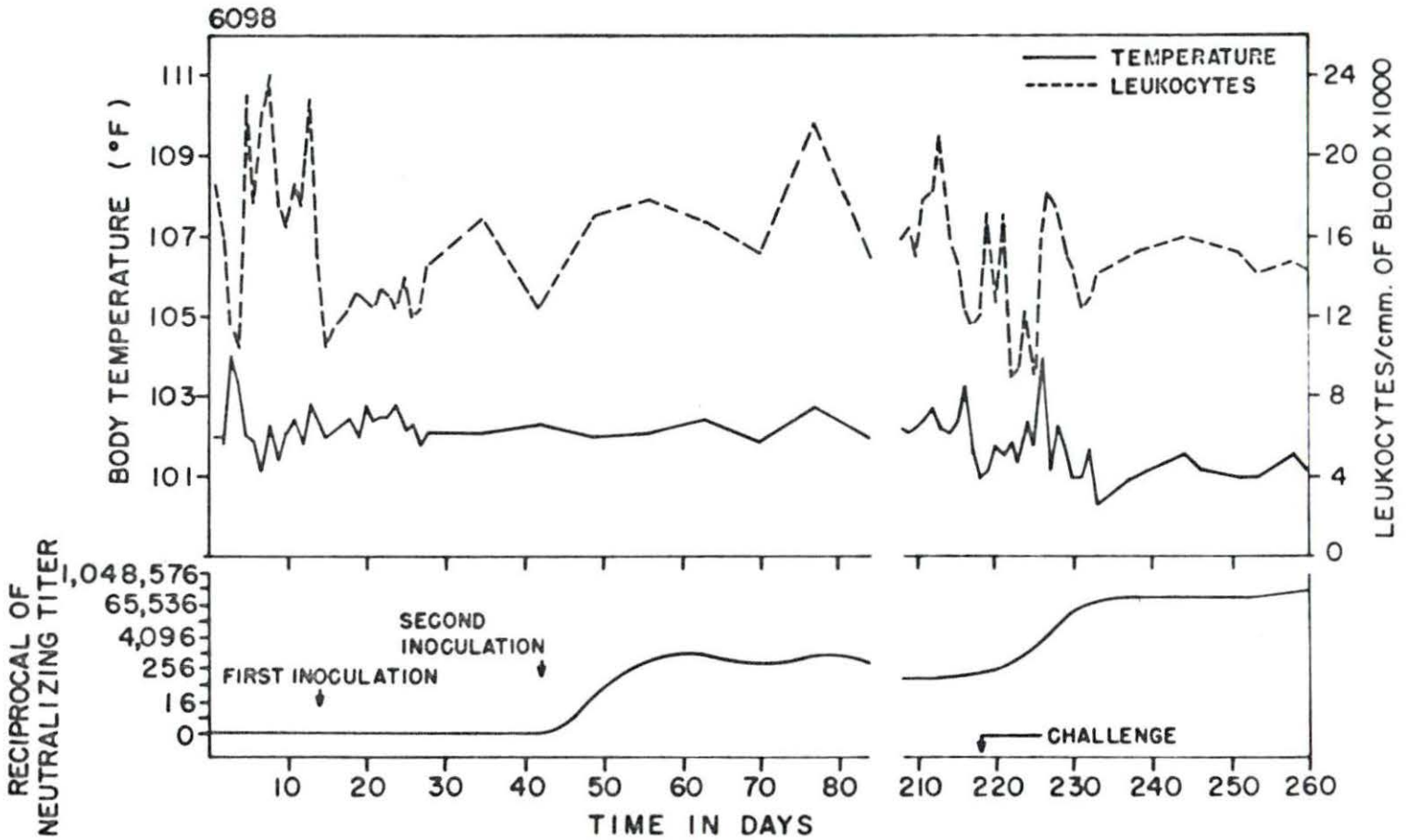

6098
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
LEUKOCYTES/cmm. OF BLOOD X 1000
24
20
16
12
8
4
0
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10 20 30 40 50 60 70 80
210 220 230 240 250 260
TIME IN DAYS

Figure 9. Calf 6099, an SPF calf, vaccinated IM with Chl vaccine and challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

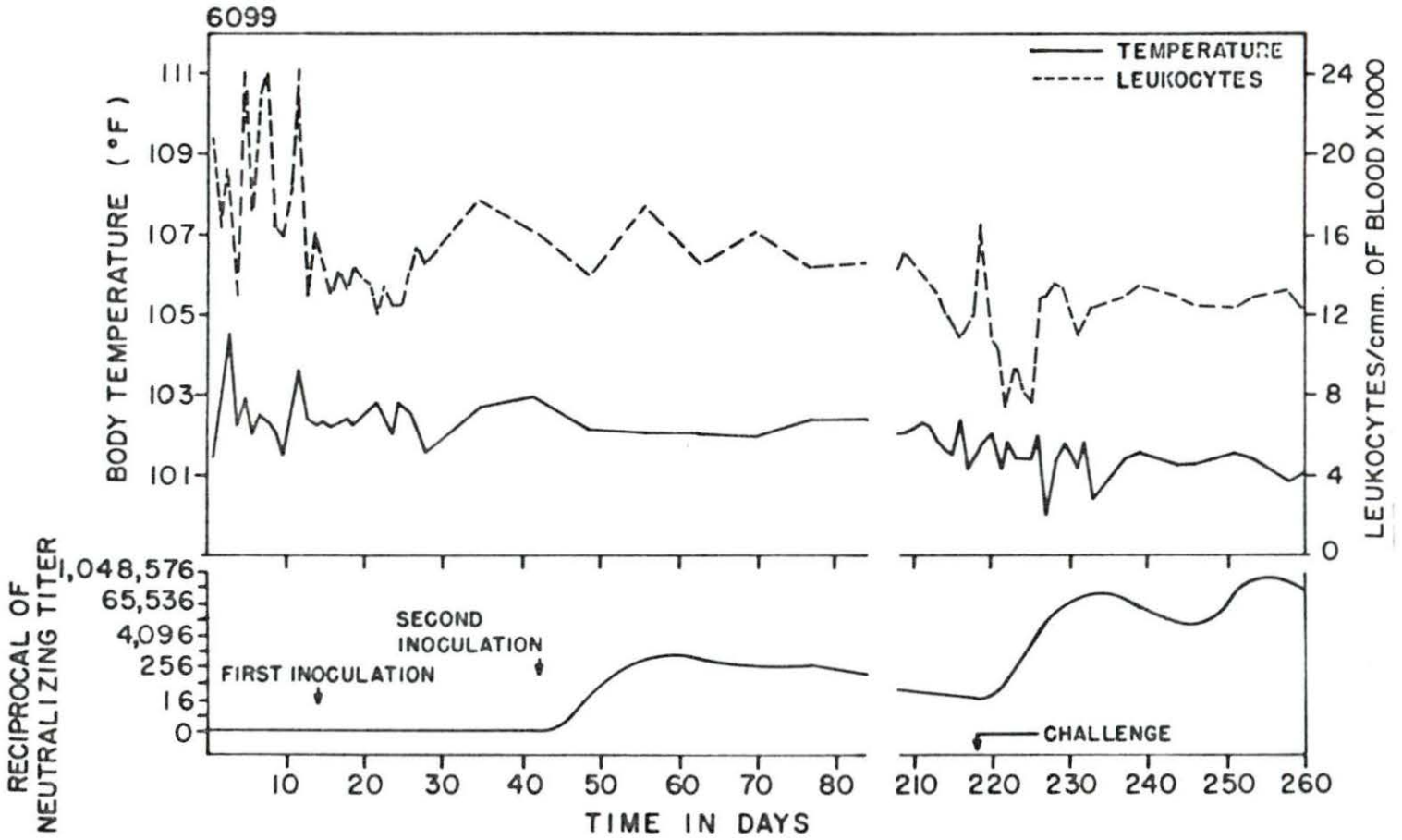

6099
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
24
20
16
12
8
4
0
LEUKOCYTES/cmm. OF BLOOD X 1000
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10 20 30 40 50 60 70 80
210 220 230 240 250 260
TIME IN DAYS

Figure 10. Calf 6066, an SPF calf, was the contact control with calves 6098 and 6099. It did not receive vaccine but was challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

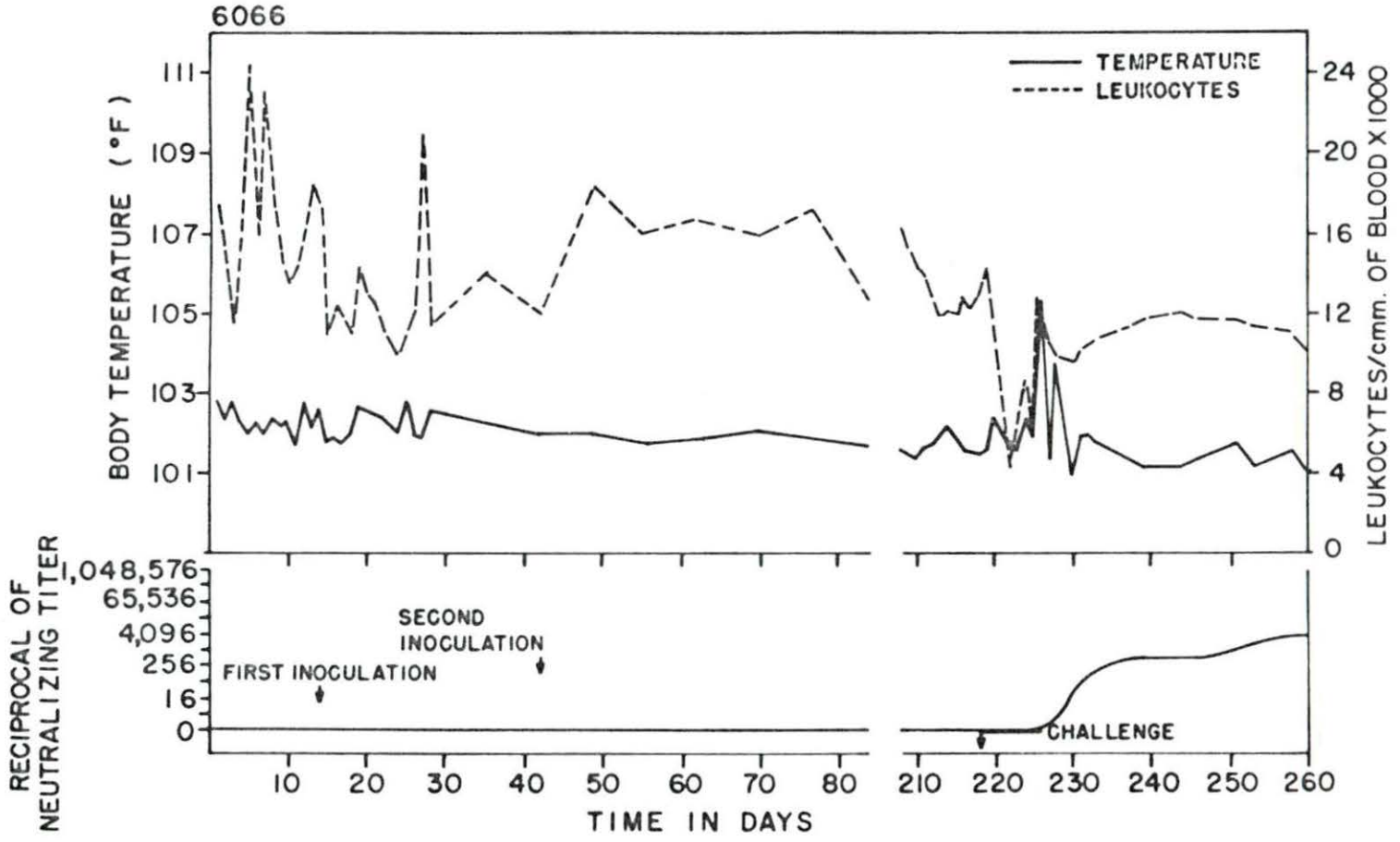

6066
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
LEUKOCYTES/cmm. OF BLOOD X 1000
RECIPROCAL OF NEUTRALIZING TITER
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
TIME IN DAYS

Figure 11. Calf 6105, an NB calf, was vaccinated IM with Chl vaccine and challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

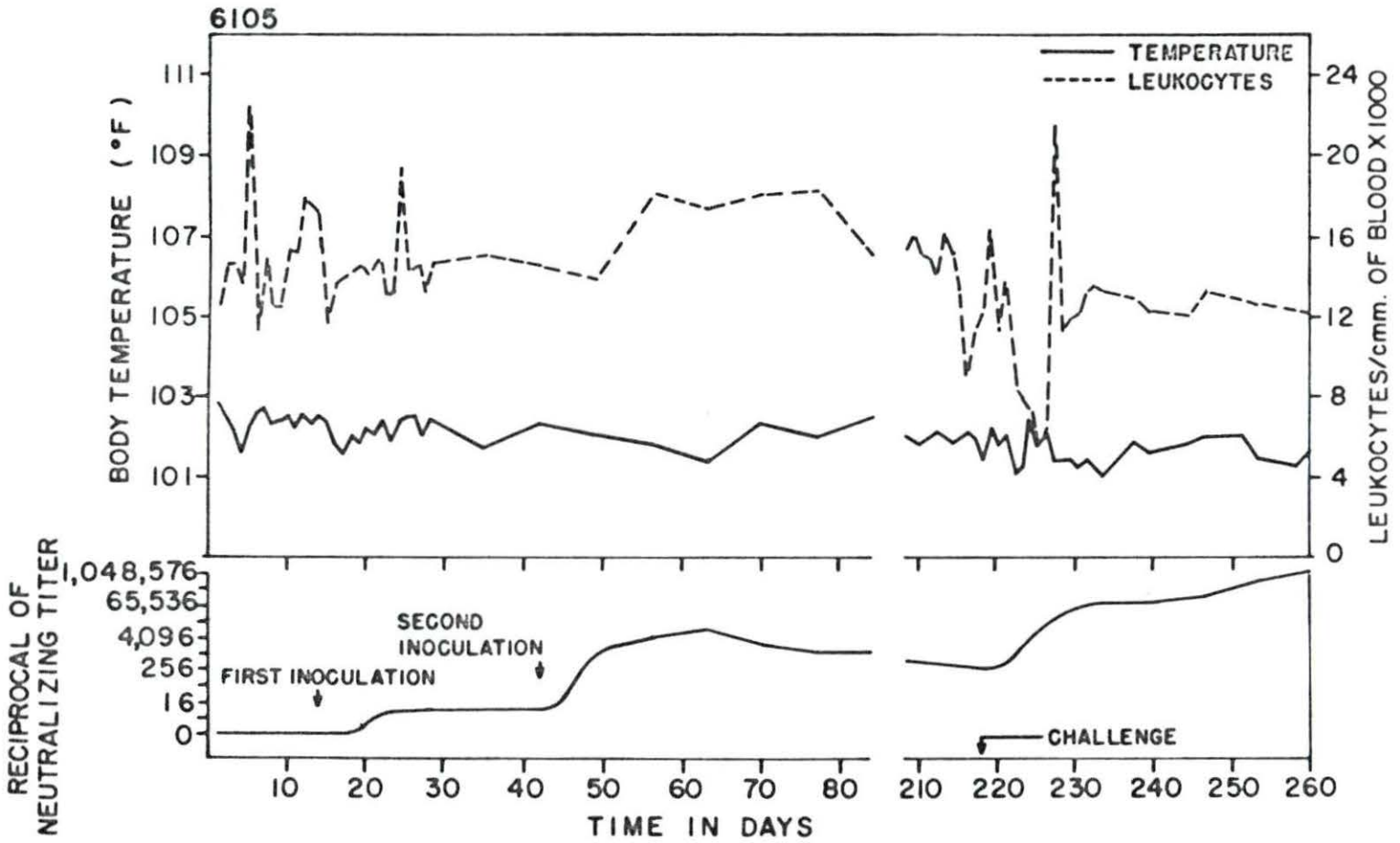

6105
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
LEUKOCYTES/cmm. OF BLOOD X 1000
RECIPROCAL OF NEUTRALIZING TITER
FIRST INOCULATION
SECOND INOCULATION
CHALLENGE
TIME IN DAYS

Figure 12. Calf 6102, an NB calf, was vaccinated IM with Chl vaccine and challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

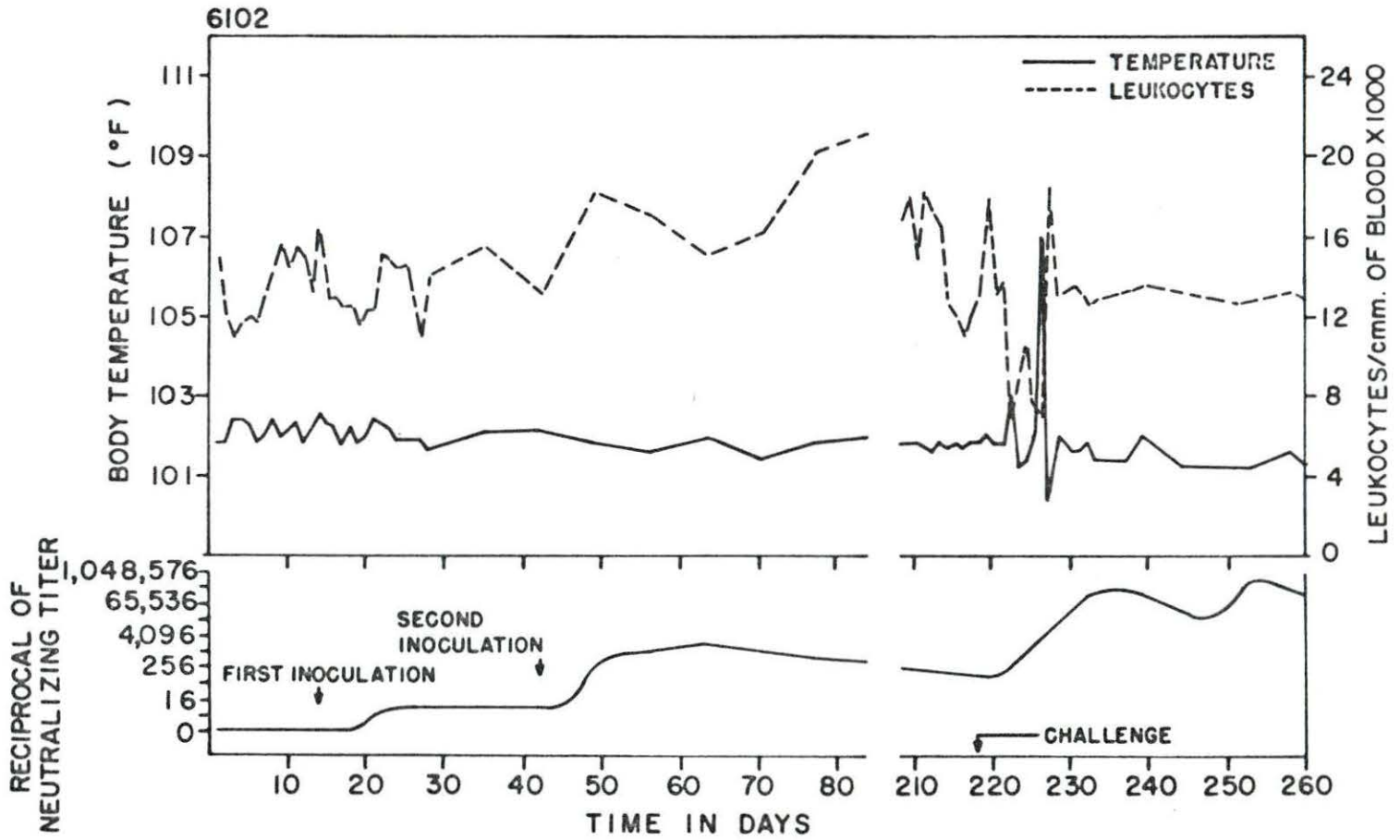

6102
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
24
20
16
12
8
4
0
LEUKOCYTES/cmm. OF BLOOD X 1000
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10 20 30 40 50 60 70 80
210 220 230 240 250 260
TIME IN DAYS

Figure 13. Calf 6087, an NB calf, was the contact control with calves 6102 and 6105. It did not receive vaccine but was challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

