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LEUKOCYTE MIGRATION INHIBITION STUDIES IN PASTEURELLOSIS

by

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INTRODUCTION

Pasteurellosis in cattle is usually associated with infection by Pasteurella hemolytica or Pasteurella multocida type 2 (or A) in combination with viral infection of the respiratory tract or debilitation of the host by stress (1, 2).

In Canada and the United States, the disease occurs most commonly in beef calves after weaning in the fall of the year and is often the most important disease occurring in cattle which have recently entered feedlot. Major economic loss results in the feedlot industry with the mortality rate reaching 0.75-1.0% (3).

Biological and chemoprophylactic preventive measures have been used extensively in attempts to control Pasteurellosis in cattle but these measures have resulted in limited success (4). The Pasteurella bacterins used may be inefficient because they do not contain the serotype most prevalent in the area at the time, or they may not be suitably antigenic and protective. The technology of producing a bacterin which contains the necessary antigens to stimulate the appropriate protective antibodies has not been developed (5).

Standardized methods for diagnosis are also needed in order to aid in differential diagnosis.

There is no simple and reliable test for Pasteurella

humoral immune reactions and only limited study of the disease has been done by cell-mediated immune reactions.

The present study was undertaken with the purpose of contributing to our understanding of pathogenesis, diagnosis, and immunity of Pasteurellosis in cattle.

The cell-mediated immune response, in cattle infected intratracheally with Pasteurella suspension or inoculated intramuscularly with Pasteurella bacterin were studied in acute and convalescent phases by the Leukocyte Migration Inhibition (LMI) test.

REVIEW OF LITERATURE

Pasteurellosis in cattle is associated with infection by Pasteurella hemolytica or Pasteurella multocida type 2 (or A) and is manifested clinically by acute broncho-pneumonia (1).

The disease most commonly affects young growing cattle ranging in age from 6 months to 2 years, but all age groups may be affected. The disease occurs commonly in outbreaks within 7-14 days after cattle have arrived in the feedlot following stressful transportation, and thus this disease is often called "Shipping Fever" (6). The disease is also a problem in dairy herds, especially when recent introductions have been made (1). Sporadic outbreaks of the disease can occur following fatigue, starvation, exposure to inclement weather, humid, poorly ventilated barns, and viral infections (1).

Pasteurella species have been shown to be part of the normal nasal flora of cattle but not part of the normal lung flora because of an effective lung clearance mechanism (7,8,9,10,11,12). Both Pasteurella hemolytica and Pasteurella multocida apparently can be carried in a latent state in the upper respiratory passages (13). Epidemiological studies have shown that the number of calves infected with Pasteurella in the nasopharynx increases during outbreaks of Shipping Fever. One serotype may predominate in the

nasapharynx of calves for 6-9 months and this may indicate the limited number of serotypes found in a herd (14). However, in some group of calves more than two serotypes are present and competition between strains may well occur in the nasopharynx (15).

Environmental droplet nuclei are produced by a moist cough or labored respirations associated with the disease (16,17). Increased nasal exudates may also serve to contaminate feed and water supplies with Pasteurella (17).

Inhaled aerosolized bacteria are deposited in the cranial, intermediate, and lower parts of caudal lung lobes (18). Gray studied the relationship between numbers of Pasteurella hemolytica in the nasal cavity and numbers of bacteria in tracheal air. He found that if Pasteurella hemolytica colonized the nasal cavity, 47.8% of the exhaled bacteria were in 1-5 µm micro droplet size, optimal for deep lung penetration. It seems logical to incriminate droplet nuclei as the method by which Pasteurella are spread from one nasal cavity to another (19,20).

Lillie stated that Pasteurella hemolytica is not usually found in systemic circulation during pneumonic Pasteurellosis. The disease is not considered to be septicemic (5).

In acute Pasteurellosis, a characteristic bacteriological lung isolation is massive numbers of Pasteurella hemolytica in pure culture. Wessman, in a study of Pasteurella hemolytica isolated from the respiratory tract of cattle

concluded that strains of Pasteurella hemolytica from healthy cattle may differ from those of acute Pasteurellosis cases (21). The fact that Pasteurella hemolytica can be transferred from stressed cattle to nonstressed cattle and result in clinical disease suggests that virulence of the organism can be enhanced by the proliferation of resistant strains (21).

An endotoxin of Pasteurella hemolytica has been demonstrated by Keiss (22). He concluded that signs of hypoxia and dyspnea are not only due to loss of functional lung parenchyma but also as an effect of endotoxin.

Clinical Findings of Pasteurellosis in Cattle

Pierson et al., surveyed for causes of fatal disease in 407,000 yearling feedlot cattle during a 14 month period; of the 4,260 (1%) cattle died during this period, 1,350 (32%) were categorized as "sudden-death" cases and the greatest numbers of sudden death cases were attributed to fibrinous or broncho-pneumonia (26%) (6).