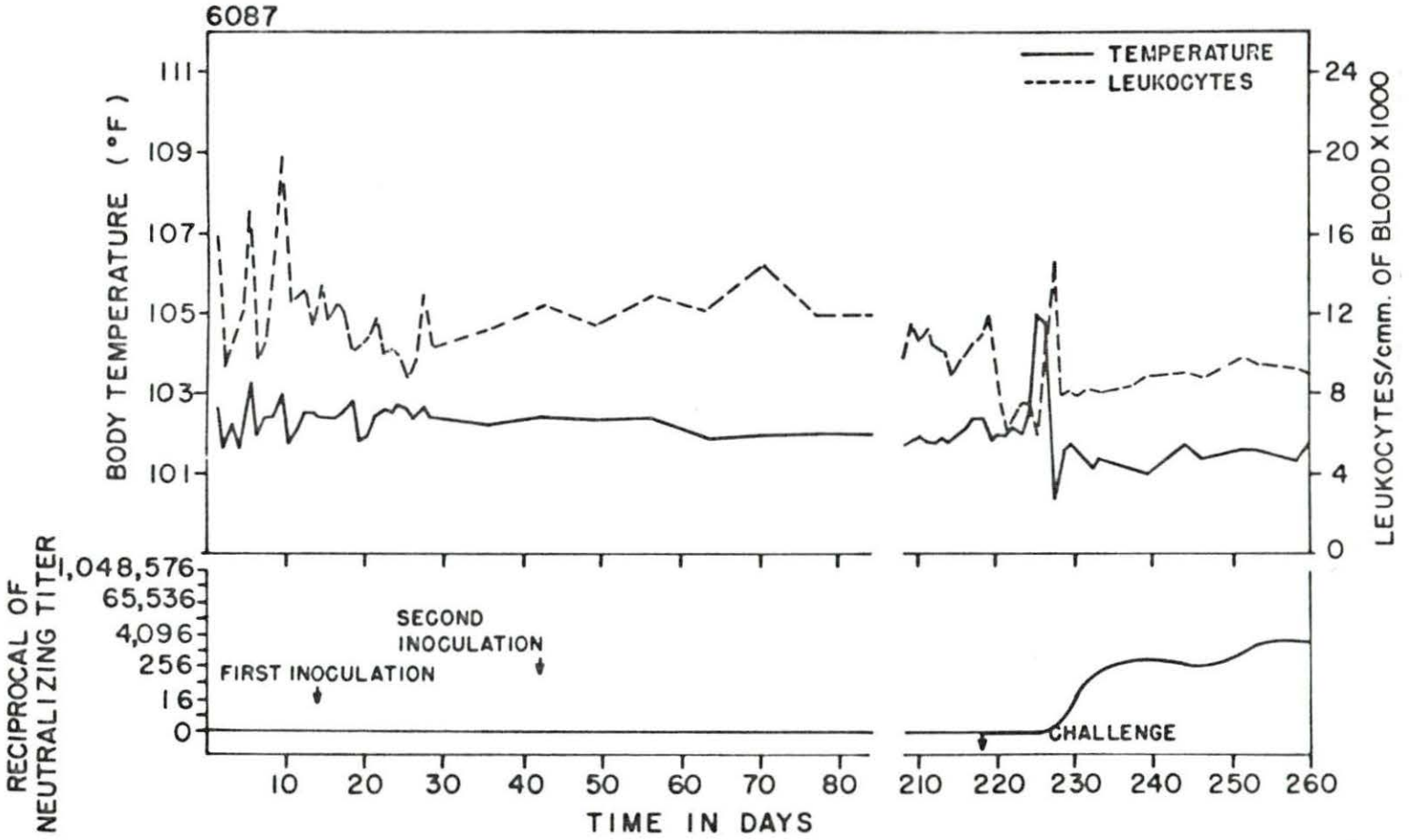

6087
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
LEUKOCYTES/cmm. OF BLOOD X 1000
RECIPROCAL OF NEUTRALIZING TITER
FIRST INOCULATION
SECOND INOCULATION
CHALLENGE
TIME IN DAYS

Figure 14. Calf 6080, an SPF calf, was vaccinated IM with BPL vaccine and challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

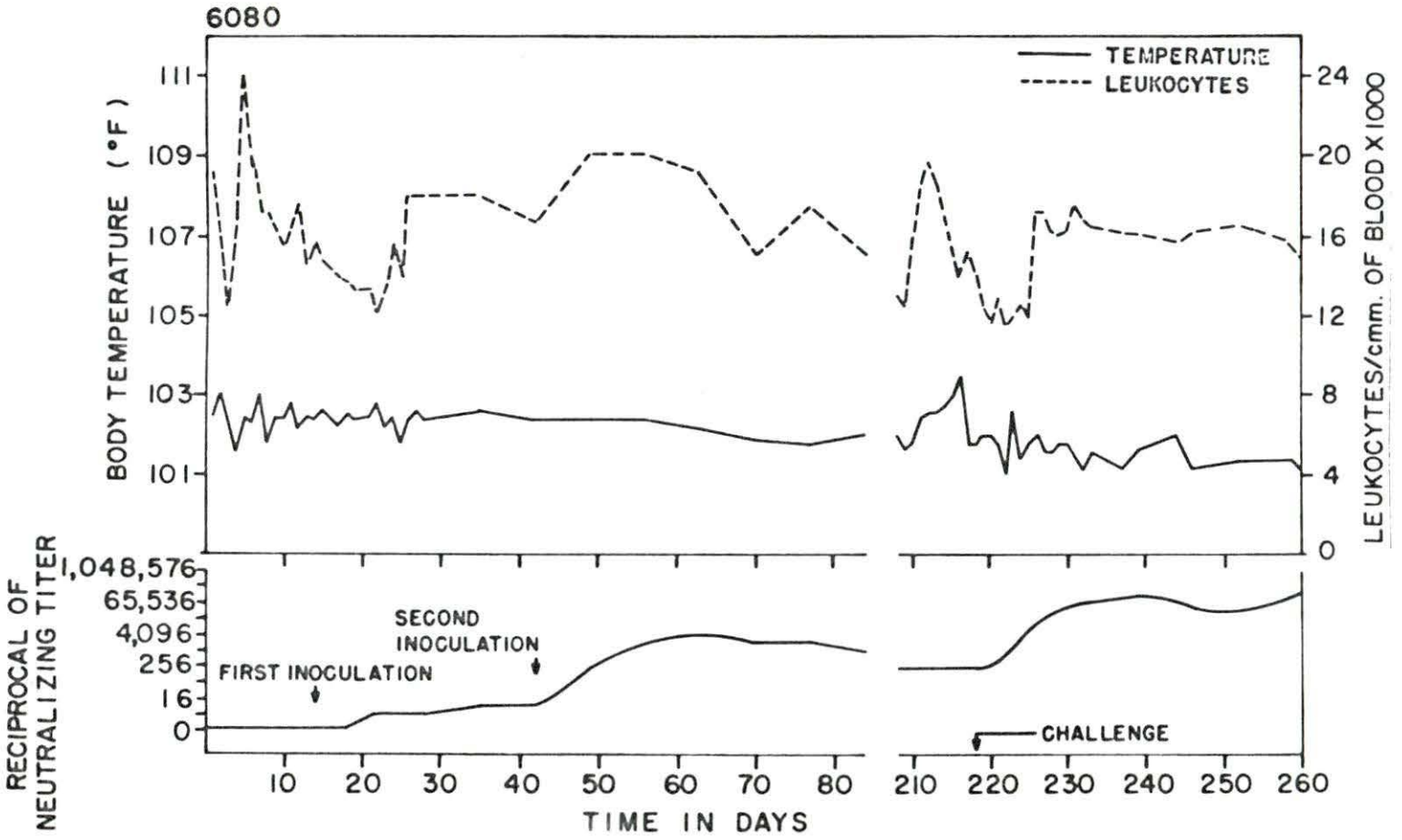

6080
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
24
20
16
12
8
4
0
LEUKOCYTES/cmm. OF BLOOD X1000
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10 20 30 40 50 60 70 80
210 220 230 240 250 260
TIME IN DAYS

Figure 15. Calf 6060, an SPF calf, was vaccinated IM with BPL vaccine and challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

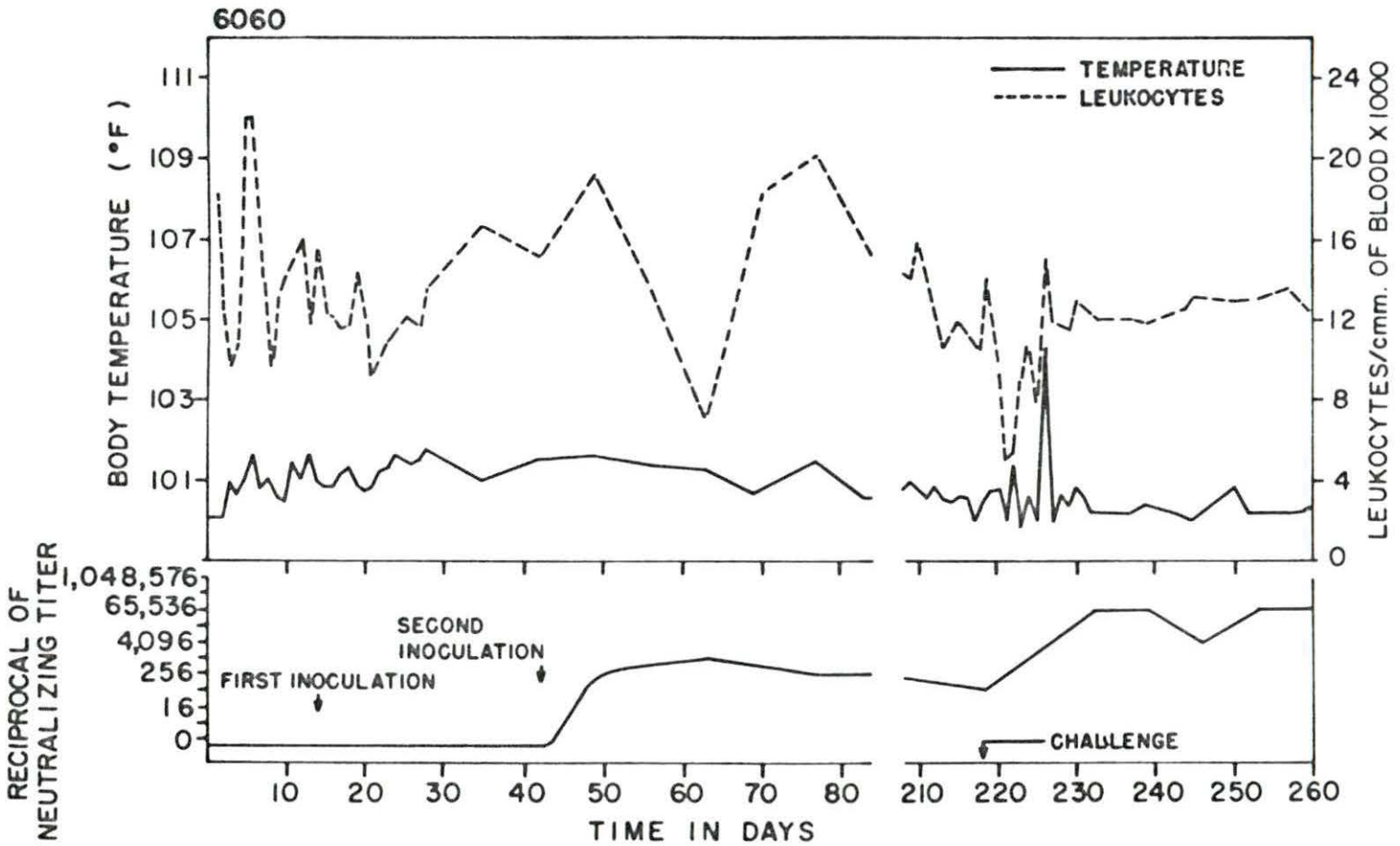

6060
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
LEUKOCYTES/cmm. OF BLOOD X 1000
24
20
16
12
8
4
0
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10 20 30 40 50 60 70 80
210 220 230 240 250 260
TIME IN DAYS

Figure 16. Calf 6088, an SPF calf, was the contact control with calves 6060 and 6080. It did not receive vaccine but was challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

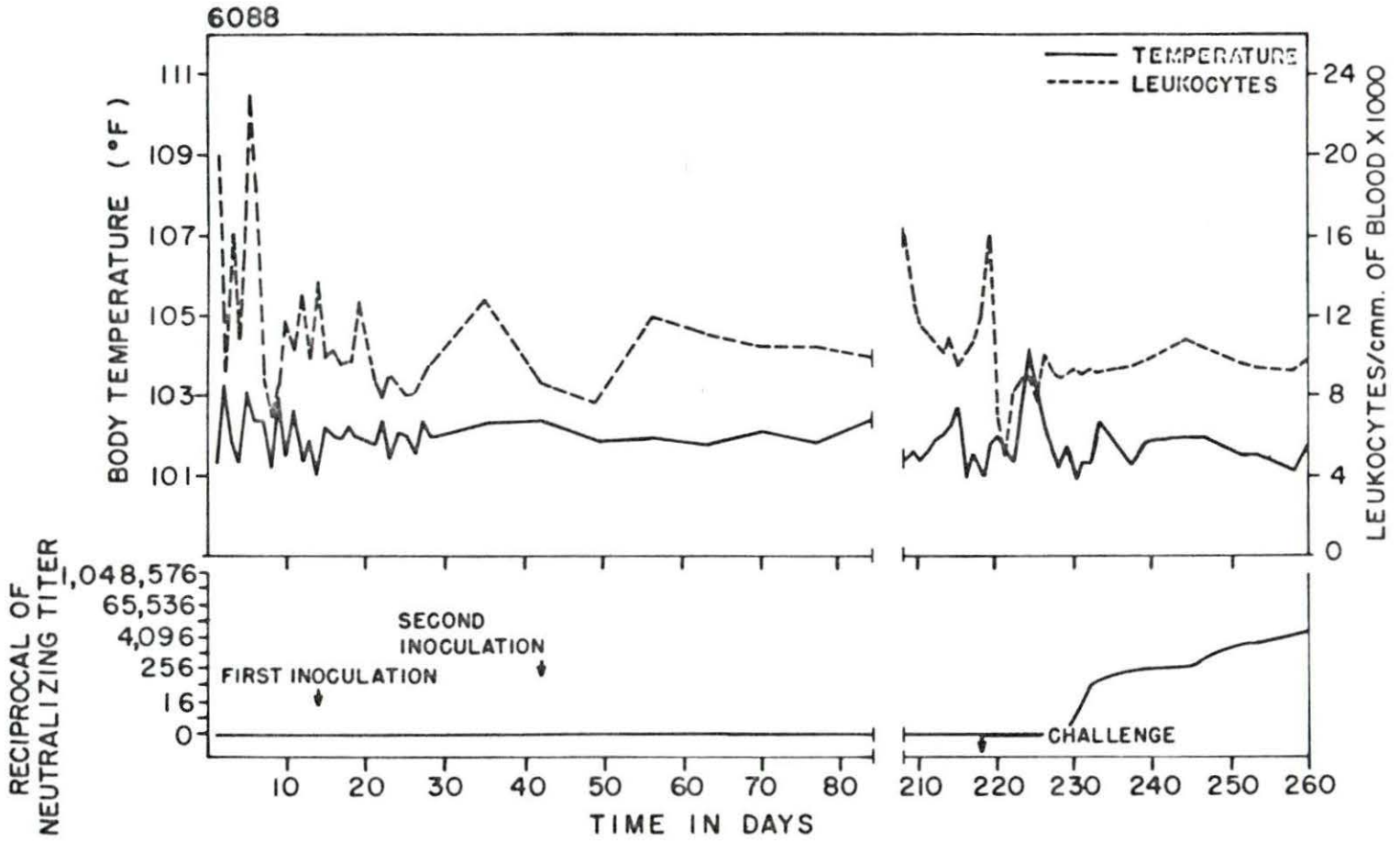

6088
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
LEUKOCYTES/cmm. OF BLOOD X 1000
RECIPROCAL OF NEUTRALIZING TITER
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
TIME IN DAYS

Figure 17. Calf 6100, an NB calf, was vaccinated IM with BPL vaccine and challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

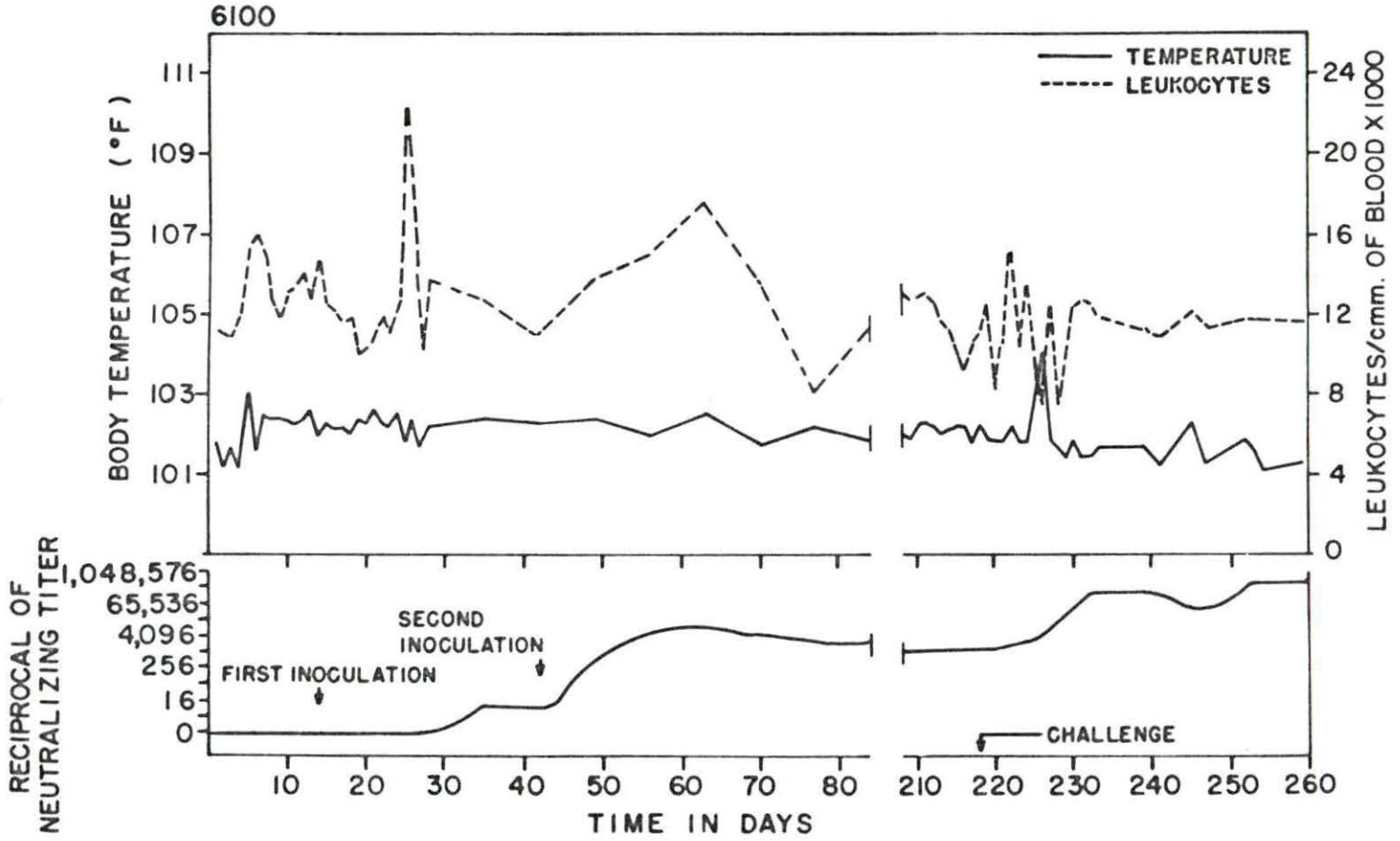

6100
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
LEUKOCYTES/cmm. OF BLOOD X 1000
RECIPROCAL OF NEUTRALIZING TITER
FIRST INOCULATION
SECOND INOCULATION
CHALLENGE
TIME IN DAYS

Figure 18. Calf 6097, an NB calf, was vaccinated IM with BPL vaccine and challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

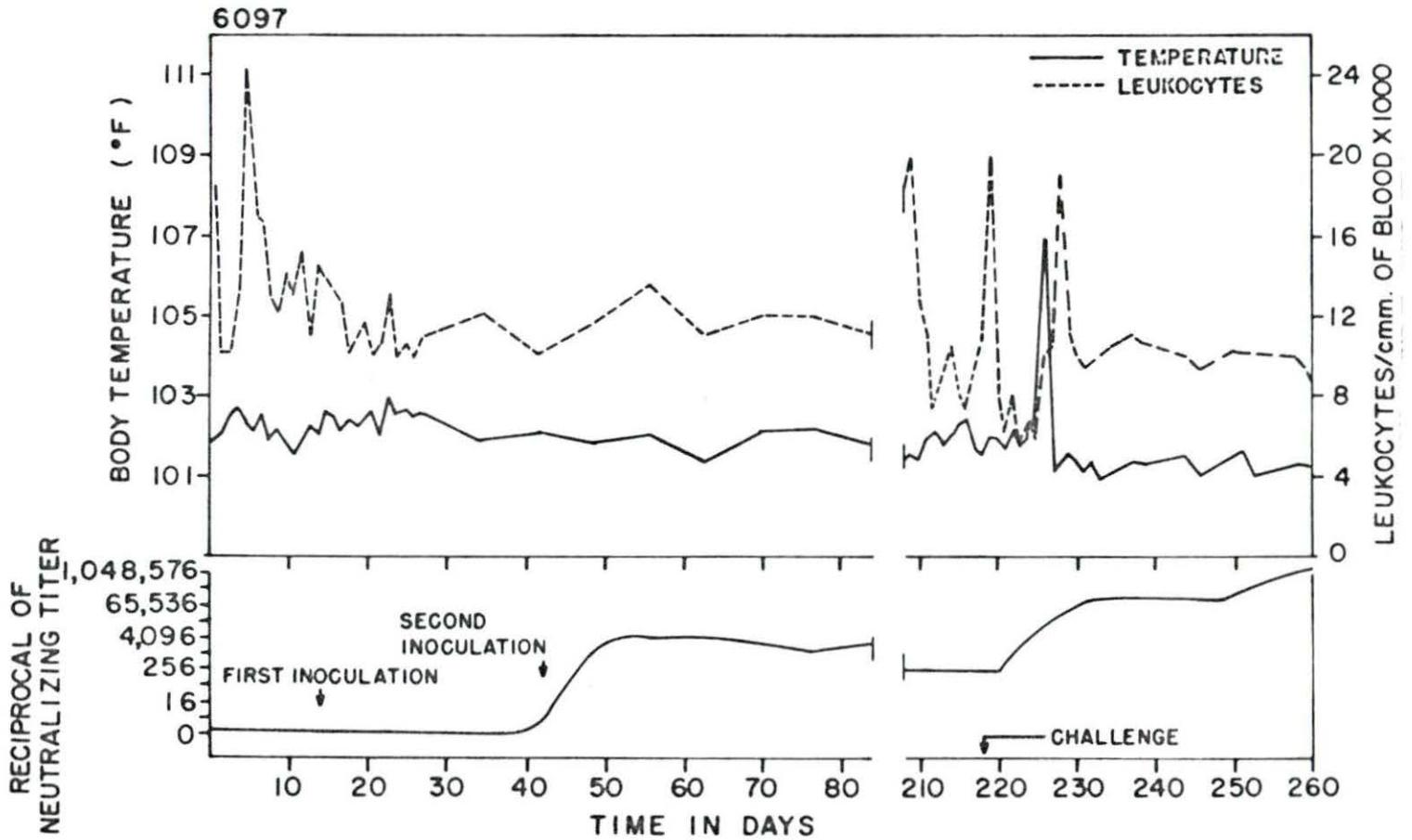

6097
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
LEUKOCYTES/cmm. OF BLOOD X 1000
RECIPROCAL OF NEUTRALIZING TITER
FIRST INOCULATION
SECOND INOCULATION
CHALLENGE
TIME IN DAYS

Figure 19. Calf 6106, an NB calf, was the contact control with calves 6097 and 6100. It did not receive vaccine but was challenged IV with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

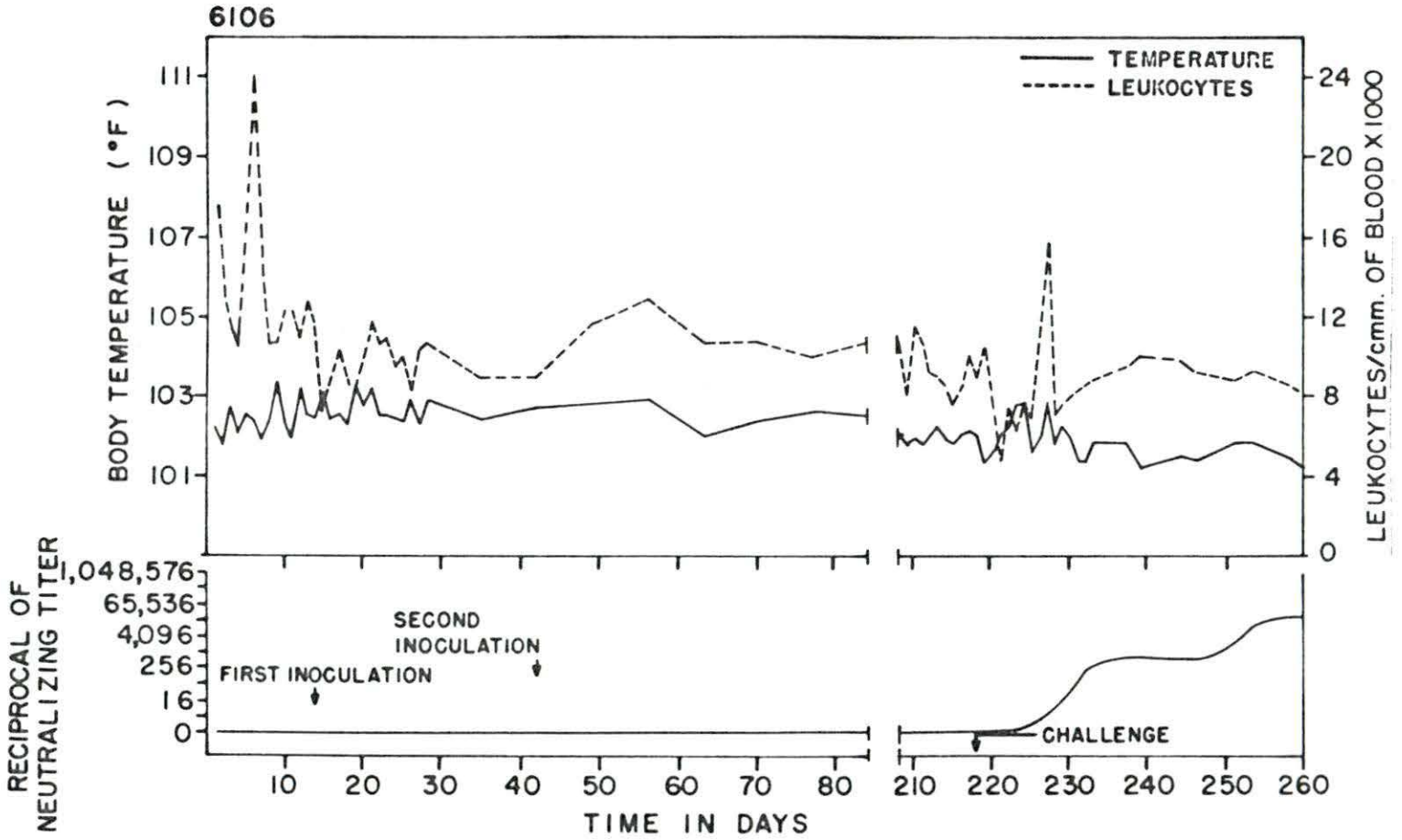

6106
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
LEUKOCYTES/cmm. OF BLOOD X 1000
RECIPROCAL OF NEUTRALIZING TITER
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
TIME IN DAYS

Figure 20. Calf 6104, an SPF calf, was a barn control animal. It did not receive vaccine, but was challenged intraocularly with third EBK passage of NADL strain BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

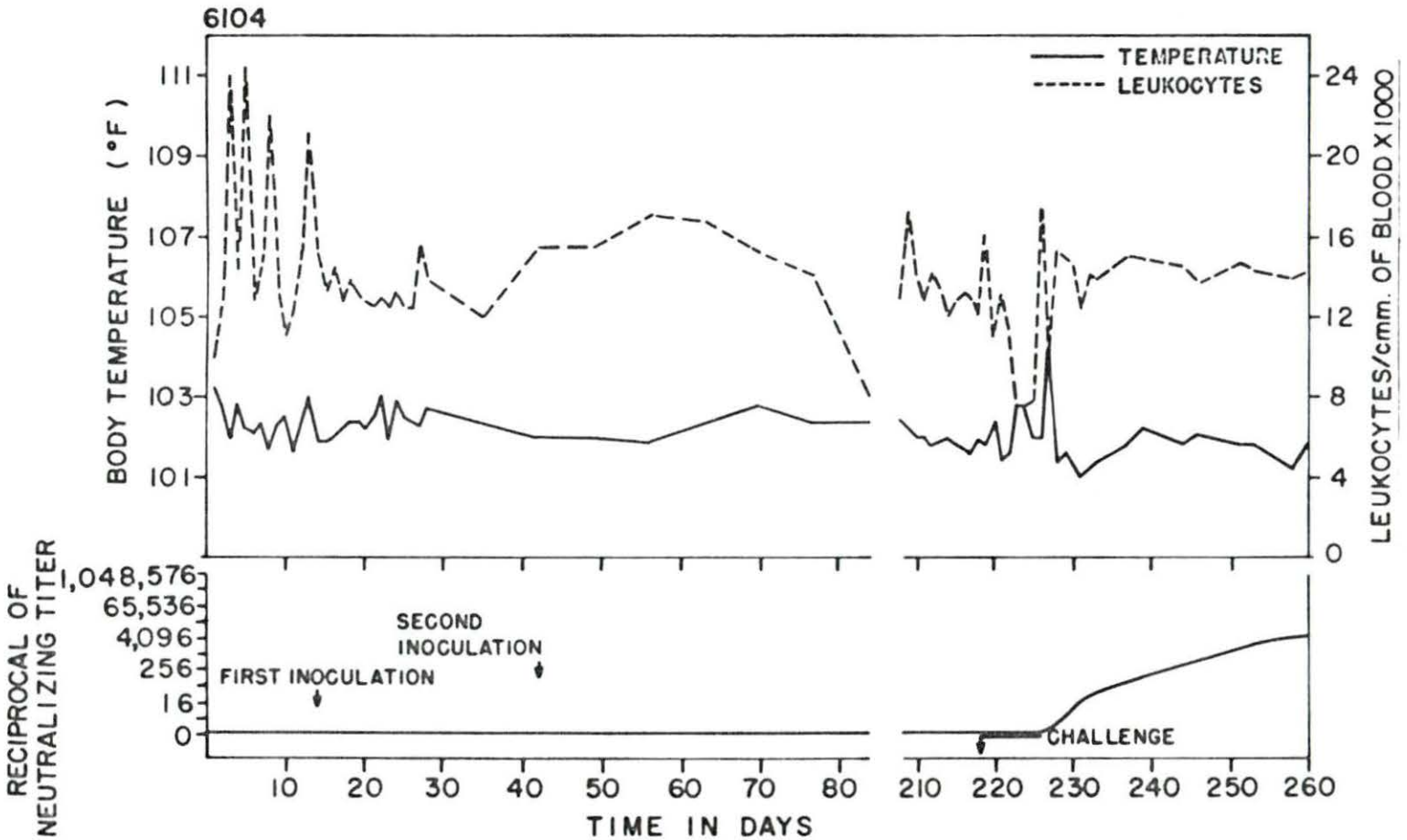

6104
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
LEUKOCYTES/cmm. OF BLOOD X1000
24
20
16
12
8
4
0
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10 20 30 40 50 60 70 80
210 220 230 240 250 260
TIME IN DAYS

Figure 21. Calf 6120, an SPF calf, was a barn control animal. It did not receive vaccine, but was challenged intraocularly with third EBK passage of NADL strain BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

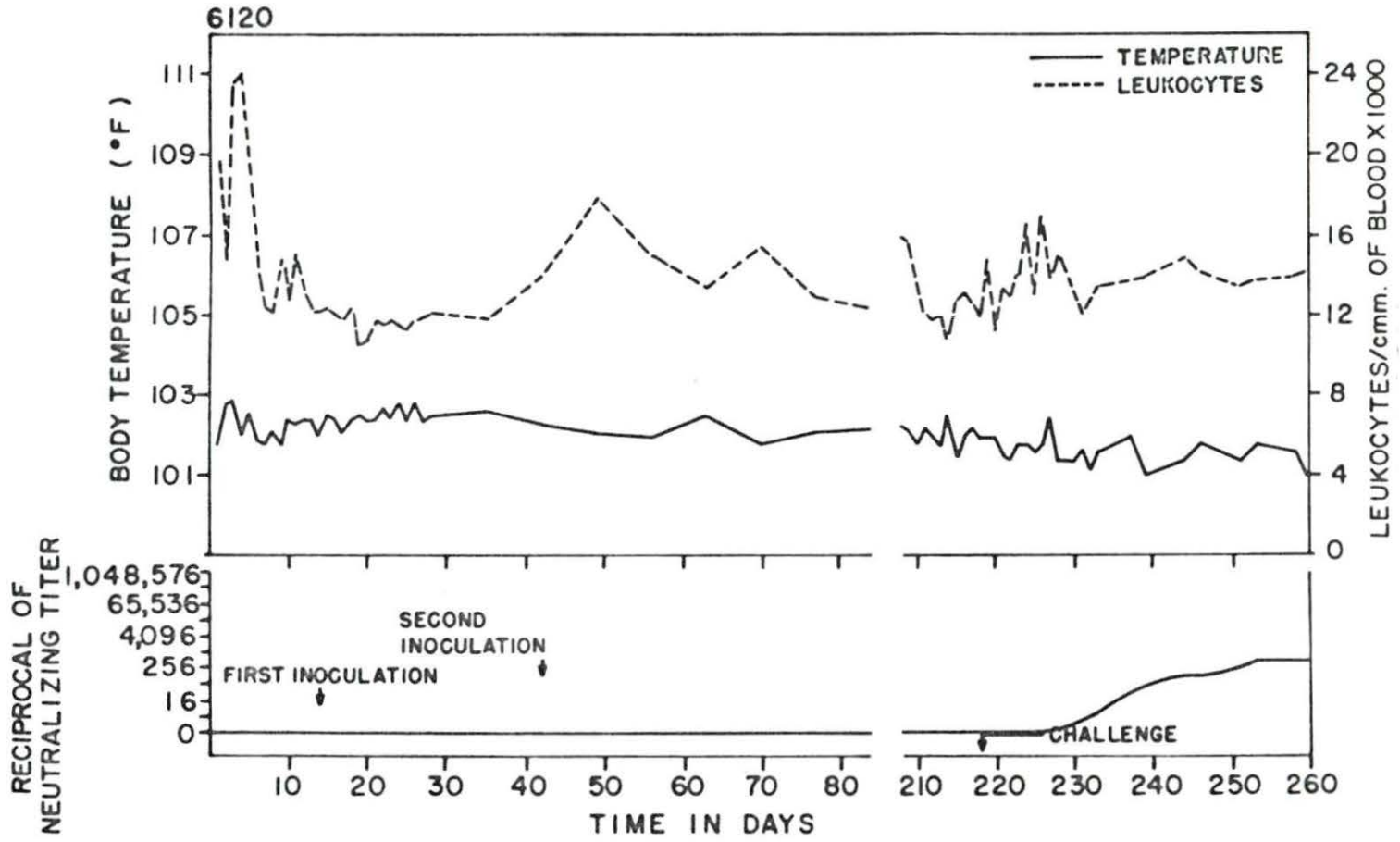

6120
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
LEUKOCYTES/cmm. OF BLOOD X 1000
24
20
16
12
8
4
0
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10 20 30 40 50 60 70 80
210 220 230 240 250 260
TIME IN DAYS

Figure 22. Calf 6127, an NB calf, was a barn control animal. It did not receive vaccine, but was challenged intranasally with third EBK passage of the NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

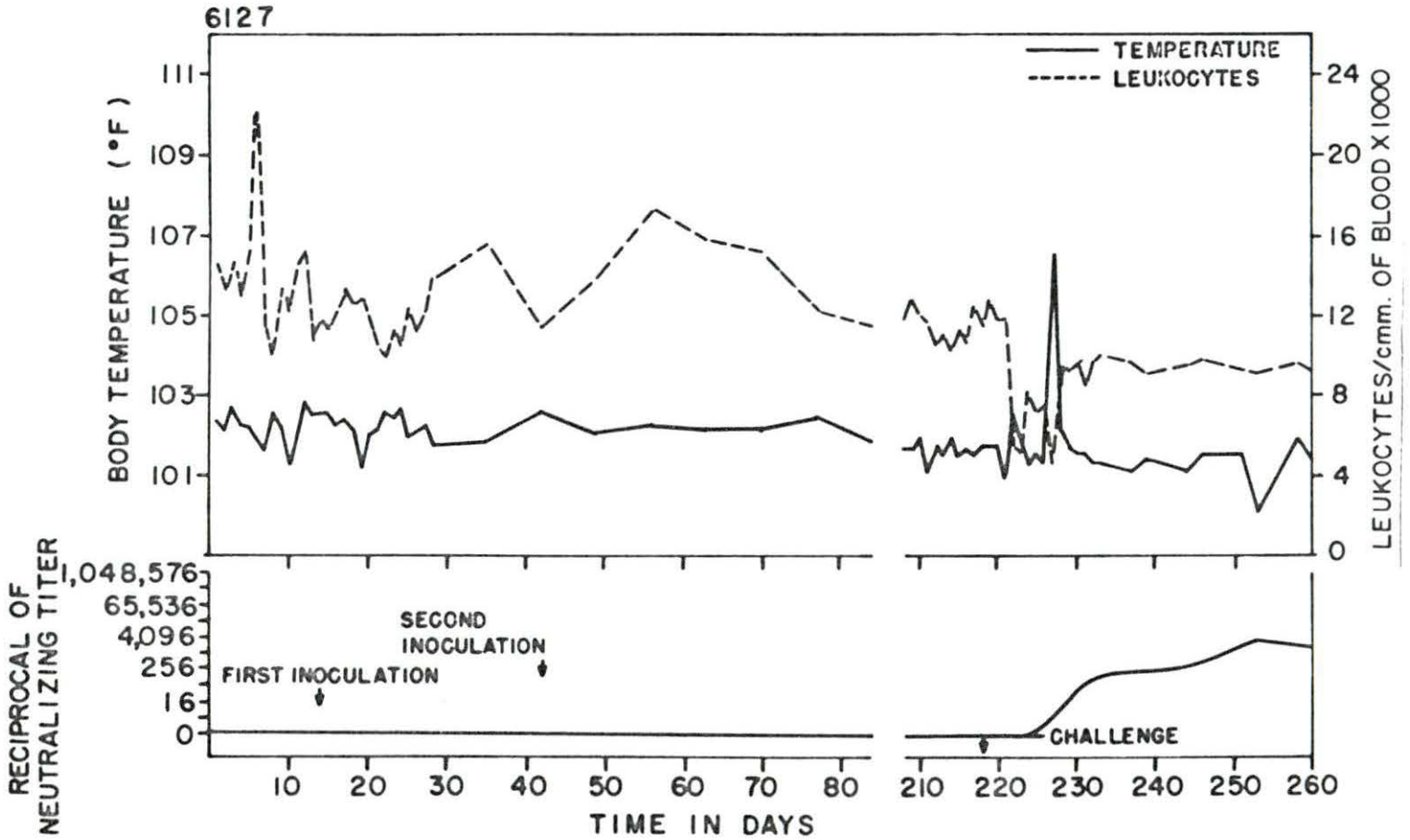

6127
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
111
109
107
105
103
101
24
20
16
12
8
4
0
LEUKOCYTES/cmm. OF BLOOD X 1000
RECIPROCAL OF NEUTRALIZING TITER
1,048,576
65,536
4,096
256
16
0
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
10 20 30 40 50 60 70 80
210 220 230 240 250 260
TIME IN DAYS

Figure 23. Calf 6128, an NB calf, was a barn control animal. It did not receive vaccine, but was challenged intranasally with third EBK passage of the NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.