Pasteurellosis usually develops in cattle within 7-14 days after they have been stressed (1,6). Sudden deaths without previous signs may be the first sign of the disease outbreak (23). Affected cattle often become depressed, gaunt, exhibit rapid and shallow respirations, and have a cough which becomes pronounced if forced to walk. Also observed is a mucopurulent nasal discharge, ocular discharge and sometimes a crusty nose (1).

Close examination often reveals a fever of 40-41 C and evidence of broncho-pneumonia. In the early stages of disease there are increased vesicular sounds and bronchial tones which are audible over the anterior and ventral parts of the lung field. As the disease progresses the bronchial tones and moist rales become louder and extend over a greater lung area. These sounds are followed by squeaky and musical dry rales in a few days especially if the disease becomes chronic. Pleuritic friction rubs may also be audible, but their absence does not preclude the presence of extensive pleuritis.

The course of disease is usually short. If treated early, affected cattle recover in 24-48 hours, but peracute cases and those which have been ill for several days before being treated may die or become chronically affected in spite of intensive therapy (1).

Outbreaks of the disease in feedlots may last 2-3 weeks or longer, and is dependent on condition of cattle when first affected. The course may be short in nourished thrifty cattle originating from one ranch while the course in cattle mingled from many sources may last for weeks with cases appearing every few days (1).

The reason for failure of therapy include advanced pneumonia, pulmonary absess, bronchiectasis, pleurisy, inadequate therapy and antibiotic resistance.

Migration Inhibition Factor (MIF)

Reactions of cell-mediated immunity fall into two broad categories; those that involve direct participation of intact lymphocytes in the effector mechanism of the reaction and those that involve mediation by soluble lymphocyte-derived factors known as lymphokines. The first type of reaction is essentially limited to lymphocyte-dependent cytotoxicity, although certain aspects of T-cell and B-cell cooperation may fall into this category. The second category appears to comprise the bulk of so-called cell-mediated immune responses and provides a link between this system and the inflammatory system (24).

Various lymphokines have shown to exert profound influence upon inflammatory cell metabolism, cell surface properties, patterns of cell migration, and cell activation for various biologic activities involved in host defense (24).

In-vitro delayed hypersensitivity studies by Rich and Lewis (25) demonstrated that tuberculin preparations inhibited cell migration from spleen fragments or peripheral blood of actively immunized animals. This assay was simplified by George and Vaughan (26), who developed the modern capillary tube method.

Bloom and Bennett (27) and David (28) independently demonstrated that the reason for inhibition of migration of peritoneal exudate macrophages was due to the release of a soluble factor, MIF, from sensitized lymphocytes which were reacting with antigen in the exudate suspension. The migration

inhibition reaction using peritoneal exudate cells from non-immunized animals could be demonstrated, provided that a source of MIF was included in the incubating medium.

MIF is of great historical importance since it was the first non-antibody, lymphocyte-derived soluble factor demonstrated, i.e., the first lymphokine (24). These factors are relatively low molecular weight as compared to conventional immunoglobulins, and also lack immunologic specificity.

Lymphokines are generated by specific immunologic reactions between antigen and sensitized lymphocytes, and do not require interaction with antigen for the expression of biologic activity (24,29).

It is assumed that migration inhibition is a linear relationship with MIF concentration and, therefore, is a direct correlate of the degree of delayed hypersensitivity (30). MIFs are not only involved in tuberculin-type reactions of delayed hypersensitivity, but also in most other manifestations of cell-mediated immunity (24,31).

Carpenter et al. (32), demonstrated that an agar medium containing guinea-pig serum and tissue culture medium supported migration of cells from fragments or suspensions of spleen, lung, lymph node or peritoneal cells. Cell suspensions were placed in holes cut in the agar gel. Migration was predominantly between the glass agar interface. Migration of cells from sensitized animals was specifically inhibited when antigen was added to agar medium.

Clausen (33) introduced the radial migration method of polymorphonuclear leukocytes, in which cells migrate in the space between the dish surface and the agarose layer. Results are expressed as the radius of the circular area covered by the migrating cells. Cells in the migration area are granulocyte and mononuclear cells which are adherent to a solid or semisolid dish surface (29).

The incubation of normal macrophages with MIF containing supernatants or partially purified MIF has profound effects on cellular morphology as observed by the light microscope. During the first hour of incubation, inhibition of macrophage spreading is commonly reported and by 24 hours, the macrophages have become round, lost pseudopodia, and are not motile (28). Following 48 hours of incubation, the cells exhibit increased spreading, with formation of long pseudopods and cellular aggregates are commonly seen (34). By 72 hours, the macrophages are enlarged, exhibit enhanced adherence to surfaces, and demonstrate increased membrane ruffling and ameboid movements (35).

The search for a suitable in-vitro correlate of cell-mediated immune reactions in cattle has recently been intensified. The Leukocyte Migration Agarose (LMA) test was applied to detect cell-mediated immunity in the course of Mycobacterium avium infection in cattle (36). Bendixen (37) performed LMA test on cattle infected with paratuberculosis. Moreno-Lopes (38) performed LMI test on cattle infected with Parainfluenza-3 virus.