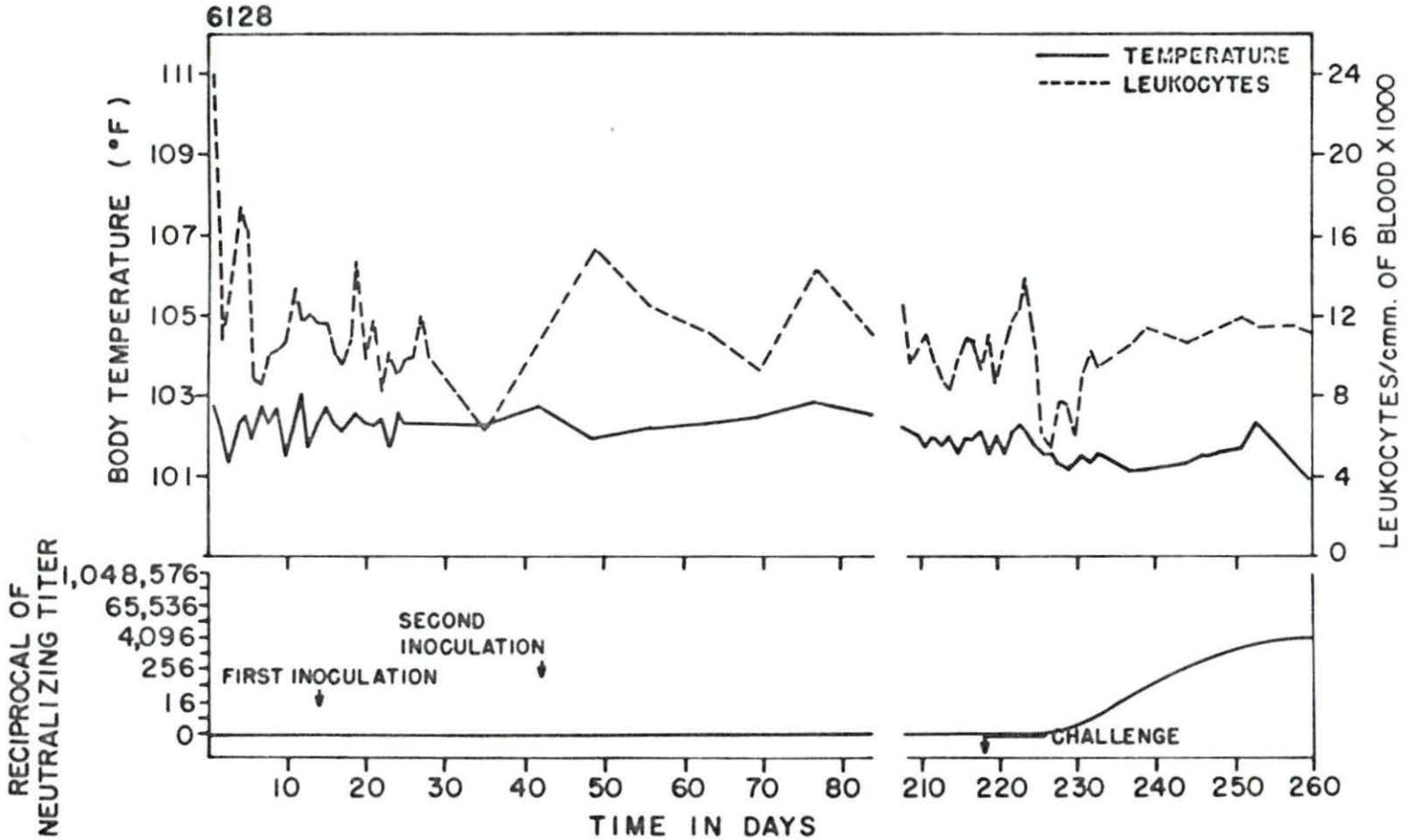
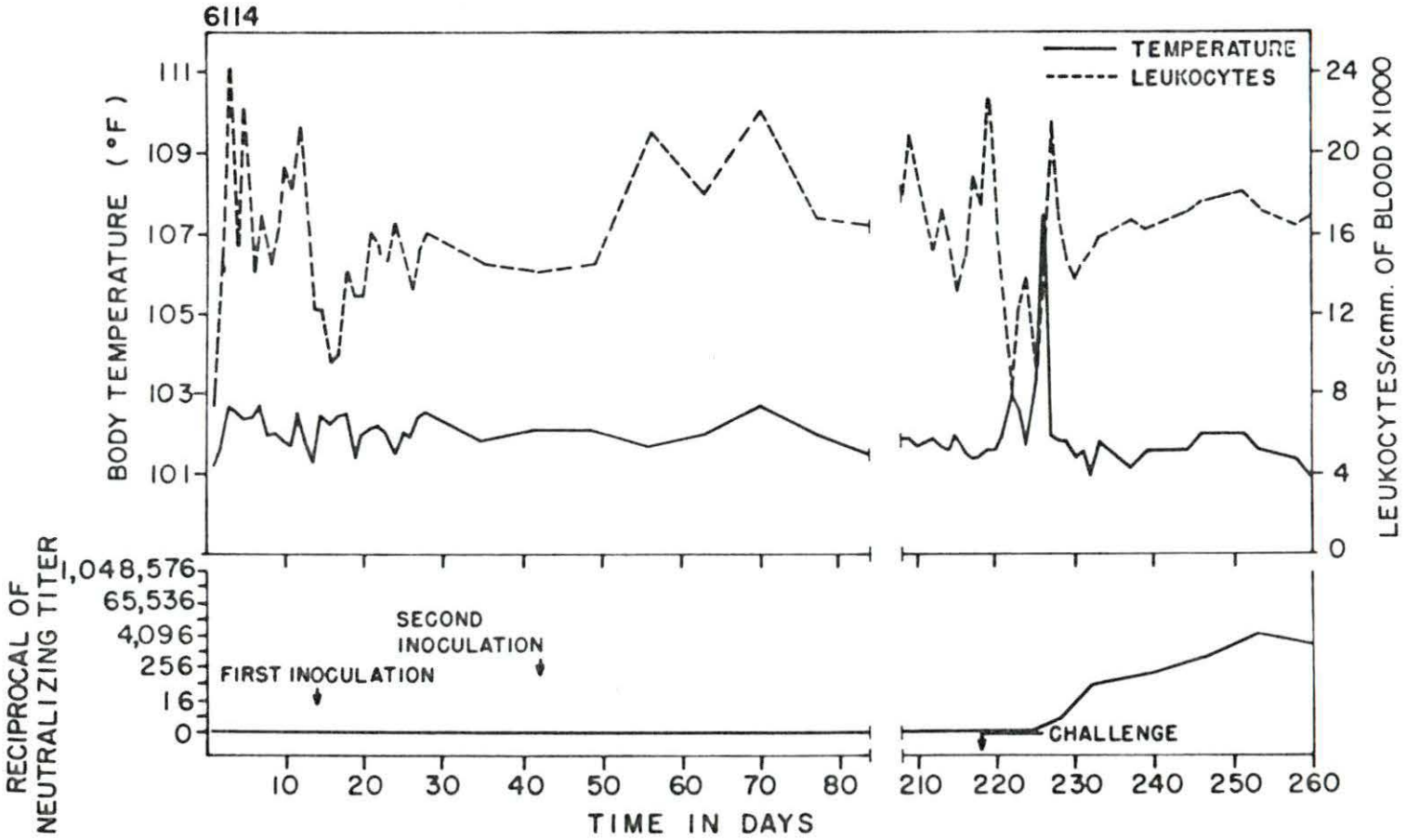

6128
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
LEUKOCYTES/cmm. OF BLOOD X 1000
RECIPROCAL OF NEUTRALIZING TITER
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
TIME IN DAYS

Figure 24. Calf 6114, an NB calf, was a barn control animal. It did not receive vaccine, but was challenged orally with third EBK passage NADL strain of BVD virus. The graph shows neutralizing antibody titer of the serum, body temperature and leukocytes per cmm. of blood.


6114
TEMPERATURE
LEUKOCYTES
BODY TEMPERATURE (°F)
LEUKOCYTES/cmm. OF BLOOD X1000
RECIPROCAL OF NEUTRALIZING TITER
SECOND INOCULATION
FIRST INOCULATION
CHALLENGE
TIME IN DAYS

V. DISCUSSION

The results of this research indicate that all 3 of the experimental inactivated BVD-MD vaccines are immunogenic in cattle, as evidenced by the induction of high levels of circulating neutralizing antibodies following the second inoculation of vaccine. There appears to be a correlation between the presence of these antibodies and protection, as most of the vaccinated calves failed to develop clinical signs of illness following challenge with low-passage homologous virus. It also appears that the immunity may last for 6 to 12 months, which would be long enough to get most feedlot animals to market.

In comparing the three vaccines one may note that the peak antibody titers and the titers at the time of challenge are highest for the SA-vaccinated cattle. This may be a result of the quality of the antigen injected or of a quantitative difference as this vaccine was concentrated approximately 10 times prior to the separation of soluble antigens by density gradient centrifugation.

The next best response was found in the calves vaccinated with BPL vaccine and the calves vaccinated with Chl vaccine had the lowest peak titers and the lowest titers at the time of challenge. It would appear that, from a practical point of view, the BPL vaccine would be the best one of the 3 for possible commercial production because the SA vaccine would be more costly to produce.

When comparing the immune response of NB, colostrum-fed calves, with the SPF, colostrum-deprived calves, it was noted that the average antibody titer was higher in the NB calves when BPL or Chl vaccine was

used. When SA vaccine was used the antibody titers were approximately the same in both the NB and SPF calves. It may have been either the quality or the larger quantity of antigen in the SA vaccine that evoked a better immune response. In the case of calves receiving Chl or BPL vaccines the colostrum-fed calves may be more immunologically competent than the SPF calves. These findings may not be significant because of the small number of calves used.

Although there were differences in antigenicity between the 3 vaccines, all 3 vaccines were immunogenic. With each of the vaccines used, some vaccinates developed a transitory leukopenia and temperature elevation following challenge, but in general, any postchallenge clinical signs of illness observed in vaccinates were so slight that they would not be noticed under field conditions. The small number of calves used precluded the possibility of varying the dosage of challenge virus given, therefore, a valid comparison of the relative protection of the 3 vaccines could not be made. In the challenge part of the vaccine evaluation it was obvious, however, that the vaccinated animals were better protected than the controls.

It was interesting to note that the control animal, calf 6114, which was also the oldest and largest animal, evidenced the most acute signs of illness. This animal was also the only animal that was challenged orally. Under natural conditions animals are probably infected orally or intranasally and the cells lining the respiratory or digestive tract may therefore be more readily infected. The resulting cellular changes could render cells more susceptible to subsequent invasion by secondary

bacteria. The barn controls, 6104 and 6120, showed the least clinical evidence of illness. This may have been a result of the low dosage of virus received because of the intraocular route of inoculation. Perhaps the intravenous route of inoculation is not the most desirable route for challenge because the virus is cleared relatively soon by the reticuloendothelial system.

Calf 6119, vaccinated with SA vaccine, developed a high level of antibodies but became ill following challenge. The fact that this animal became ill was not surprising because this calf had developed a chronic respiratory condition prior to the first inoculation of vaccine and appeared ill at other times during the experiment. The fact that the control calf remained BVD-MD negative until challenge indicates that calf 6119 did not have a BVD-MD infection. Because BVD-MD virus was not recovered from this animal during the postchallenge period, it was felt that the primary cause of illness was probably some other agent and that challenge potentiated the effect of the pre-existing infection.

One may note that very few of the control animals developed a diarrhea while a transitory respiratory involvement was not uncommon after challenge. In previous experiments the respiratory symptoms were also observed more often than diarrhea. This resembles the observations in the field and justifies the inclusion of BVD-MD virus as a cause of respiratory disease in cattle.

The choice of an inactivating agent was not easy because viruses may be inactivated by a number of physical and/or chemical methods. It

was decided to use chemical inactivation in this experiment because of the narrow range between loss of infectivity and complete denaturation when physical methods are used.

In the past formalin has been the most widely used chemical inactivating agent, but more recently it has been shown that comparable or better results are obtained by the use of other agents such as BPL, acetylethyleneimine, and perchloroethylene. Beta-propiolactone was used because it was available, easy to work with, and preliminary tests indicated that it could be used successfully to inactivate BVD-MD virus and maintain some antigenicity.

Ether or chloroform will inactivate BVD-MD virus and disrupt the intact virion releasing soluble antigens. It was decided to use chloroform for the inactivating agent for a second vaccine because it is easier and safer to work with than ether. The Chl vaccine could also be used for preparing the soluble antigens following procedures used by Gutekunst and Malmquist (41).

In the SA vaccine the nucleic acids were probably left with the protein fractions of the virus. With the nucleic acids present in a vaccine there is the possibility of replication taking place, so in future production of a soluble antigen vaccine it would be more desirable to further purify the vaccine by separating and discarding the nucleic acids.

Earlier experiences with residual virus prompted the use of large samples (20%) from each batch of vaccine for testing and the 4 passages of vaccine samples on EBK cells without evidence of CPE before considering the samples free of live virus.

Results of an earlier experiment indicated that calves could be infected with a Chl vaccine that was mixed with chloroform for only 48 hours. The vaccine appeared free of residual live virus after 1% of the vaccine was passed once on EBK cells without evidence of CPE.

In a second preliminary test of a Chl vaccine, the virus was mixed with the chloroform for 96 hours and more extensive in vitro tests for residual virus indicated that the vaccine did not contain infective virus. Subsequent tests in calves verified the fact that the vaccine was inactivated without complete loss of antigenicity.

It was observed that EBK cells did not appear normal during the first passage of the Chl vaccine. It appears that a very small quantity of residual chloroform can affect the condition of EBK cells. The lack of typical BVD-MD CPE may have been from complete inactivation of the virus or from some alteration in the cells. Later tests did show that such abnormal appearing cells can be infected by the virus. The fact that the second and third passages did not affect the appearance of the cells would indicate that the abnormal appearance of the cells during the first passage was a result of the chloroform rather than from virus. The added procedures for removing the residual chloroform, the slower inactivation time and the idea that inactivation by chloroform may be due only to the action on the outer envelope indicated that chloroform was not the inactivating agent of choice.

Since primary EBK cells were used throughout the experiment there was always the possibility of contamination with adventitious non-CPE-producing BVD-MD viruses. Such viruses might render the EBK cells

resistant to infection by the CPE-producing strain of virus being used, thereby giving false negative tests for residual virus. To guard against this possibility all batches of EBK cells were tested for non-CPE adventitious BVD-MD viruses by use of the interference test using NADL strain of virus to detect the presence of interfering virus.

In this experiment the inactivated vaccines were tested for infective virus before the addition of the adjuvant. In the future, tests should be made after the adjuvant is added in order to detect possible re-activation of the virus. The adjuvant if complexed with the virus could enhance attachment and entry into the EBK cells or the body cells of the vaccinated animal, Rouhandeh et al. (80).

Although the extensive in vitro testing indicated the vaccines were killed it was gratifying to note that none of the contact controls developed BVD-MD antibody titers prior to challenge. The relatively weak primary response was further circumstantial evidence that the vaccines did not contain residual virus. One might compare the primary response to the vaccines with the primary response of the control animals to challenge virus and note the differences in the curves. The first contact with live virus induces a more prompt response with higher antibody titers than the first contact with a killed virus.

Occasional samples of feces and nasal mucous were collected and tested for local BVD-MD neutralizing antibodies. There was no evidence of antibodies in the feces but this sampling was done late in the experiment and such antibodies, if ever present, could have disappeared by the time the samples were taken.

The nasal mucous from all vaccinated animals did contain a neutralizing substance against the BVD-MD virus and it was not present in the control animals prior to challenge. This material has not been characterized. It was not determined if this substance was produced locally or if it was a result of extravasation of serum from the capillaries. It was found that the titer of this substance was several times higher when nasal mucous was collected by the use of tampons inserted into the nose and removed 2 to 24 hours later, than if collected by a direct low vacuum aspiration method. Because of the obvious irritation, the tampon method was completely unsatisfactory. If these neutralizing substances are, in fact, locally produced immunoglobulins of the IgA class, it would be an interesting finding that could contribute to our understanding of the mechanism of protection against BVD-MD infection introduced by way of the respiratory route.

The results of this project indicate that it may be practical to use inactivated BVD-MD vaccines. The duration of immunity is long enough but a more prompt induction of immunity would be desirable. The time that is required to induce protection might be reduced by giving the second inoculation of vaccine sooner, increasing the amount of adjuvant used, using a different adjuvant, or increasing the dosage of the vaccine.

Only live attenuated BVD-MD vaccines are commercially available and they are usually satisfactory. They have certain advantages over killed vaccines, such as:

1. They induce immunity more promptly.

2. Protection lasts longer.
3. They protect against a greater number of strains of the virus.
4. Local immunity is usually better.
5. They are less expensive to produce.
6. One inoculation is usually adequate.

Although live vaccines have some important advantages they also have some disadvantages and potential hazards that might be eliminated through the use of inactivated vaccines. It has been mentioned earlier that killed vaccines have been used successfully against a number of animal diseases. Some of the potential hazards that might be eliminated are:

1. Possible reversion to a more virulent form.
2. Transmission of disease to susceptible animals.
3. Induction of severe reactions in some vaccinates.
4. Transmission of adventitious agents.
5. Induction of abortion in pregnant animals.
6. Production of fetal abnormalities.
7. Potential transmission to man.
8. Stress potentiation of currently existing infection.
9. Deterioration of vaccine during use or storage.
10. The perpetuation of the live virus which could render control programs ineffective.

Although the added cost of production may preclude the general usage of these BVD-MD vaccines, they could be used to vaccinate valuable pregnant animals or very young calves, because the use of the presently available

BVD-MD vaccines is not recommended for such animals. If control programs are ever attempted it might be desirable to use inactivated vaccines for a few years to reduce the number of possible carriers of BVD-MD virus.

The future acceptance and usage of the inactivated BVD-MD vaccines will depend on a number of economic and educational factors as well as possible regulations concerning the use of a live vaccine.

VI. SUMMARY

Three experimental chemically inactivated vaccines were developed. One of the vaccines was inactivated by BPL, the second by chloroform, and the third was a chloroform-inactivated soluble antigen vaccine. Aluminum hydroxide was used as the adjuvant with all 3 vaccines.

Large samples (20%) from each batch of vaccine were negative to *in vitro* tests for residual virus. None of the controls developed neutralizing antibodies prior to challenge which was further evidence that the vaccines did not contain live virus.

The immunogenicity of the vaccines was evaluated in 12 serologically BVD-MD negative calves. Each vaccine was tested in 4 SPF, colostrum-deprived calves and in 4 naturally-born calves that had received colostrum.

All vaccinates were given two 5-ml intramuscular injections of vaccine, the first at approximately 4 months of age and the second 4 weeks later. None of the 6 contact or 5 barn control calves were vaccinated. Six months after the second inoculation all calves were challenged with a third passage NADL strain of BVD-MD virus.

All 12 of the vaccinates developed high titers of circulating neutralizing antibodies against BVD-MD virus and the titers persisted for at least 6 months.

Following challenge, 10 of the 11 control animals developed signs of illness, body temperature elevation and leukopenia while over 90% of the vaccinates were protected.

VII. CONCLUSIONS

1. All 3 of the experimental inactivated BVD-MD vaccines are capable of inducing the production of neutralizing antibodies in serologically negative calves.

2. Both BPL and chloroform are suitable inactivating agents.

3. The duration of immunity induced by these vaccines is at least 6 months and the antibody titer at this time is still high.

4. Over 90% of the vaccinates are protected against acute clinical BVD-MD after challenge with a low passage of the homologous virus.

5. Following the second injection of vaccine all calves had a strong secondary immune response.

6. The SA vaccine induces the highest titers of antibodies.

7. The BPL vaccine is more antigenic than the Chl vaccine.

VIII. LITERATURE CITED

1. Olafson, P., MacCallum, A. D., and Fox, F. H. An apparently new transmissible disease of cattle. Cornell Veterinarian 36: 205-213. 1946.

2. Ramsey, F. K. and Chivers, W. H. Mucosal disease of cattle. North American Veterinarian 34: 629-633. 1953.

3. Pritchard, W. R. The mucosal disease of cattle: epizootiology, symptomatology, and experimental studies. American Veterinary Medical Association Proceedings 1955: 37-42. 1955.

4. Gillespie, J. H. and Baker, J. A. Studies on virus diarrhea. Cornell Veterinarian 49: 439-443. 1959.

5. Schipper, I. A., Eveleth, D. F., Shumard, R. F., and Richards, S. H. Mucosal disease of cattle. Veterinary Medicine 50: 431-434. 1955.

6. Shope, R. E., Jr. Experimental studies on virus diarrhea of cattle. Unpublished Ph.D. thesis. Minneapolis, Minnesota, Library, University of Minnesota. 1964.

7. Lambert, G. The role of a bovine viral diarrhea virus in neonatal calf enteritis. Unpublished M.S. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology. 1966.

8. Olafson, P. and Rickard, C. G. Further observations on the virus diarrhea (new transmissible disease of cattle). Cornell Veterinarian 37: 104-106. 1947.

9. Gillespie, J. H., Coggins, L., Thompson, J. and Baker, J. A. Comparison by neutralization tests of strains of virus diarrhea and mucosal disease. Cornell Veterinarian 51: 155-159. 1961.

10. Ramsey, F. K. The pathology of mucosal disease of cattle. Printed monograph. Ames, Iowa, Department of Veterinary Pathology, Iowa State University of Science and Technology. 1956.

11. Tyler, D. E. and Ramsey, F. K. Comparative pathologic, immunologic, and clinical responses produced by selected agents of the bovine mucosal disease-virus diarrhea complex. American Journal of Veterinary Research 26: 903-913. 1965.

12. Mills, J. H. L., Luginbuhl, R. E. and Nielsen, S. W. Transmission of bovine mucosal disease using virus recovered from urine. Research in Veterinary Science 9: 500-505. 1968.

13. Hermodsson, S. and Dinter, Z. Properties of bovine virus diarrhoea virus. Nature 194: 893-894. 1962.

14. Ditchfield, J. and Doane, F. W. The properties and classification of bovine viral diarrhea virus. Canadian Journal of Comparative Medicine and Veterinary Science 28: 148-152. 1964.

15. Gutekunst, D. E. Characterization of a bovine viral diarrhea agent. Unpublished M.S. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology. 1964.

16. Fernelius, A. L. Characterization of bovine viral diarrhea viruses by their interactions with susceptible host cells. Unpublished Ph.D. dissertation. Ames, Iowa, Library, Iowa State University of Science and Technology. 1967.

17. Pritchard, W. R. The bovine viral diarrhea-mucosal disease complex. Advances in Veterinary Science 8: 1-47. 1963.

18. Dutta, S. K., Shope, R. E. and Pomeroy, B. S. Electron microscopic structure of bovine virus diarrhea virus. (Abstract) Bacteriological Proceedings 1964: 111. 1964.

19. Feldman, H. A. and Wang, S. S. Sensitivity of various viruses to chloroform. Society for Experimental Biology and Medicine Proceedings 106: 736-738. 1961.

20. Gillespie, J. H., Madin, S. H. and Darby, N. B., Jr. Studies on virus diarrhea virus of cattle, with special reference to its growth in tissue culture and its ether susceptibility. Cornell Veterinarian 53: 276-282. 1963.

21. Underdahl, N. R., Grace, O. D. and Hoerlein, A. B. Cultivation in tissue-culture of cytopathogenic agent from bovine mucosal disease. Society for Experimental Biology and Medicine Proceedings 94: 795-797. 1957.

22. Gratzek, J. B. Characteristics of two bovine viral diarrhea agents. Unpublished Ph.D. thesis. Madison, Wisconsin, Library, University of Wisconsin. 1962.

23. Dinter, Z. Relationship between bovine viral diarrhea virus and hog cholera virus. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene 188: 475-486. 1963.

24. Cheng, P. -Y. The inactivation of group B arthropod-borne animal viruses by proteases. Virology 6: 120-136. 1958.

25. Diderholm, H. and Dinter, Z. Infectious RNA derived from bovine virus diarrhoea virus. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abt. I: Orig. 201: 270-272. 1966.

26. Schaffer, F. L. Physical and chemical properties and infectivity of RNA from animal viruses. Cold Spring Harbor Symposia on Quantitative Biology 27: 89-99. 1962.

27. Gillespie, J. H., Baker, J. A. and McEntee, K. A cytopathogenic strain of virus diarrhea virus. Cornell Veterinarian 50: 73-79. 1960.

28. Kniazeff, A. J. and Pritchard, W. R. Antigenic relationships in the bovine viral diarrhea-mucosal disease complex. United States Livestock Sanitary Association Proceedings 1960: 344-350. 1961.

29. _____, Huck, R. A., Jarrett, W. F. H., Pritchard, W. R., Ramsey, F. K., Schipper, I. A., Stober, V. M., and Liess, B. Antigenic relationships between some bovine viral diarrhea-mucosal disease viruses from the United States, Great Britain, and West Germany. Veterinary Record 73: 768-769. 1961.

30. Gutekunst, D. E. and Malmquist, W. A. Complement fixing and neutralizing antibody response to bovine viral diarrhea and hog cholera antigens. Canadian Journal of Comparative Medicine and Veterinary Science 28: 19-23. 1964.

31. Gillespie, J. H., Madin, S. H., and Darby, N. B., Jr. Cellular resistance in tissue culture, induced by noncytopathogenic strains to a cytopathogenic strain of virus diarrhea virus of cattle. Society for Experimental Biology and Medicine Proceedings 110: 248-250. 1962.

32. Inaba, Y., Omori, T. and Kumagai, T. Detection and measurement of non-cytopathogenic strains of virus diarrhea of cattle by the END method. Archiv. für die Gesamte Virusforschung 13: 425-429. 1963.

33. Darbyshire, J. H. Agar gel diffusion studies with a mucosal disease of cattle. I. Preliminary results with the technique. Research in Veterinary Science 3: 118-124. 1962.

34. Beckenhauer, W. H., Brown, A. L., Lidolph, A. A., and Norden, C. J., Jr. Immunization of swine against hog cholera with a bovine enterovirus. Veterinary Medicine 56: 108-112. 1961.

35. Darbyshire, J. H. Agar gel diffusion studies with a mucosal disease of cattle. II. A serological relationship between a mucosal disease and swine fever. Research in Veterinary Science 3: 125-128. 1962.

36. Sheffy, B. E., Coggins, L. and Baker, J. A. Relationship between hog cholera virus and virus diarrhea virus of cattle. Society for Experimental Biology and Medicine Proceedings 109: 349-352. 1962.

37. Snowdon, W. A. and French, E. L. The bovine mucosal disease-swine fever complex in pigs. Australian Veterinary Journal 44: 179-184. 1968.

38. Tomoglia, T. W., Tellejohn, A. L., Philips, C. E., and Wilkinson, F. B. Further evaluation of hog cholera immunizing agents bovine virus diarrhea and hog cholera vaccine, MlV, TCO. United States Livestock Sanitary Association Proceedings 1965: 385-389. 1966.

39. Mengeling, W. L., Gutekunst, D. E., Fernelius, A. L., and Pirtle, E. C. Demonstration of an antigenic relationship between hog cholera and bovine viral diarrhea viruses by immunofluorescence. Canadian Journal of Comparative Medicine and Veterinary Science 27: 162-164. 1963.

40. Fernelius, A. L. Noncytopathogenic bovine viral diarrhea viruses detected and titrated by immunofluorescence. Canadian Journal of Comparative Medicine and Veterinary Science 28: 121-126. 1964.

41. Gutekunst, D. E. and Malmquist, W. A. Separation of a soluble antigen and infectious particles of bovine viral diarrhea viruses and their relationship to hog cholera. Canadian Journal of Comparative Medicine and Veterinary Science 27: 121-123. 1963.

42. Fernelius, A. L. and Packer, R. A. Serological reactions of bovine diarrhoea viruses with anticellular and antivirus sera produced in rabbits. Journal of General Virology 5: 243-249. 1969.

43. Baker, J. A., York, C. J., Gillespie, J. H. and Mitchell, G. Virus diarrhea in cattle. American Journal of Veterinary Research 15: 531-535. 1954.

44. _____, Gillespie, J. H., Sheffy, B. E. and Marshall, V. Simultaneous immunization of cattle against leptospirosis, virus diarrhea, and infectious bovine rhinotracheitis. Cornell Veterinarian 48: 207-213. 1958.

45. York, C. J., Rosner, S. F. and MacLean, G. J. Evaluation of vaccines for virus diarrhea of cattle. United States Livestock Sanitary Association Proceedings 1960: 339-343. 1961.

46. Coggins, L., Gillespie, J. H., Robson, D. S., Thompson, J. D., Phillips, W. V., Wagner, W. C. and Baker, J. A. Attenuation of virus diarrhea virus (strain Oregon C24V) for vaccine purposes. Cornell Veterinarian 51: 539-545. 1961.

47. Malmquist, W. A., Fernelius, A. L. and Gutekunst, D. E. Interference of bovine viral diarrhea virus by hog cholera virus in swine kidney cell cultures. American Journal of Veterinary Research 26: 1316-1327. 1965.

48. Marcus, S. J. and Moll, T. Adaptation of bovine viral diarrhea virus to Madin-Darby bovine kidney cell line. American Journal of Veterinary Research 29: 817-819. 1968.

49. Bittle, J. L., Gutekunst, D. E., Sweat, R. L. and Holper, J. C. Vaccination for bovine viral diarrhea-mucosal disease. American Veterinary Medical Association Journal 152: 861-870. 1968.

50. _____ and York, C. J. Combination vaccines for control of bovine respiratory diseases. American Veterinary Medical Association Journal 152: 889-893. 1968.

51. Gutekunst, D. E. Comments on vaccination for bovine viral diarrhea-mucosal disease. American Veterinary Medical Association Journal 152: 865-866. 1968.

52. Peter, C. P., Tyler, D. E. and Ramsey, F. K. Characteristics of a condition following vaccination with bovine virus diarrhea vaccine. American Veterinary Medical Association Journal 150: 46-52. 1967.

53. Kirkpatrick, K. J. Comments on combination vaccines for bovine respiratory diseases. American Veterinary Medical Association Journal 152: 896-897. 1968.

54. Rosner, S. F. Complications following vaccination of cattle against infectious bovine rhinotracheitis, bovine viral diarrhea-mucosal disease, and parainfluenza type 3. American Veterinary Medical Association Journal 152: 898-902. 1968.

55. Brown, L. N. and Ramsey, F. K. Comments on complications following vaccination of cattle against bovine respiratory diseases. American Veterinary Medical Association Journal 152: 903-904. 1968.

56. Fuller, D. A. Comments on complications following vaccination against bovine respiratory disease. American Veterinary Medical Association Journal 152: 904-905. 1968.

57. Peacock, G. V. Comments on complications that arise after vaccination against bovine respiratory diseases. American Veterinary Medical Association 152: 905-907. 1968.

58. Holper, J. C. Comments on bovine viral diarrhea-mucosal disease. American Veterinary Medical Association Journal 152: 868-869. 1968.

59. Clark, J. G. Comments on bovine viral diarrhea-mucosal disease. American Veterinary Medical Association Journal 152: 868-869. 1968.

60. Clark, F. R. Immunologic aspects of mucosal disease. Modern Veterinary Practice Dec. 38-43. 1968.

61. Simonyi, E., Bognár, K., Biró, J. and Palatka, Z. Immunization experiments against bovine viral diarrhoea. I. The production and application of hyperimmune serum. Acta Veterinaria Academiae Scientiarum Hungaricae Tomus 18: 237-243. 1968.

62. _____, _____, _____ and _____. Immunization experiments against bovine viral diarrhoea. II. Active immunization. Acta Veterinaria Academiae Scientiarum Hungaricae Tomus 18: 245-250. 1968.

63. Lambert, G., Boylan, C. G. and Rea, C. Immunogenicity of bovine rhinotracheitis-virus diarrhea vaccine in cattle inoculated simultaneously with concentrated homologous antiserum. Veterinary Medicine - Small Animal Clinician Nov. 1087-1088. 1967.

64. _____, Moeller, D. H. and Welter, C. J. Preliminary field evaluation of antiserum for the prevention of the neonatal calf pneumonia-enteritis complex. Submitted to Veterinary Medicine and Small Animal Clinician Mar. 1970.

65. LoGrippo, G. A. and Hartman, F. W. Antigenicity of β-propiolactone-inactivated virus vaccines. Journal of Immunology 75: 123-128. 1955.

66. _____. Investigations of the use of beta-propiolactone in virus inactivation. The New York Academy of Sciences, Annals. 83: 578-594. 1960.

67. Mack, W. N. and Chotisen, A. Beta-propiolactone as a virus altering agent for Newcastle disease vaccine. Poultry Science 34: 1010-1013. 1955.

68. Gill, E., Sullivan, J. F., Stone, H. D. and Hundemann, A. S. Roll of adjuvants in immunogenicity of killed Newcastle disease vaccines. American Journal of Veterinary Research 20: 357-365. 1959.

69. Hofstad, M. S. An evaluation of commercial inactivated Newcastle disease vaccines. Avian Diseases 11: 198-202. 1967.

70. _____. Comparative immunogenicity of three strains of Newcastle disease virus used in inactivated vaccines. Avian Diseases 12: 665-669. 1968.

71. Chang, P. W., Yates, V. J., Pendleton, J. A. and Flanagan, T. D. Immune response of chickens to six strains of Newcastle disease virus as measured by hemagglutination-inhibition test. Avian Diseases 13: 46-52. 1969.

72. Stone, H. D. and Boney, W. A. Vaccination of congenitally-immune chicks against Newcastle disease virus. Society for Experimental Biology and Medicine Proceedings 128: 525-530. 1968.

73. _____, Ritchie, A. E. and Boney, W. A. Immunization of chickens against Newcastle disease with beta-propiolactone-killed virus antigen administered in drinking water. Avian Diseases 13: 568-578. 1969.

74. Winterfield, R. W. Immunity response from an inactivated infectious bronchitis vaccine. Avian Diseases 11: 446-451. 1967.

75. Price, W. H. and Thind, I. S. Live and inactivated vaccines of Group B arboviruses: role of neutralizing antibody and serum protective factor. Nature 222: 1294-1295. 1969.

76. Pilchard, E. I. Experimental inactivated-virus hog cholera vaccines. American Journal of Veterinary Research 28: 915-923. 1967.

77. Mirchamsy, H. and Taslimi, H. Inactivated African horse sickness virus cell culture vaccine. Immunology 14: 81-88. 1968.

78. Fellowes, O. N. Comparison of the inactivation and antigenicity of foot-and-mouth disease virus by acetylethyleneimine and by combined effect of ultraviolet light and beta-propiolactone. Journal of Immunology 95: 1100-1106. 1965.

79. Walker, R. V. L., Griffiths, H. J., Shope, R. E., Maurer, F. D. and Jenkins, D. L. Rinderpest III. Immunization experiments with inactivated bovine tissue vaccines. American Journal of Veterinary Research 7: 145-151. 1946.

80. Rouhandeh, H., Brinkman, M. L. and Sells, L. L. Reactivation of ether-inactivated infectious bovine rhinotracheitis virus. Journal of Infectious Diseases 117: 237-241. 1967.

81. Crick, J. and Brown, F. Viral subunits for rabies vaccination. Nature 222: 92. 1969.

82. Brown, F., Cartwright, B. and Almeida, J. D. The antigens of vesicular stomatitis virus. The Journal of Immunology 96: 537-545. 1966.

83. Davenport, F. M. Antigenic enhancement of ether-extracted influenza virus vaccines by $AlPO_4$. Society for Experimental Biology and Medicine Proceedings 127: 587-590. 1968.

84. Kasel, J. A., Huber, M., Loda, F., Banks, P. A. and Knight, V. Immunization of volunteers with soluble antigens of adenovirus type 1. Society for Experimental Biology and Medicine Proceedings 117: 186-190. 1964.

85. Norrby, E. C. J., Lagercrantz, R. and Gard, S. Measles vaccination. IV. Responses to two different types of preparations given as fourth dose of vaccine. British Medical Journal 5438: 813-817. 1965.

86. Younger, J. S. Monolayer tissue culture. I. Preparation and standardization of suspensions of trypsin-dispersed monkey kidney cells. Society for Experimental Biology and Medicine Proceedings 85: 202-205. 1954.

87. Reed, L. J. and Muench, H. A simple method of estimating fifty per cent end points. American Journal of Hygiene 27: 493-497. 1938.

IX. ACKNOWLEDGEMENTS

I wish to thank Dr. R. A. Packer for his counsel and guidance and many valuable suggestions throughout this project. I wish to thank him most of all for encouraging me to return to school after being in general practice for 10 years.

I also wish to thank the other members of my committee: Drs. C. J. Mare', Donald Beitz, L. O. Mott and A. L. Fernelius for their time and interest.

Special thanks are given to Dr. Fernelius for the use of his fine collection of reference material, technical help, patience, guidance and close friendship the past two years while working together at NADL.

The assistance and services of the following people at the National Animal Disease Laboratory are acknowledged:

Mr. H. L. Keech and the members of Central Services for providing glassware, media, reagents and cell cultures.

Dr. P. J. Matthews and his Animal Services staff for providing and caring for experimental animals.

Mr. R. M. Glazier and his Photographic and Illustrative Services staff for graphs and photographs.

Mr. R. L. Smith for his faithful and conscientious assistance and many helpful suggestions in the many laboratory procedures and animal work.

Mrs. M. J. Eernisse for typing rough drafts of this manuscript.

Mrs. A. L. Bates for her excellent typing of the final draft of this thesis.

My most heartfelt thanks and appreciation are given to my wife Charlotte, and sons Nicholas, Charles, Harold and Craig, for their patience, understanding, and personal sacrifices made in order that their husband and father could return to school and work on this project.

This project was conducted, in part, under the U. S. Government Employees Training Act, P.L. 85-507.

X. APPENDIX

Data from calf 6080 Vaccine - BPL

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		102.5	19,000	-	-	-	-	
2		103.0	16,200					
3		102.4	12,500					
4		101.6	16,800					
5		102.5	24,000					
6		102.3	19,200					
7		103.0	17,100					
8		101.8	17,000	-	-	-	-	
9		102.4	16,250					
10		102.4	15,500					
11		102.8	16,500					
12		102.2	17,800					
13		102.4	16,500					
14	Vaccinated	102.4	17,500	-	-	-	-	
15		102.6	14,500					
16		102.4	15,600		-	-	-	
17		102.2	14,700					
18		102.5	13,000					
19		102.4	14,000		-	-	-	
20		102.4	13,600					
21		102.5	13,200	4				
22		102.8	13,250		-	-	-	
23		102.3	13,100					
24		102.4	12,250					
25		101.8	13,400					
26		102.4	15,500					
27		102.6	14,000					
28		102.4	17,900	4	-	-	-	
35		102.6	18,000	8				
42	2nd Vaccination	102.4	16,700	8				

Data from calf 6080 (continued)

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49		102.4	19,900	256				
56		102.4	20,000	2,048				
63		102.2	19,000	4,096				
70		101.9	15,000	2,048				
77		101.8	17,500	2,048				
84		102.0	15,000	1,024				
91		102.1	12,000	2,048				
98		101.9	16,500	2,048				
105		102.4	11,750	1,024				
112		101.5	16,000	256				
119		101.7	15,200	2,048				
126		101.8	16,200	2,048				
133		101.9	18,000	1,024				
140		101.6	16,100	512				
147		101.8	14,750	128				
154		102.0	15,600	512				
161		102.1	16,300	1,024				
168		102.4	17,000	1,024				
175		102.1	15,400	512				
208		102.0	13,000	512				
209		101.7	12,500	512				
210		101.8	15,600	512				
211		102.4	18,200	256				
212		102.6	19,500	256				
213		102.7	18,600					
214		102.8	17,000					
215		102.9	15,400					
216		103.5	14,000					
217		101.8	15,200					
218	Challenged	101.8	14,200		-	-	-	

Data from calf 6080 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	102.0	12,500	256				
220	102.0	11,900		-	-	-	
221	101.8	12,900					
222	101.0	11,500		-	-	-	
223	102.6	11,900					
224	101.4	12,600					
225	101.8	12,000	8,192	-	-	-	
226	102.0	17,250					
227	101.6	17,150		-	-	-	
228	101.6	16,250					
229	101.8	16,000					
230	101.8	16,200		-	-	-	
231	101.4	17,500					
232	101.2	16,800	65,536	-	-	-	
233	101.6	16,500					
237	101.2	16,250		-	-	-	
239	101.6	16,150	131,072	-	-	-	
244	102.0	15,800		-	-	-	
246	101.4	16,200	32,768	-	-	-	
251	101.4	16,500		-	-	-	
253	101.4	16,400	32,768	-	-	-	
258	101.4	15,750		-	-	-	
260	101.2	14,800	131,072	-	-	-	

Data from calf 6060 Vaccine - BPL

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		101.0	20,000	-	-	-	-	
2		101.0	14,400					
3		101.9	11,600					
4		101.6	12,700					
5		102.0	24,000					
6		102.6	24,000					
7		101.8	15,800					
8		102.0	11,600	-	-	-	-	
9		101.6	15,000					
10		101.5	16,250					
11		102.4	17,000					
12		102.0	18,000					
13		102.6	13,600					
14	Vaccinated	102.0	17,500	-	-	-	-	
15		101.9	14,300					
16		101.9	14,100		-	-	-	
17		102.1	13,500					
18		102.3	13,700					
19		101.9	16,250		-	-	-	
20		101.7	14,000					
21		101.8	11,250	-				
22		102.2	12,000		-	-	-	
23		102.3	12,750					
24		102.8	13,250					
25		102.5	14,200					
26		102.4	14,000					
27		102.5	13,700					
28		102.7	15,500	-	-	-	-	
35		102.0	18,500	-				
42		102.5	17,100	-				

Data from calf 6060 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49	102.6	21,250	256				
56	102.4	19,500	1,024				
63	102.3	15,500	2,048				
70	101.8	9,000	1,024				
77	102.5	20,000	512				
84	101.6	22,000	512				
91	102.2	17,000	512				
98	101.8	13,000	512				
105	101.5	16,000	512				
112	101.6	16,000	512				
119	101.4	15,200	1,024				
126	101.6	14,700	1,024				
133	101.7	14,000					
140	101.5	17,200					
147	101.9	19,500	128				
154	102.2	20,000					
161	102.3	18,200	96				
168	101.9	17,800					
175	101.6	16,000					
208	101.8	16,500					
209	102.0	16,000	128				
210	101.8	17,800	128				
211	101.6	15,900	256				
212	101.9	14,500	256				
213	101.6	12,500					
214	101.5	13,600					
215	101.6	14,000					
216	101.6	13,250					
217	101.0	12,700					
218	101.5	12,500		-	-	-	

Data from calf 6060 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.8	16,000	128				
220	101.8	12,000		-	-	-	
221	101.0	7,000					
222	102.4	7,500		-	-	-	
223	100.8	11,000					
224	101.6	12,800					
225	101.0	10,000	8,182	-	-	-	
226	105.0	17,000					
227	101.0	18,250		-	-	-	
228	101.6	14,100					
229	101.4	13,800					
230	101.8	13,400		-	-	-	
231	101.6	15,000					
232	101.2	14,700	131,072	-	-	-	
233	101.2	14,300					
237	101.2	14,100		-	-	-	
239	101.4	13,900	131,072	-	-	-	
244	101.2	14,600		-	-	-	
246	101.0	15,200	8,192	-	-	-	
251	101.8	15,000		-	-	-	
253	101.2	14,900	131,072	-	-	-	
258	101.2	15,600		-	-	-	
260	101.2	14,750	131,072	-	-	-	

Data from calf 6087 Vaccine - None, Contact Control

Days		Temperature	Leukocytes	Virus Isolation from Titer	Nose	Rectum	Blood	Symptoms
1		102.6	16,000	-	-	-	-	
2		101.6	9,400					
3		102.2	10,600					
4		101.6	12,100					
5		103.3	17,300					
6		102.0	9,600					
7		102.4	10,700					
8		102.4	14,800	-	-	-	-	
9		103.0	19,800					
10		101.7	12,500					
11		102.1	12,800					
12		102.5	13,200					
13		102.5	11,400					
14	Vaccinated	102.4	13,400	-	-	-	-	
15		102.4	11,500					
16		102.4	12,500		-	-	-	
17		102.5	11,900					
18		102.8	10,200					
19		101.8	10,400		-	-	-	
20		101.9	10,750					
21		102.4	11,800	-				
22		102.6	10,100		-	-	-	
23		102.5	10,250					
24		102.7	9,900					
25		102.6	8,750					
26		102.4	9,800					
27		102.6	13,000					
28		102.4	10,500	-	-	-	-	
35		102.2	11,750	-				
42	2nd Vaccination	102.4	12,400	-				

Data from calf 6087 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49	102.4	11,500	-				
56	102.4	13,000	-				
63	101.9	12,250	-				
70	102.0	14,500	-				
77	102.0	12,000	-				
84	102.0	12,000	-				
91	101.5	12,500	-				
98	101.8	14,600	-				
105	101.7	13,300	-				
112	101.9	11,900	-				
119	101.8	14,000	-				
126	102.0	12,200	-				
133	101.7	11,800	-				
140	101.8	10,900	-				
147	101.7	12,200	-				
154	102.0	10,750	-				
161	101.9	11,400	-				
168	101.9	10,900	-				
175	101.7	10,800	-				
208	101.7	10,000	-				
209	101.9	11,500	-				
210	102.0	10,750	-				
211	101.9	11,200					
212	101.8	10,500					
213	101.9	10,200					
214	101.8	9,000					
215	102.0	9,700					
216	102.1	10,250					
217	102.4	10,600					
218 Challenged	102.6	11,000	-	-	-	-	

Data from calf 6087 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.8	12,000					
220	102.0	8,000		-	-	-	
221	102.0	6,250					D, A
222	102.2	7,000		-	-	-	D, A
223	102.0	7,600					D, A
224	102.6	7,300					D, A
225	105.0	5,900	-	-	+	-	D, A
226	104.8	10,600					D, A
227	100.4	15,000		-	-	+	
228	101.6	8,000					
229	101.8	8,200					
230	101.6	8,000		-	-	-	
231	101.4	8,300					
232	101.2	8,400	128	-	-	-	
233	101.4	8,200					
237	101.2	8,600		-	-	-	
239	101.0	9,000	512	-	-	-	
244	101.8	9,200		-	-	-	
246	101.4	9,000	256	-	-	-	
251	101.6	10,000		-	-	-	
253	101.6	9,600	2,048	-	-	-	
258	101.4	9,400		-	-	-	
260	101.6	9,200	2,048	-	-	-	

D depression
A anorexia

Data from calf 6105 Vaccine - Chl

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		102.8	12,500	-	-	-	-	
2		102.5	14,500					
3		102.2	14,600					
4		101.6	13,500					
5		102.3	22,500					
6		102.6	11,200					
7		102.7	15,100					
8		102.3	12,400					
9		102.4	12,400	-	-	-	-	
10		102.5	15,250					
11		102.2	15,000					
12		102.5	17,750					
13		102.3	17,400					
14	Vaccinated	102.5	17,000	-	-	-	-	
15		102.4	11,600					
16		101.8	13,500		-	-	-	
17		101.6	13,900					
18		102.0	14,200					
19		101.8	14,500		-	-	-	
20		102.2	14,000					
21		102.1	14,900	4				
22		102.4	13,000		-	-	-	
23		101.9	13,200					
24		102.4	19,200					
25		102.5	14,200					
26		102.5	14,500					
27		102.0	13,200					
28		102.4	14,600	8	-	-	-	
35		101.7	15,000	8				
42	2nd Vaccination	102.3	14,500	8				

Data from calf 6105 (continued)

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49		102.0	13,800	1,024				
56		101.8	18,000	4,096				
63		101.4	17,300	8,192				
70		102.3	18,000	2,048				
77		102.0	18,200	1,024				
84		102.5	15,000	1,024				
91		101.5	18,200	2,048				
98		102.1	12,000	2,048				
105		101.5	12,500	1,024				
112		101.8	12,000	2,048				
119		101.7	14,500	1,024				
126		101.8	12,700	1,024				
133		101.9	11,800	1,024				
140		101.6	11,200	512				
147		102.1	12,800	256				
154		101.9	14,200	512				
161		102.1	15,000	1,024				
168		102.1	14,500	512				
175		101.8	15,500	512				
208		102.0	15,200	512				
209		101.9	16,000	256				
210		101.8	15,100	256				
211		102.0	14,800	256				
212		102.1	14,000					
213		102.0	16,000	512				
214		101.8	15,000					
215		101.9	13,200					
216		102.1	9,000					
217		102.0	11,200					
218	Challenged	101.4	12,300		-	-	-	

Data from calf 6105 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	102.2	16,000	256				
220	101.8	11,250		-	-	-	
221	102.0	13,500					
222	101.0	8,400		-	-	-	
223	101.2	8,000					
224	102.4	7,200					
225	101.8	5,400	8,182	-	-	-	
226	102.0	6,400					
227	101.4	21,500		-	-	-	
228	101.4	11,250					
229	101.4	11,800					
230	101.2	12,000		-	-	-	
231	101.4	13,000					
232	101.2	13,500	65,536	-	-	-	
233	101.0	13,200					
237	101.8	12,800		-	-	-	
239	101.6	12,200	65,536	-	-	-	
244	101.8	12,000		-	-	-	
246	102.0	13,000	131,072	-	-	-	
251	102.0	12,700		-	-	-	
253	101.4	12,500	524,288	-	-	-	
258	101.2	12,200		-	-	-	
260	101.6	12,000	1,048,576	-	-	-	

Data from calf 6102 Vaccine - Chl

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		101.8	14,900	-	-	-	-	
2		101.8	12,000					
3		102.4	10,800					
4		102.4	11,600					
5		102.3	11,900					
6		101.8	11,500					
7		102.0	13,000					
8		102.4	14,200	-	-	-	-	
9		102.0	15,750					
10		102.2	14,500					
11		102.3	15,500					
12		101.8	14,900					
13		102.2	13,200					
14	Vaccinated	102.6	16,200	-	-	-	-	
15		102.3	12,800					
16		102.2	12,900		-	-	-	
17		101.8	12,400					
18		102.2	12,400					
19		101.8	11,400		-	-	-	
20		102.0	12,200					
21		102.4	12,300	4				
22		102.3	15,000		-	-	-	
23		102.2	14,700					
24		101.9	14,300					
25		101.9	14,500					
26		101.9	12,700					
27		101.9	11,000					
28		101.6	14,000	8	-	-	-	
35		102.1	15,500	8				
42	2nd Vaccination	102.1	12,900	8				

Data from calf 6102 (continued)

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49		101.8	18,100	384				
56		101.6	17,000	1,024				
63		102.0	15,000	2,048				
70		101.4	16,200	1,024				
77		101.8	20,000	512				
84		102.0	21,000	512				
91		101.6	14,800	512				
98		101.4	17,500	256				
105		101.6	14,500	256				
112		101.5	16,500	384				
119		101.4	17,000	512				
126		101.8	14,900	512				
133		101.8	15,200	512				
140		101.6	16,200	256				
147		101.5	15,800	64				
154		101.8	15,200	128				
161		101.4	14,800	128				
168		101.6	16,100	128				
175		101.6	15,800	128				
208		101.8	16,900	128				
209		101.8	18,000	128				
210		101.8	14,800	128				
211		101.7	18,100	256				
212		101.6	17,200	256				
213		101.8	16,400					
214		101.7	12,800					
215		101.8	12,000					
216		101.7	11,000					
217		101.8	12,200					
218	Challenged	101.8	13,000		-	-	-	

Data from calf 6102 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	102.0	18,000	128				
220	101.8	13,000		-	-	-	
221	101.8	13,750					
222	103.0	7,000		-	-	-	
223	101.2	8,800					
224	101.4	10,500					
225	102.0	7,800	2,048	-	-	-	
226	107.0	7,000					D
227	100.4	18,600		-	-	-	
228	102.0	13,000					
229	101.8	13,200					
230	101.6	13,500		-	-	-	
231	101.6	13,100					
232	101.8	12,500	131,072	-	-	-	
233	101.4	12,800					
237	101.4	13,200		-	-	-	
239	102.0	13,500	131,072	-	-	-	
244	101.2	13,200		-	-	-	
246	101.2	13,000	16,384	-	-	-	
251	101.2	12,700		-	-	-	
253	101.2	12,900	524,288	-	-	-	
258	101.6	13,200		-	-	-	
260	101.2	12,800	131,072	-	-	-	

D depression

Data from calf 6116 — Vaccine - SA

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		102.4	12,400	-	-	-	-	
2		102.1	11,000					
3		102.0	24,000					
4		101.7	12,200					
5		102.3	19,750					
6		102.7	12,400					
7		102.3	12,000					
8		102.5	13,200	-	-	-	-	
9		102.3	10,000					
10		102.3	12,500					
11		101.3	15,000					
12		102.5	14,500					
13		102.5	11,100					
14	Vaccinated	102.9	13,000	-	-	-	-	
15		102.4	11,600					
16		102.6	12,500	-	-	-	-	
17		102.6	10,200					
18		102.4	9,400					
19		102.5	10,500		-	-	-	
20		102.4	9,800		-	-	-	
21		102.4	9,800	-				
22		102.8	8,700		-	-	-	
23		102.6	9,000					
24		103.0	11,500					
25		102.3	12,800					
26		102.6	11,800					
27		102.5	11,600					
28		102.4	12,000	2	-	-	-	
35		102.4	12,700	8				
42		101.6	12,500	8				

Data from calf 6116 (continued)

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49		101.6	12,500	1,024				
56		101.9	13,500	8,192				
63		101.8	18,500	16,384				
70		102.4	16,000	4,096				
77		102.4	18,300	2,048				
84		102.0	16,500	2,048				
91		101.8	13,200	2,048				
98		101.6	11,600	2,048				
105		101.1	12,500	2,048				
112		102.5	13,300	2,048				
119		101.6	15,500	4,096				
126		102.0	9,800	4,096				
133		102.0	13,650	2,048				
140		101.8	16,500	2,048				
147		101.6	12,800	1,024				
154		102.0	13,400	1,024				
161		102.0	14,400	1,536				
168		101.6	13,200	1,024				
175		101.8	12,800	1,024				
208		102.2	11,800	1,024				
209		102.0	12,600	512				
210		101.8	13,200	1,024				
211		101.6	14,600	512				
212		102.0	15,200	512				
213		102.2	15,800					
214		102.0	16,500					
215		102.4	14,000					
216		103.4	11,500					
217		101.2	13,000					
218	Challenged	102.0	12,200		-	-	-	

Data from calf 6116 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.6	9,800	1,024				
220	102.0	11,000		-	-	-	
221	101.2	12,000					
222	102.2	11,000		-	-	-	
223	101.4	7,000					
224	101.4	8,800					
225	101.8	8,250	32,768	-	-	-	
226	102.2	9,750					
227	102.0	12,750		-	-	-	
228	101.6	14,800					
229	101.6	12,900					
230	101.2	12,400		-	-	-	
231	101.2	10,800					
232	101.2	11,000	131,072	-	-	-	
233	101.4	11,600					
237	101.4	11,500		-	-	-	
239	101.4	12,000	131,072	-	-	-	
244	102.2	12,600		-	-	-	
246	102.2	12,800	32,768	-	-	-	
251	103.2	13,200		-	-	-	
253	102.2	12,500	524,288	-	-	-	
258	102.4	12,000		-	-	-	
260	101.4	11,800	1,048,576	-	-	-	

Data from calf 6071 Vaccine - None, Contact Control

Days		Temperature	Leukocytes	Virus Isolated from Titer	Nose	Rectum	Blood	Symptoms
1		102.1	12,600	-	-	-	-	
2		101.6	13,000					
3		102.3	12,000					
4		102.3	12,900					
5		102.5	24,000					
6		102.6	19,100					
7		102.4	13,750					
8		102.0	14,500	-	-	-	-	
9		102.6	15,500					
10		102.1	16,800					
11		102.2	16,500					
12		102.4	17,400					
13		102.0	15,500					
14	Vaccinated	102.8	16,000	-	-	-	-	
15		102.7	15,000					
16		102.5	14,700		-	-	-	
17		102.7	13,500					
18		102.6	12,000					
19		102.5	9,900		-	-	-	
20		102.4	10,900					
21		102.5	11,000	-				
22		102.9	12,500		-	-	-	
23		102.4	11,600					
24		102.6	10,500					
25		102.4	15,500					
26		102.2	13,200					
27		102.4	12,600					
28		102.1	14,000	-	-	-	-	
35		102.0	14,750	-				
42	2nd Vaccination	102.6	12,750					

Data from calf 6071 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49	102.1	14,800	-				
56	101.8	19,700	-				
63	102.0	17,600	-				
70	102.0	18,250	-				
77	102.3	16,500	-				
84	102.5	16,500	-				
91	102.1	17,500	-				
98	102.2	16,400	-				
105	102.1	13,200	-				
112	102.4	11,400	-				
119	102.2	12,400	-				
126	102.0	13,200	-				
133	102.1	12,750	-				
140	102.0	11,400	-				
147	102.4	16,000	-				
154	102.1	14,600	-				
161	102.0	15,800	-				
168	102.3	17,100	-				
175	101.9	18,250	-				
208	102.0	15,000	-				
209	102.3	17,200	-				
210	102.1	15,900	-				
211	102.0	14,800					
212	102.2	12,750					
213	102.0	11,500					
214	101.8	10,450					
215	101.9	11,700					
216	101.5	12,100					
217	101.4	11,200					
218 Challenged	101.5	12,000		-	-	-	

Data from calf 6071 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.4	20,000					
220	101.6	12,800		-	-	-	
221	103.0	6,250					D
222	102.4	8,200		-	+	-	D
223	102.6	7,700					D, A
224	101.8	9,100					
225	102.0	8,000	-	-	-	-	
226	102.0	12,500					
227	101.0	16,250		-	-	+	
228	101.6	11,000					
229	101.4	11,300					
230	101.4	11,400		-	-	-	
231	101.4	11,600					
232	101.4	11,800	256	-	-	-	
233	101.8	12,200					
237	101.4	12,500		-	-	-	
239	101.4	12,300	512	-	-	-	
244	101.4	12,000		-	-	-	
246	101.6	12,500	2,048	-	-	-	
251	101.0	12,800		-	-	-	
253	101.8	13,000	8,192	-	-	-	
258	100.8	12,600		-	-	-	
260	101.0	12,900	16,384	-	-	-	

D depression
A anorexia

Data from calf 6098 Vaccine - Chl

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		102.0	18,500	-	-	-	-	
2		102.0	15,900					
3		104.0	11,500					
4		103.4	10,400					
5		102.0	23,000					
6		101.9	17,600					
7		101.2	22,000					
8		102.3	24,000	-	-	-	-	
9		101.4	17,400					
10		102.1	16,500					
11		102.4	18,500					
12		101.8	17,500					
13		102.9	22,900					
14	Vaccinated	102.4	15,400	-	-	-	-	
15		102.0	10,500					
16		102.2	11,500		-	-	-	
17		102.3	12,000					
18		102.4	12,500					
19		102.0	13,300		-	-	-	
20		102.8	12,800					
21		102.4	12,500	-				
22		102.5	13,500		-	-	-	
23		102.5	13,200					
24		102.8	12,500					
25		102.2	14,000					
26		102.3	12,000					
27		101.8	12,500					
28		102.1	14,600	-	-	-	-	
35		102.1	16,900					
42	2nd Vaccination	102.3	12,400	-				

Data from calf 6098 (continued)

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49		102.0	17,000	32				
56		102.1	17,800	512				
63		102.4	16,750	1,024				
70		101.9	15,100	512				
77		102.8	21,500	1,024				
84		102.0	15,000	512				
91		101.8	11,250	512				
98		101.5	14,500	256				
105		101.4	15,500	256				
112		101.8	15,100	128				
119		102.2	14,700	512				
126		102.5	14,200	256				
133		102.0	15,500	256				
140		102.4	17,400	128				
147		101.9	16,800	256				
154		102.2	16,400	128				
161		102.3	15,500	128				
168		102.0	16,000	128				
175		102.4	15,100	128				
208		102.2	16,000	128				
209		102.1	16,500	64				
210		102.2	15,000	128				
211		102.4	17,800	128				
212		102.7	18,200	128				
213		102.2	20,750					
214		102.1	15,800					
215		102.4	14,600					
216		103.3	12,500					
217		101.6	11,700					
218	Challenged	101.0	12,200		-	-	-	

Data from calf 6098 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.2	16,500	256				
220	101.8	12,750		-	-	-	
221	101.6	17,000					
222	101.8	9,000		-	-	-	
223	101.4	9,400					
224	102.4	12,400					
225	101.8	9,250	2,048	-	-	-	
226	104.0	15,900					
227	101.2	18,250		-	-	-	
228	102.2	17,500					
229	101.8	15,200					
230	101.0	14,500		-	-	-	
231	101.0	12,500					
232	101.6	13,000	131,072	-	-	-	
233	100.4	14,200					
237	101.0	14,800		-	-	-	
239	101.2	15,200	131,072	-	-	-	
244	101.6	16,000		-	-	-	
246	101.2	15,800	131,072	-	-	-	
251	101.0	15,200		-	-	-	
253	101.0	14,200	131,072	-	-	-	
258	101.6	14,800		-	-	-	
260	101.2	14,500	262,144	-	-	-	

Data from calf 6099 Vaccine - Chl

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		101.4	20,600	-	-	-	-	
2		102.8	16,200					
3		104.5	19,000					
4		102.2	12,900					
5		102.9	24,000					
6		102.0	17,100					
7		102.5	22,500					
8		102.4	24,000	-	-	-	-	
9		102.0	16,200					
10		101.5	15,800					
11		102.7	18,000					
12		103.6	24,000					
13		102.4	12,900					
14	Vaccinated	102.2	16,000	-	-	-	-	
15		102.3	14,250					
16		102.2	13,000		-	-	-	
17		102.3	14,200					
18		102.4	13,250					
19		102.2	14,200		-	-	-	
20		102.5	13,800					
21		102.7	13,500	-				
22		102.8	11,900		-	-	-	
23		102.4	13,400					
24		102.0	12,400					
25		102.8	12,500					
26		102.6	14,000					
27		102.2	15,200					
28		101.5	14,500	-	-	-	-	
35		102.7	17,500	-				
42	2nd Vaccination	102.9	16,000	-				

Data from calf 6099 (continued)

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49		102.1	13,800	32				
56		102.0	17,300	512				
63		102.0	14,400	512				
70		101.9	16,000	256				
77		102.4	14,300	256				
84		102.4	14,500	128				
91		102.0	16,000	256				
98		101.5	12,800	256				
105		102.0	12,200	128				
112		101.5	14,500	64				
119		102.0	16,500	256				
126		101.8	15,500	128				
133		101.7	13,200					
140		101.9	14,600					
147		101.8	14,600	32				
154		102.0	15,200					
161		102.1	14,500	64				
168		101.8	13,200					
175		101.7	13,600					
208		102.0	14,200					
209		102.0	15,000	128				
210		102.1	14,400	32				
211		102.3	14,100	32				
212		102.2	13,600	32				
213		101.8	13,000					
214		101.6	12,000					
215		101.5	11,500					
216		102.4	10,750					
217		101.1	11,400					
218	Challenged	101.4	12,000		-	-	-	

Data from calf 6099 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.8	16,500	16				
220	102.0	10,800		-	-	-	
221	101.1	10,250					
222	101.8	7,400		-	-	-	
223	101.4	9,400					
224	101.4	8,300					
225	101.4	7,500	2,048	-	-	-	
226	102.0	12,750					
227	100.0	12,750		-	-	-	
228	101.4	13,500					
229	101.8	13,300					
230	101.6	12,200		-	-	-	
231	101.2	10,800					
232	101.8	11,700	131,072	-	-	-	
233	100.4	12,200					
237	101.4	12,800		-	-	-	
239	101.5	13,400	32,768	-	-	-	
244	101.2	12,900		-	-	-	
246	101.2	12,500	8,192	-	-	-	
251	101.5	12,300		-	-	-	
253	101.4	12,750	524,288	-	-	-	
258	100.8	13,200		-	-	-	
260	101.0	12,500	131,072	-	-	-	

Data from calf 6066 Vaccine - None, Contact Control

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		102.8	16,700	-	-	-	-	
2		102.4	13,900					
3		102.8	11,500					
4		102.3	15,500					
5		102.0	24,500					
6		102.3	16,000					
7		102.0	23,000					
8		102.4	17,500	-	-	-	-	
9		102.2	14,500					
10		102.3	13,500					
11		101.7	14,500					
12		102.8	16,200					
13		102.2	18,500					
14	Vaccinated	102.6	17,500	-	-	-	-	
15		101.8	11,000					
16		101.9	12,400		-	-	-	
17		101.8	11,800					
18		102.0	11,000					
19		102.7	14,500		-	-	-	
20		102.6	13,100					
21		102.5	12,600	-				
22		102.4	9,600		-	-	-	
23		102.2	10,500					
24		102.0	10,000					
25		102.8	11,000					
26		102.0	12,200					
27		101.8	21,000					
28		102.6	11,400	-	-	-	-	
35		102.3	14,000	-				
42	2nd Vaccination	102.0	12,000	-				

Data from calf 6066 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49	102.0	18,500	-				
56	101.8	16,100	-				
63	101.9	16,800	-				
70	102.1	16,000	-				
77	101.9	15,500	-				
84	101.7	12,800	-				
91	101.8	12,200	-				
98	102.0	12,100	-				
105	102.1	11,200	-				
112	101.9	12,800	-				
119	101.7	14,100	-				
126	101.8	15,400	-				
133	101.6	16,200	-				
140	101.5	15,100	-				
147	101.6	14,900	-				
154	101.9	14,100	-				
161	102.0	14,400	-				
168	101.6	13,800	-				
175	101.8	13,500	-				
208	101.6	16,500	-				
209	101.5	15,250	-				
210	101.4	14,400	-				
211	101.7	13,900					
212	101.8	13,100					
213	102.0	12,000					
214	102.2	12,250					
215	102.0	12,000					
216	101.6	12,800					
217	101.6	12,400					
218 Challenged	101.5	13,000		-	-	-	

Data from calf 6066 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.6	15,250					
220	102.4	9,750		-	-	+	
221	102.0	8,100					
222	101.8	4,500		-	-	-	
223	101.6	6,500					
224	102.4	8,500					
225	102.0	6,000	-	-	-	+	D, A
226	105.0	12,750					
227	101.4	10,800		-	-	-	
228	103.8	10,100					D
229	102.2	9,800					
230	101.0	9,500		-	-	-	
231	102.0	10,200					
232	102.0	10,600	128	-	+	-	
233	101.8	10,800					
237	101.4	11,400		-	-	-	
239	101.2	11,800	512	-	-	-	
244	101.2	12,200		-	-	-	
246	101.4	11,800	512	-	-	-	
251	101.8	11,900		-	-	-	
253	101.2	11,500	2,048	-	-	-	
258	101.6	11,200		-	-	-	
260	101.2	10,700	4,096	-	-	-	

D depression
A anorexia

Data from calf 6123 Vaccine - SA

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		102.6	22,000	-	-	-	-	
2		102.6	15,400					
3		102.2	24,000					
4		101.6	15,000					
5		104.7	21,000					
6		104.8	14,000					
7		102.9	18,000					
8		101.8	22,600	-	-	-	-	
9		102.5	16,000					
10		102.0	14,800					
11		102.7	14,800					
12		102.5	15,000					
13		102.2	14,500					
14	Vaccinated	102.4	18,900	-	-	-	-	
15		102.0	10,700					
16		102.2	11,200		-	-	-	
17		102.5	10,000					
18		102.5	11,300					
19		102.2	11,200		-	-	-	
20		102.6	10,800					
21		102.4	10,000	4				
22		102.9	9,500		-	-	-	
23		102.6	10,200					
24		103.4	9,000					
25		102.7	9,000					
26		102.4	10,500					
27		102.1	10,200					
28		102.3	10,200	4	-	-	-	
35		101.5	11,900	4				
42	2nd vaccination	102.5	13,100	32				

Data from calf 6123 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49	102.2	10,700	2,048				
56	102.4	12,500	16,384				
63	102.3	12,000	16,384				
70	102.6	13,000	4,096				
77	102.3	9,800	8,192				
84	102.4	14,200	4,096				
91	102.6	13,000	4,096				
98	102.1	12,300	4,096				
105	102.5	16,000	4,096				
112	102.4	12,800	2,048				
119	102.2	11,800	4,096				
126	102.6	13,000	4,096				
133	101.8	14,800	4,096				
140	102.2	15,200	2,048				
147	102.5	13,800	1,024				
154	101.9	11,800	1,024				
161	101.8	10,900	1,024				
168	102.0	12,200	1,024				
175	102.2	13,400	1,024				
208	102.2	14,800	1,024				
209	102.5	16,000	1,024				
210	102.0	14,800	1,024				
211	101.9	15,300	512				
212	102.0	17,800	512				
213	101.8	16,000					
214	101.6	15,200					
215	101.8	12,800					
216	101.7	11,500					
217	101.9	12,600					
218 Challenged				-	-	-	

Data from calf 6123 (continued)

Days Postchallenge	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	102.0	14,750	1,024				
220	102.2	13,250		-	-	-	
221	101.9	12,500					
222	102.4	13,500		-	-	-	
223	103.0	7,500					
224	102.0	8,700					
225	102.4	9,250	2,048	-	-	-	
226	101.6	8,650					
227	101.2	18,250		-	-	-	
228	101.6	17,500					
229	101.8	15,200					
230	101.8	14,500		-	-	-	
231	101.4	12,500					
232	101.8	13,200	131,072	-	-	-	
233	101.8	13,500					
237	102.0	14,200		-	-	-	
239	102.0	14,600	131,072	-	-	-	
244	102.0	14,300		-	-	-	
246	101.4	13,800	32,768	-	-	-	
251	101.6	13,500		-	-	-	
253	101.4	13,800	262,144	-	-	-	
258	100.8	14,200		-	-	-	
260	101.6	13,900	524,288	-	-	-	

Data from calf 6119 Vaccine - SA

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		102.0	13,100	-	-	-	-	
2		101.0	14,400					
3		102.7	17,700					D, R
4		101.6	11,400					D, R
5		103.5	23,400					D, R
6		101.8	17,500					
7		102.3	15,000					
8		101.7	11,900	-	-	-	-	
9		102.6	17,000					
10		102.0	12,000					
11		102.4	22,000					
12		102.3	18,500					
13		102.6	11,000					D
14	Vaccinated	101.8	12,500	-	-	-	-	
15		102.6	12,500					
16		102.5	12,800		-	-	-	
17		102.4	11,250					
18		102.8	14,900					D
19		102.4	13,200		-	-	-	
20		102.6	14,000					
21		102.3	15,500	4				
22		103.4	13,500		-	-	-	D
23		102.4	12,800					
24		102.8	10,400					D
25		102.4	9,200					
26		102.6	11,400					
27		102.5	14,200					
28		102.4	20,200	8	-	-	-	
35		102.2	14,000	8				
42	2nd Vaccination	102.3	13,100	32				

Data from calf 6119 (continued)

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49		101.4	11,300	8,192				
56		101.6	14,000	8,192				
63		103.3	16,350	16,384				D
70		102.4	15,000	16,384				
77		102.0	14,000	16,384				
84		102.4	16,200	8,192				
91		101.8	14,500	8,192				
98		102.6	16,500	8,192				D
105		102.4	18,000	8,192				
112		102.2	13,500	8,192				
119		101.8	12,500	4,096				
126		101.8	13,500	4,096				
133		102.0	14,200	4,096				
140		102.4	15,100	2,048				
147		101.8	14,600	1,024				
154		102.2	15,400	2,048				
161		102.0	14,600	2,048				
168		101.8	13,600	2,048				
175		102.2	14,700	2,048				
208		102.4	15,200	2,048				
209		102.4	15,700	2,048				
210		101.5	14,800	2,048				
211		101.8	17,500	1,024				
212		101.6	15,400	512				
213		101.8	14,800					
214		101.7	13,600					
215		102.0	14,300					
216		102.1	13,000					
217		101.6	14,200					
218	Challenged	101.9	12,800		-	-	-	

Data from calf 6119 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.8	15,750	2,048				D,A,R cough, dep. anorexia
220	102.2	11,500		-	-	-	D,A,R
221	101.6	12,000					D,A,R
222	102.8	12,000		-	-	-	D,A,R
223	103.2	6,400					D,A,R
224	102.6	10,400					D,A,R
225	101.6	10,000	4,096	-	-	-	D,A,R
226	106.0	8,750					D,A,R
227	102.4	11,750		-	-	-	D,A,R
228	101.6	12,750					D,A,R
229	101.4	12,500					D,A,R
230	102.0	12,000		-	-	-	D,A,R
231	101.4	11,500					D
232	102.0	12,000	131,072	-	-	-	D
233	101.8	12,400					D
237	101.4	12,200		-	-	-	D
239	102.0	11,800	131,072	-	-	-	D
244	101.4	11,400		-	-	-	D
246	101.2	10,800	32,768	-	-	-	D
251	102.0	10,400		-	-	-	D
253	101.8	11,000	131,144	-	-	-	D
258	101.6	11,400		-	-	-	D
260	101.2	12,000	522,288	-	-	-	D

D depression
A anorexia
R respiratory (cough)

Data from calf 6121 Vaccine - None, Contact Control

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		101.8	19,800	-	-	-	-	
2		102.4	15,400					
3		102.4	15,000					
4		101.3	16,300					
5		102.4	24,000					
6		102.2	19,100					
7		102.5	24,000					
8		101.7	21,000	-	-	-	-	
9		102.0	13,500					
10		101.8	14,100					
11		101.9	14,000					
12		102.1	11,200					
13		102.4	17,100					
14	Vaccinated	102.2	14,750	-	-	-	-	
15		101.9	11,500					
16		101.8	12,600					
17		102.2	11,900	-	-	-	-	
18		101.8	12,500					
19		102.4	12,800					
20		102.2	12.100	-	-	-	-	
21		102.1	12,700					
22		102.7	10,500					
23		102.1	11,000	-	-	-	-	
24		103.4	10,250					
25		102.3	11,250					
26		102.4	10,400					
27		103.4	9,200					
28		102.4	12,200	-	-	-	-	
35		102.1	12,600	-				
42	2nd Vaccination	102.2	14,000	-				

Data from calf 6121 (continued)

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49		102.4	13,300					
56		102.6	15,100	-				
63		102.6	16,800	-				
70		103.1	15,200	-				
77		102.4	18,500	-				
84		101.6	12,750	-				
91		102.5	13,500	-				
98		102.2	12,750	-				
105		102.1	13,100	-				
112		101.9	12,250	-				
119		101.8	13,300	-				
126		101.6	14,000	-				
133		101.7	14,200	-				
140		101.8	15,200	-				
147		101.5	16,700	-				
154		101.5	15,000	-				
161		101.7	14,800	-				
168		101.8	13,900	-				
175		101.6	13,700	-				
208		101.8	20,000	-				
209		101.6	18,700	-				
210		101.5	17,100	-				
211		101.9	15,200					
212		102.0	13,100					
213		102.6	12,400					
214		102.8	10,500					
215		101.6	11,700					
216		101.5	12,600					
217		101.6	12,900					
218	Challenged	101.5	12,000		-	-	-	

Data from calf 6121 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.4	15,000					
220	102.0	12,500		-	-	-	
221	101.4	8,400					D, A
222	102.2	10,200		-	-	-	
223	102.4	9,800					D,A,R
224	101.0	10,300					
225	101.4	10,000	-	-	-	+	
226	104.0	16,500					D, A
227	101.8	15,600		-	-	-	
228	102.2	14,500					
229	101.6	14,000					
230	101.0	13,400		-	-	-	
231	101.2	14,750					
232	101.8	14,500	64	-	-	-	
233	102.4	14,200					
237	101.2	15,000		-	-	-	
239	101.2	15,400	128	-	-	-	
244	101.4	15,200		-	-	-	
246	101.2	14,800	512	-	-	-	
251	100.0	14,200		-	-	-	
253	101.4	14,500	2,048	-	-	-	
258	101.4	14,700		-	-	-	
260	101.0	14,400	1,024	-	-	-	

D depression
A anorexia
R respiratory (cough)

Data from calf 6086 Vaccine - SA

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		101.7	13,500	-	-	-	-	
2		101.0	8,000					
3		102.2	9,000					
4		101.8	10,300					
5		102.2	12,400					
6		102.5	18,000					
7		101.4	12,400					
8		102.6	10,000	-	-	-	-	
9		102.4	19,800					
10		101.8	12,000					
11		101.5	12,400					
12		102.4	12,600					
13		102.2	12,600					
14	Vaccinated	102.0	11,800	-	-	-	-	
15		102.2	11,200					
16		102.0	10,200	-	-	-	-	
17		102.1	9,600					
18		101.6	9,600					
19		101.8	9,300		-	-	-	
20		102.2	9,000					
21		102.2	9,000	4				
22		102.4	8,500		-	-	-	
23		102.1	10,300					
24		102.2	10,000					
25		102.0	13,400					
26		102.4	12,700					
27		102.2	12,400					
28		102.3	11,900	8	-	-	-	
35		101.8	11,300	8				
42	2nd Vaccination	102.2	11,200	32				

Data from calf 6086 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49	101.7	9,400	8,192				
56	101.7	15,500	16,384				
63	101.6	19,100	16,384				
70	101.8	13,300	16,384				
77	101.6	12,500	16,384				
84	101.8	12,500	8,192				
91	101.8	15,900	16,384				
98	101.8	8,800	16,384				
105	101.0	10,800	8,192				
112	102.1	9,800	16,384				
119	102.0	11,500	16,384				
126	101.8	11,800	16,384				
133	102.0	13,200	8,192				
140	102.0	12,500	4,096				
147	102.2	12,400	2,048				
154	101.8	11,800	4,096				
161	101.6	12,000	4,096				
168	102.2	10,600	2,048				
175	102.4	10,000	2,048				
208	102.3	9,000	2,048				
209	101.8	10,600	2,048				
210	102.2	11,500	2,048				
211	102.2	11,800	2,048				
212	102.2	12,000	2,048				
213	102.1	11,400					
214	102.2	11,800					
215	101.5	11,500					
216	101.8	9,800					
217	101.8	10,200					
218 Challenged	101.5	11,000		-	-	-	

Data from calf 6086 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.8	12,000	2,048				
220	101.6	11,300		-	-	-	
221	101.2	10,500					
222	102.0	13,400		-	-	-	
223	102.0	12,800					
224	102.0	12,700					
225	101.8	12,500	2,048	-	-	-	
226	102.0	14,500					
227	102.0	18,800		-	-	-	
228	101.8	13,000					
229	101.8	13,200					
230	101.6	13,500		-	-	-	
231	101.2	13,400					
232	101.4	13,800	4,096	-	-	-	
233	101.0	13,200					
237	101.2	14,000		-	-	-	
239	101.2	13,600	4,096	-	-	-	
244	101.4	13,200		-	-	-	
246	101.5	12,800	2,048	-	-	-	
251	101.6	13,600		-	-	-	
253	101.2	13,200	8,192	-	-	-	
258	101.2	12,700		-	-	-	
260	101.0	11,900	4,096	-	-	-	

Data from calf 6088 Vaccine - None, Contact Control

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		101.4	20,000	-	-	-	-	
2		103.2	9,400					
3		101.9	16,000					
4		101.4	11,000					
5		103.1	23,000					
6		102.4	17,200					
7		102.4	9,250					
8		101.3	7,100	-	-	-	-	
9		103.0	8,500					
10		101.5	11,750					
11		102.7	10,500					
12		101.4	13,000					
13		101.9	10,100					
14	Vaccinated	101.1	13,600	-	-	-	-	
15		102.2	10,000					
16		102.0	10,400	-	-	-	-	
17		101.9	9,600					
18		102.2	9,800					
19		102.0	12,750		-	-	-	
20		101.9	10,700					
21		101.8	9,000	-				
22		102.4	8,000		-	-	-	
23		101.5	9,100					
24		102.1	8,500					
25		102.0	8,000					
26		101.6	8,200					
27		102.4	9,100					
28		102.0	9,500	-	-	-	-	
35		102.3	12,750	-				
42	2nd Vaccination	102.4	8,700	-				

Data from calf 6088 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49	101.9	9,700	-				
56	102.0	12,000	-				
63	101.8	11,200	-				
70	102.1	10,600	-				
77	101.8	10,500	-				
84	102.4	10,000	-				
91	102.3	12,600	-				
98	102.0	11,400	-				
105	102.2	10,800	-				
112	102.1	10,400	-				
119	102.1	10,700	-				
126	101.9	11,000	-				
133	101.7	11,400	-				
140	102.0	10,900	-				
147	101.8	12,100	-				
154	101.5	12,400	-				
161	101.7	15,000	-				
168	101.6	16,000	-				
175	101.7	15,400	-				
208	101.5	16,000	-				
209	101.6	12,750	-				
210	101.4	11,800	-				
211	101.6	11,250	-				
212	101.9	10,750					
213	102.0	10,200					
214	102.2	10,900					
215	102.7	9,600					
216	101.0	10,200					
217	101.5	11,000					
218 Challenged	101.0	12,100		-	-	-	

Data from calf 6088 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.8	16,000	-				
220	102.0	7,250		-	-	+	
221	101.8	6,250					D, A
222	101.4	8,200		-	-	+	D, A
223	102.4	9,000					D, A
224	104.2	9,200					D, R
225	103.0	8,000	-	-	-	+	L stiff
226	102.4	10,200					L stiff
227	101.8	9,250		-	+	-	
228	101.4	9,000					
229	101.8	9,300					
230	101.0	9,500		-	-	-	
231	101.4	9,200					
232	101.4	9,500	64	-	-	+	
233	102.4	9,300					
237	101.4	9,600		-	-	-	
239	101.9	10,000	256	-	-	-	
244	102.0	11,000		-	-	-	
246	102.0	10,600	512	-	-	-	
251	101.6	9,800		-	-	-	
253	101.6	9,600	2,048	-	-	-	
258	101.2	9,500		-	-	-	
260	101.8	9,800	4,096	-	-	-	

D depression
A anorexia
L laminitis
R respiratory (cough)

Data from calf 6100 Vaccine - BPL

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		101.8	11,200					
2		101.2	11,000	-	-	-	-	
3		101.7	10,400					
4		101.2	11,900					
5		103.0	15,200					
6		101.6	16,000					
7		102.5	14,900					
8		102.4	12,900	-	-	-	-	
9		102.4	11,750					
10		102.4	13,200					
11		102.3	13,500					
12		102.4	14,000					
13		102.6	12,700					
14	Vaccinated	102.0	14,600	-	-	-	-	
15		102.3	12,500					
16		102.2	12,000		-	-	-	
17		102.2	11,600					
18		102.1	11,800					
19		102.4	10,100		-	-	-	
20		102.3	10,300					
21		102.6	11,200	-				
22		102.3	11,800		-	-	-	
23		102.1	11,000					
24		102.5	12,750					
25		101.8	22,200					
26		102.4	14,200					
27		101.8	10,200					
28		102.2	13,800	-	-	-	-	
35		102.4	12,800	8				
42	2nd Vaccination	102.3	11,000	8				

Data from calf 6100 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49	102.4	13,800	384				
56	102.0	15,000	4,096				
63	102.5	17,500	8,192				
70	101.7	13,500	4,096				
77	102.2	8,000	2,048				
84	101.9	11,250	2,048				
91	102.3	12,000	2,048				
98	101.5	11,800	1,024				
105	101.7	10,600	1,536				
112	101.8	8,750	1,536				
119	101.7	10,000	1,024				
126	101.9	11,100	2,048				
133	102.3	11,800	1,024				
140	101.9	12,600	1,024				
147	102.2	12,800	1,024				
154	101.8	11,900	1,024				
161	101.9	13,000	1,024				
168	102.2	14,200	512				
175	102.3	15,000	512				
208	102.1	14,000	512				
209	101.8	12,500	256				
210	102.3	12,800	256				
211	102.3	13,000	512				
212	102.1	12,500	1,024				
213	102.0	11,700					
214	102.1	11,200					
215	102.2	10,200					
216	102.2	9,000					
217	101.8	10,400					
218 Challenged	102.2	11,000		-	-	-	

Data from calf 6100 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.8	12,500	1,024				
220	101.8	8,750		-	-	-	
221	101.8	10,300					
222	102.2	15,000		-	-	-	
223	101.8	10,300					
224	101.8	13,500					
225	103.0	8,500	2,048	-	-	-	D
226	104.0	7,750					D
227	101.8	12,250		-	-	-	
228	101.6	7,500					
229	101.4	10,000					
230	101.8	12,400		-	-	-	
231	101.4	12,600					
232	101.4	12,400	131,072	-	-	-	
233	101.6	11,800					
237	101.6	11,200		-	-	-	
239	101.2	10,800	131,072	-	-	-	
244	102.2	12,000		-	-	-	
246	101.2	11,800	32,768	-	-	-	
251	101.8	12,000		-	-	-	
253	101.0	11,400	262,144	-	-	-	
258	101.0	11,800		-	-	-	
260	101.2	11,800	262,144	-	-	-	

D depression

Data from calf 6097 Vaccine - BPL

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		101.8	17,000	-	-	-	-	
2		102.0	10,000					
3		102.4	10,100					
4		102.6	12,500					
5		102.2	24,000					
6		102.0	24,000					
7		102.4	16,800					
8		101.8	16,500	-	-	-	-	
9		102.1	12,600					
10		101.8	12,000					
11		101.5	14,000					
12		101.8	13,000					
13		102.2	15,000					
14	Vaccinated	102.0	11,000	-	-	-	-	
15		102.5	14,500					
16		102.4	13,600		-	-	-	
17		102.1	13,000					
18		102.3	12,500					
19		102.2	10,100		-	-	-	
20		102.4	10,900					
21		102.5	11,600	-				
22		102.0	10,000		-	-	-	
23		102.9	10,500					
24		102.5	13,000					
25		102.6	9,800					
26		102.4	10,500					
27		102.5	9,800					
28		102.4	10,900					
35		101.8	11,900	-	-	-	-	
42	2nd Vaccination	102.0	10,000	2				

Data from calf 6097 (continued)

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49		101.8	11,700	1,024				
56		102.0	13,600	4,096				
63		101.5	11,000	4,096				
70		102.1	12,000	2,048				
77		102.2	12,000	1,024				
84		101.9	11,250	2,048				
91		101.6	9,600	2,048				
98		102.4	9,000	1,024				
105		101.7	7,500	1,024				
112		101.6	9,200	1,536				
119		101.9	9,000	2,048				
126		101.8	10,400	2,048				
133		102.0	11,000	1,024				
140		101.6	11,300	1,024				
147		101.7	12,400	512				
154		101.5	11,900	512				
161		101.8	13,000	256				
168		101.9	15,200	256				
175		101.5	18,100	128				
208		101.5	17,900	256				
209		101.6	20,000	128				
210		101.5	13,000	256				
211		102.0	11,200	256				
212		102.2	7,600	256				
213		101.9	9,200					
214		102.1	9,600					
215		102.4	8,400					
216		102.5	7,600					
217		101.8	9,000					
218	Challenged	101.6	11,000		-	-	-	

Data from calf 6097 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	102.0	20,000	256				
220	102.0	8,900		-	-	-	
221	101.8	6,300					
222	102.4	8,200		-	-	-	
223	102.0	5,800					
224	102.0	6,900					
225	102.8	6,000	8,192	-	-	-	D, E
226	107.0	10,200					D
227	101.2	19,250		-	-	-	
228	101.4	11,750					
229	101.6	10,200					
230	101.4	9,600		-	-	-	
231	101.2	9,800					
232	101.4	10,200	131,072	-	-	-	
233	101.0	10,600					
237	101.4	11,200		-	-	-	
239	101.4	10,800	131,072	-	-	-	
244	101.6	10,200		-	-	-	
246	101.2	9,600	131,072	-	-	-	
251	101.8	10,400		-	-	-	
253	101.2	10,400	524,288	-	-	-	
258	101.4	10,200		-	-	-	
260	101.4	9,800	>1,048,576	-	-	-	

D depression
E enteritis

Data from calf 6106 Vaccine - None, Contact Control

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		102.2	17,500	-	-	-	-	
2		101.8	13,000					
3		102.8	11,600					
4		102.1	10,600					
5		102.6	17,000					
6		102.4	24,000					
7		101.9	14,000					
8		102.4	10,700	-	-	-	-	
9		103.4	10,850					
10		102.4	12,300					
11		102.0	12,250					
12		103.2	11,000					
13		102.6	12,800					
14	Vaccinated	102.5	11,800	-	-	-	-	
15		103.1	7,500					
16		102.5	8,900		-	-	-	
17		102.6	10,400					
18		102.3	9,000					
19		103.2	8,200		-	-	-	
20		102.8	9,800					
21		103.2	11,750	-				
22		102.6	10,750		-	-	-	
23		102.6	10,900					
24		102.5	9,500					
25		102.4	10,000					
26		102.9	8,300					
27		102.0	10,400					
28		102.9	10,750	-	-	-	-	
35		102.4	9,000	-				
42	2nd Vaccination	102.7	8,900	-				

Data from calf 6106 (continued)

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49		102.8	11,600	-				
56		102.9	12,750	-				
63		102.0	10,900	-				
70		102.4	10,750	-				
77		102.6	10,000	-				
84		102.5	10,000	-				
91		102.4	10,700	-				
98		102.0	11,000	-				
105		101.9	9,000	-				
112		102.1	9,900	-				
119		101.9	8,000	-				
126		101.8	8,500	-				
133		102.1	9,250	-				
140		101.8	10,250	-				
147		101.6	11,000	-				
154		102.0	10,500	-				
161		102.1	9,600	-				
168		101.9	9,200	-				
175		101.7	8,500	-				
208		102.0	10,500	-				
209		101.8	8,000	-				
210		101.9	11,500	-				
211		101.8	10,750					
212		102.0	9,250					
213		102.2	9,000					
214		101.9	8,500					
215		101.8	7,750					
216		102.0	8,200					
217		102.1	10,000					
218	Challenged	102.0	9,000		-	-	-	

Data from calf 6106 (continued)

Days	Temperature	Leukocytes	Virus Isolation from Titer	Nose	Rectum	Blood	Symptoms
219	101.4	10,500					
220	101.6	7,500		-	-	-	
221	102.0	4,500					D
222	102.2	7,400		-	-	+	D
223	102.8	6,200					D
224	102.8	7,400					D, E
225	101.6	7,000	-	+	+	+	D, E
226	102.0	11,000					
227	102.8	15,500		-	-	+	
228	101.8	7,000					
229	102.2	7,600					
230	101.9	8,000		-	-	-	
231	101.4	8,200					
232	101.4	8,600	256	-	-	-	
233	101.8	8,900					
237	101.8	9,400		-	-	-	
239	101.2	10,000	512	-	-	-	
244	101.5	9,800		-	-	-	
246	101.4	9,200	512	-	-	-	
251	101.8	8,800		-	-	-	
253	101.8	9,200	8,182	-	-	-	
258	101.4	8,500		-	-	-	
260	101.4	8,400	16,384	-	-	-	

D depression
E enteritis

Data from calf 6104 Vaccine - None, Barn Control

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		103.2	10,000	-	-	-	-	
2		102.8	12,500					
3		102.0	24,000					
4		102.8	14,300					
5		102.2	24,500					
6		102.1	12,750					
7		102.3	15,200					
8		101.7	22,000	-	-	-	-	
9		102.3	13,500					
10		102.5	11,000					
11		101.6	12,500					
12		102.3	15,000					
13		103.0	21,000					
14	Vaccinated	101.9	15,300	-	-	-	-	
15		101.9	13,250					
16		102.0	14,500		-	-	-	
17		102.2	12,750					
18		102.4	13,750					
19		102.4	13,100		-	-	-	
20		102.2	12,750					
21		102.5	12,500	-				
22		103.0	13,000		-	-	-	
23		102.0	12,500					
24		102.9	13,250					
25		102.5	12,500					
26		102.4	12,400					
27		102.3	15,500					
28		102.7	13,800	-	-	-	-	
35		102.3	12,000	-				
42	2nd Vaccination	102.0	15,500	-				

Data from calf 6104 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49	102.0	15,500	-				
56	101.9	17,000	-				
63	102.4	16,700	-				
70	102.8	15,200	-				
77	102.4	14,000	-				
84	102.4	8,000	-				
91	102.5	12,000	-				
98	102.0	14,500	-				
105	102.2	10,800	-				
112	102.1	7,800	-				
119	102.2	10,000	-				
126	102.4	12,600	-				
133	102.2	14,800	-				
140	102.4	16,000	-				
147	102.5	14,500	-				
154	102.0	16,200	-				
161	102.4	15,500	-				
168	102.2	16,000	-				
175	102.4	17,000	-				
208	102.5	13,000	-				
209	102.2	17,300	-				
210	102.0	14,000	-				
211	102.0	12,750					
212	101.8	14,200					
213	101.9	13,600					
214	102.0	12,000					
215	101.8	12,800					
216	101.7	13,200					
217	101.6	12,750					
218 Challenged	101.9	12,000		-	-	-	

Data from calf 6104 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.8	16,000					
220	102.4	11,000		+	-	+	
221	101.4	13,100					D
222	101.6	11,200		-	-	-	D
223	102.8	7,600					D
224	102.8	7,600					D
225	102.0	7,800	-	-	+	-	
226	102.0	17,750					
227	104.2	9,750		-	-	-	
228	101.4	15,250					
229	101.6	14,800					
230	101.4	14,500		-	-	-	
231	101.0	12,400					
232	101.2	14,000	32	-	-	+	
233	101.4	13,750					
237	101.8	15,000		-	-	-	
239	102.2	14,800	128	-	-	-	
244	101.8	14,500		-	-	-	
246	102.0	13,700	512	-	-	-	
251	101.8	14,600		-	-	-	
253	101.8	14,200	2,048	-	-	-	
258	101.2	13,900		-	-	-	
260	101.8	14,200	4,096	-	-	-	

D depression

Data from calf 6120 Vaccine - None, Barn Control

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		101.8	19,700	-	-	-	-	
2		102.8	14,800					
3		102.9	23,500					
4		102.0	24,000					
5		102.5	19,800					
6		101.9	14,300					
7		101.8	12,400					
8		102.1	12,250	-	-	-	-	
9		101.8	14,800					
10		102.4	12,800					
11		102.3	15,000					
12		102.4	13,250					
13		102.4	12,250					
14	Vaccinated	102.0	12,250	-	-	-	-	
15		102.5	12,500					
16		102.4	12,100		-	-	-	
17		102.1	11,750					
18		102.4	12,450					
19		102.5	10,600		-	-	-	
20		102.4	10,750					
21		102.4	11,700	-				
22		102.7	11,500		-	-	-	
23		102.5	11,800					
24		102.8	11,500					
25		102.4	11,300					
26		102.8	11,750					
27		102.4	12,000					
28		102.5	12,250	-	-	-	-	
35		102.6	11,750	-				
42	2nd Vaccination	102.3	14,000	-				

Data from calf 6120 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49	102.1	17,900	-				
56	102.0	15,000	-				
63	102.5	13,500	-				
70	101.8	15,500	-				
77	102.1	13,000	-				
84	102.2	12,500	-				
91	102.0	13,200	-				
98	102.2	10,250	-				
105	101.8	11,600	-				
112	102.2	12,200	-				
119	102.0	11,900	-				
126	101.7	10,900	-				
133	102.0	12,600	-				
140	102.2	11,200	-				
147	102.5	12,750	-				
154	102.0	11,800	-				
161	101.8	12,900	-				
168	101.9	14,200	-				
175	102.4	13,100	-				
208	102.3	16,000	-				
209	102.2	15,700	-				
210	101.8	14,300	-				
211	102.2	12,200					
212	102.0	11,800					
213	101.8	12,000					
214	102.5	11,000					
215	101.5	12,800					
216	102.0	13,200					
217	102.2	12,750					
218 Challenged	102.0	12,000					

Data from calf 6120 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	102.0	15,000	-				
220	102.0	11,400		-	-	+	
221	101.5	13,500					
222	101.4	13,000		+	+	-	
223	101.8	14,100					
224	101.8	16,700					
225	101.6	13,000	-	-	-	+	D
226	101.8	17,250					D
227	102.4	13,750		-	-	+	D
228	101.4	15,000					
229	101.4	14,200					
230	101.4	13,000		-	-	+	
231	101.6	12,500					
232	101.2	12,900	4	-	-	-	
233	101.6	13,400					
237	102.0	13,750		-	-	-	
239	101.0	14,000	64	-	-	-	
244	101.4	15,000		-	-	-	
246	101.8	14,200	128	-	-	-	
251	101.4	13,500		-	-	-	
253	101.8	13,800	512	-	-	-	
258	101.6	14,000		-	-	-	
260	101.0	14,200	512	-	-	-	

D depression

Data from calf 6127 Vaccine - None, Barn Control

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		102.4	14,600	-	-	-	-	
2		102.2	12,700					
3		102.7	14,700					
4		102.3	13,000					
5		102.2	15,400					
6		102.0	22,000					
7		101.7	11,600					
8		102.6	10,100	-	-	-	-	
9		102.3	13,400					
10		101.3	12,250					
11		102.1	14,500					
12		102.9	15,500					
13		102.6	10,900					
14	Vaccinated	102.6	11,750	-	-	-	-	
15		102.6	11,400					
16		102.3	12,200		-	-	-	
17		102.4	13,400					
18		102.2	12,600					
19		101.2	13,100		-	-	-	
20		102.1	11,800					
21		102.2	10,500	-				
22		102.6	10,000					
23		102.5	11,600		-	-	-	
24		102.8	10,500					
25		102.0	12,400					
26		102.1	11,200					
27		102.3	12,400					
28		101.8	14,000	-	-	-	-	
35		101.9	15,800	-				
42	2nd Vaccination	102.7	11,500	-				

Data from calf 6127 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49	102.1	13,900	-				
56	102.3	17,750	-				
63	102.2	16,000	-				
70	102.2	15,300	-				
77	102.5	12,500	-				
84	101.9	11,500	-				
91	102.0	16,000	-				
98	102.1	14,500	-				
105	101.8	16,200	-				
112	101.9	18,300	-				
119	101.9	15,500	-				
126	102.0	14,000	-				
133	101.7	12,500	-				
140	101.6	11,250	-				
147	101.9	12,200	-				
154	101.8	11,800	-				
161	101.8	12,250	-				
168	102.0	13,500	-				
175	101.7	12,500	-				
208	101.7	12,000	-				
209	101.7	13,000	-				
210	102.0	12,200	-				
211	101.2	11,800					
212	101.8	10,600					
213	101.6	11,200					
214	102.0	10,500					
215	101.6	11,600					
216	101.7	10,900					
217	101.6	12,500					
218 Challenged	101.8	11,600		-	-	-	

Data from calf 6127 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.8	17,000	-				
220	101.8	11,900		+	-	-	
221	101.0	12,000					
222	102.6	5,700		-	-	-	D
223	102.0	5,300					D
224	101.4	8,200					D, R
225	101.6	7,300	-	-	+	-	D
226	101.4	7,500					D
227	106.6	4,750				+	D, A
228	102.2	9,500					D, A
229	101.8	9,400					
230	101.6	9,800		-	-	+	
231	101.6	8,750					
232	101.4	9,800	128	-	-	-	
233	101.4	10,200					
237	101.2	9,800		-	-	-	
239	101.5	9,200	256	-	-	-	
244	101.2	9,600		-	-	-	
246	101.6	10,000	512	-	-	-	
251	101.6	9,500		-	-	-	
253	100.2	9,400	4,096	-	-	-	
258	102.0	9,800		-	-	-	
260	101.4	9,500	2,048	-	-	-	

D depression
A anorexia
R respiratory (cough)

Data from calf 6128 Vaccine - None, Barn Control

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		102.8	24,000	-	-	-	-	
2		102.2	11,000					
3		101.4	14,000					
4		102.4	17,500					
5		102.6	16,500					
6		102.0	9,000					
7		102.8	8,700					
8		102.4	10,200	-	-	-	-	
9		102.7	10,400					
10		101.6	10,750					
11		102.4	13,600					
12		103.1	11,800					
13		101.8	12,200					
14	Vaccinated	102.4	11,800	-	-	-	-	
15		102.8	11,800					
16		102.4	10,200		-	-	-	
17		102.2	9,700					
18		102.4	11,000					
19		102.6	14,400		-	-	-	
20		102.4	9,800					
21		102.3	11,750	-				
22		102.5	8,250		-	-	-	
23		101.8	10,200					
24		102.7	9,200					
25		102.4	9,900					
26		102.4	10,000					
27		102.4	12,000					
28		102.4	10,000	-	-	-	-	
35		102.3	6,300	-				
42	2nd Vaccination	102.8	10,800	-				

Data from calf 6128 (continued)

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49		102.0	15,400					
56		102.3	12,500	-				
63		102.4	11,600	-				
70		102.5	9,400	-				
77		102.9	14,500	-				
84		102.6	11,250	-				
91		101.8	12,800	-				
98		102.0	13,500	-				
105		102.1	12,700	-				
112		102.3	14,200	-				
119		102.2	12,800	-				
126		102.0	12,200	-				
133		102.3	11,600	-				
140		102.2	12,600	-				
147		102.0	11,200	-				
154		102.3	10,200	-				
161		102.2	10,800	-				
168		102.3	9,800	-				
175		102.0	11,000	-				
208		102.3	12,600	-				
209		102.2	9,750	-				
210		102.1	10,600	-				
211		101.8	11,200					
212		102.0	9,750					
213		101.8	9,000					
214		102.0	8,500					
215		101.6	10,000					
216		102.0	11,000					
217		102.0	10,750					
218	Challenged	102.1	9,400		-	-	-	

Data from calf 6128 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.6	11,250	-				D,A,E
220	102.0	8,750		+	-	-	D,A,E
221	101.6	10,250					
222	102.2	11,800		-	+	-	
223	102.4	12,300					
224	102.2	13,800					D,R
225	101.8	10,500	-	-	+	+	D
226	101.6	6,250					D
227	101.6	5,450		+	+	-	
228	101.4	7,750					
229	101.2	7,600					
230	101.4	6,300		-	-	-	
231	101.6	9,300					
232	101.4	10,400	4	-	+	-	
233	101.6	9,600					
237	101.2	10,800		-	-	+	
239	101.2	11,500	64	-	-	-	
244	101.4	10,800		-	-	-	
246	101.6	11,200	512	-	-	-	
251	101.8	12,000		-	-	-	
253	102.4	11,500	2,048	-	-	-	
258	101.4	11,600		-	-	-	
260	101.0	11,400	4,096	-	-	-	

D depression
A anorexia
E enteritis
R respiratory (cough)

Data from calf 6114 Vaccine - None, Barn Control

Days		Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
1		101.2	7,500	-	-	-	-	
2		101.6	14,500					
3		102.6	24,000					
4		102.5	15,400					
5		102.3	22,000					
6		102.4	14,000					
7		102.7	16,750					
8		101.9	14,500	-	-	-	-	
9		102.0	16,100					
10		101.8	19,000					
11		101.7	18,200					
12		102.5	21,000					
13		101.8	16,500					
14	Vaccinated	101.3	12,200	-	-	-	-	
15		102.4	12,200					
16		102.2	9,600		-	-	-	
17		102.4	10,000					
18		102.4	7,000					
19		101.4	14,100		-	-	-	
20		101.9	12,800					
21		102.1	16,000	-				
22		102.2	15,000		-	-	-	
23		102.0	14,600					
24		101.5	16,500					
25		102.0	15,250					
26		101.9	13,250					
27		102.4	15,000					
28		102.5	15,900	-	-	-	-	
35		101.8	14,500	-				
42	2nd Vaccination	102.1	14,000	-				

Data from calf 6114 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
49	102.1	14,500	-				
56	101.7	21,000	-				
63	102.0	18,000	-				
70	102.7	22,000	-				
77	102.0	16,800	-				
84	101.5	16,500	-				
91	102.4	19,500	-				
98	102.2	17,500	-				
105	102.3	16,200	-				
112	102.6	13,000	-				
119	102.5	15,800	-				
126	102.3	16,500	-				
133	102.0	16,000	-				
140	102.2	18,200	-				
147	102.1	17,600	-				
154	102.0	15,900	-				
161	101.9	15,000	-				
168	101.7	17,250	-				
175	101.9	18,500	-				
208	101.9	18,000	-				
209	101.9	20,750	-				
210	101.7	18,500	-				
211	101.8	16,750					
212	101.9	15,250					
213	101.7	17,250					
214	101.6	15,500					
215	102.0	13,250					
216	101.6	14,900					
217	101.4	18,750					
218 Challenged	101.5	17,500		-	-	-	

Data from calf 6114 (continued)

Days	Temperature	Leukocytes	Titer	Virus Isolation from Nose	Rectum	Blood	Symptoms
219	101.6	22,500					
220	101.6	16,500		-	+	-	
221	102.0	13,500					
222	103.0	8,000		-	-	+	D
223	102.6	12,100					D, E
224	101.8	13,800					D, E
225	103.4	9,500	-	+	+	-	D,A,E,Bloody & Mucous
226	107.4	14,000					D,A,E,Arched Back
227	102.0	22,250		-	+	-	D, A
228	101.8	16,800					
229	101.8	14,800					
230	101.4	13,800		-	-	+	
231	101.6	14,500					
232	101.0	15,000	64	-	+	-	
233	101.8	15,800					
237	101.2	16,600		-	-	-	
239	101.6	16,200	128	-	-	-	
244	101.6	17,000		-	-	-	
246	102.0	17,600	512	-	-	-	
251	102.0	18,000		-	-	-	
253	101.6	17,200	4,096	-	-	-	
258	101.4	16,500		-	-	-	
260	101.0	16,800	2,048	-	-	-	

D depression
A anorexia
E enteritis

Hanks balanced salt solution (HBSS):

NaCl	8.00 grams per liter
KCl	0.40 " " "
$MgSO_4 \cdot 7H_2O$	0.20 " " "
$Na_2HPO_4 \cdot H_2O$	0.06 " " "
Glucose	1.00 " " "
KH_2PO_4	0.06 " " "
$CaCl_2$	0.14 " " "
$NaHCO_3$	0.35 " " "

Earle balanced salt solution (EBSS):

NaCl	6.80 grams per liter
KCl	.40 " " "
$MgSO_4$	.10 " " "
NaH_2PO_4	.125 " " "
$NaHCO_3$	2.20 " " "
Glucose	1.00 " " "
$CaCl_2$	10.0 " " "
Lactalbumin hydrolysate	5.0 " " "

Eagle basal medium (EBM):

NaCl	6.8 grams per liter
KCl	.4 " " "
$NaH_2PO_4 \cdot H_2O$	.14 " " "
$NaHCO_3$	2.2 " " "

Eagle basal medium (EBM): (Continued)

$CaCl_2$	0.2	grams per liter
$MgCl_2.6H_2O$	0.17	" " "
Glucose	1.0	" " "
Arginine	.021	" " "
Cystine	.012	" " "
Histidine	.008	" " "
Isoleucine	.026	" " "
Leucine	.026	" " "
Lysine	.026	" " "
Methionine	.008	" " "
Phenylalanine	.016	" " "
Threonine	.024	" " "
Tryptophan	.004	" " "
Tyrosine	.018	" " "
Valine	.024	" " "
Glutamine	.300	" " "
Biotin	1.0	milligrams per liter
Choline	1.0	" " "
Folic acid	1.0	" " "
Nicotinamide	1.0	" " "
Pantothenic acid	1.0	" " "
Pyridoxal	1.0	" " "
Thiamin	1.0	" " "
Riboflavin	0.1	" " "
Phenol red	.04	" " "

GKN solution:

NaCl	8.0	grams per liter
KCl	0.4	" " "
Glucose	1.0	" " "

Trypsin solution:

Trypsin	2.0	grams per liter
NaCl	8.0	" " "
KCl	0.4	" " "
Glucose	1.0	" " "
$NaHCO_3$	0.84	" " "

Dulbecco phosphate-buffered saline (PBS):

NaCl	8.0	grams per liter
KCl	0.2	" " "
Na_2HPO_4	1.15	" " "
KH_2PO_4	0.2	" " "
$CaCl_2$	0.1	" " "
$MgCl_2.6H_2O$	0.1	" " "

Ammonium and potassium oxalate:

Ammonium oxalate	1.2 grams
Potassium oxalate	0.8 grams
Distilled water q.s.	100.0 ml.

Veronal-buffered saline:

NaCl	8.5	grams per liter
Na-5,5-diethyl barbiturate	.0375	" " "

Veronal-buffered saline: (Continued)

5,5-diethyl barbituric acid .0575 grams per liter

Add 0.5 ml of a stock solution containing 1.00 M $MgCl_2$ and 0.30 M $CaCl_2$.