REFERENCES

1. BLOOD, HENDERSON, and RADOSTITS. Veterinary Medicine, 5th ed., 488. 1979.

2. COLLIER, J. R. Pasteurellosis in Bovine Respiratory Disease. J.A.V.M.A., 152, 6:824-827. March 15, 1968.

3. Livestock and Meat Situation. Washington, D. C., Economics. Statistics and Cooperative Service, U. S. Dept. of Agriculture, LMS, 222. Aug., 1978.

4. KAHRS, U. F. Rational Basis for an Immunization Program Against the Common Diseases of the Bovine Respiratory Tract. Can. Vet. J., 15, 9:252-255. Sep., 1974.

5. LILLIE, L. E. The Bovine Respiratory Disease Complex. Can. Vet. J., 15, 9:233-242. Sep., 1974.

6. JENSEN, R. et al. Shipping Fever in Yearling Feedlot Cattle. J.A.V.M.A. 169, 15:500-506. Sep., 1976.

7. COLLIER, J. R. Significance of Bacteria in Bovine Respiratory Disease. J.A.V.M.A., 153:1645-1651. 1968.

8. COLLIER, J. R. et al. Microflora of Apparently Healthy Lung Tissue of Cattle. Am. J. Vet. Res., 25:391-393. 1964.

9. HAMDY, A. H. and TRAPP, A. L. Investigation of Nasal Microflora of Feedlot Calves Before and After Weaning. Am. J. Vet. Res., 28:1019-1025. 1967.

10. MAGWEED, S. E. et al. Nasal Bacterial Flora of Calves in Healthy and Prone Herds. Can. J. Comp. Med., 33:237-243. 1969.

11. WESSMAN, G. E. Characterization of Pasteurella hemolytica Isolated from the Respiratory Tract of Cattle. Can. J. Comp. Med., 32:498-504. 1968.

12. THOMSON, R. G. et al. Pneumonic Pasteurellosis of Cattle: Microbiology and Immunology. Can. J. Comp. Med., 30:194-206. 1969.

13. MERCHANT and PACKER. Veterinary Bacterioloty, 2nd ed. 1974.

14. THOMPSON, D. A. and WRAY, C. An Epidemiological Study of Pasteurella hemolytica in Calves. Br. Vet. J., 129:116-122. 1973.

15. BIBERSTEIN, E. L. et al. Variation in Carrier Rates of Pasteurella hemolytica in Sheep Flocks. J. Comp. Path., 80:499-507. 1970.

16. WELLS, W. F. Air-borne Infection: Droplets and Droplet Nuclei. Am. J. Hyg., 20:611-618. 1934.

17. CARTER, G. R. Some Remarks on Shipping Fever in Canada. Can. J. Comp. Med., 20:289-293. 1956.

18. LAURENZI, G. A. et al. Clearance of Bacteria by the Lower Respiratory Tract. Science, 142:1572-1573. 1963.

19. WRIGHT, G. W. Structure and Function of Respiratory Tract in Relation to Infection. Bac. Rev., 25:219-227. 1961.

20. GREY, C. L. and THOMSON, R. G. Pasteurella hemolytica in the Trachial Air of Calves. Can. J. Comp. Med., 35:121-128, 1971.

21. WESSMAN, G. E. and HILKER, G. Characterization of Pasteurella hemolytica Isolated from the Respiratory Tract of Cattle. Can. J. Comp. Med., 32:498-504. 1968.

22. KEISS, R. E. et al. Skin Toxicity and Hemodynamic Properties of Endotoxin Derived from Pasteurella hemolytica. Am. J. Vet. Res., 25:935-942. 1964.

23. PIERSON, R. E. et al. Sudden Deaths in Yearling Feedlot Cattle. J.A.V.M.A., 169, 5:527-529. Sep., 1976.

24. COHEN, S. The Role of Cell-mediated Immunology in the Induction of Inflammatory Responses. Am. J. Pathol., 88:501-528. 1977.

25. RICH, A. R. and LEWIS, M. R. Mechanism of Allergy in Tuberculosis. Proc. Soc. Exp. Biol. N.Y., 25:596. 1928.

26. GEORGE, M. and VAUGHAN, J. H. In-vitro Cell Migration as a Model for Delayed Hypersensitivity. Proc. Soc. Exp. Biol. N.Y., 111:514. 1962.

27. BLOOM, B. R. and BENNETT, B. Mechanisms of a Reaction in-vitro Associated with Delayed Type Hypersensitivity. Science (Wash., D.C.), 153:80. 1966.

28. DAVID, J. R. Delayed Hypersensitivity in-vitro: Its Mechanism by Cell Free Substances Formed by Lymphoid Cell-antigen Interaction. Proc. Natl. Acad., Science, U.S.A., 56:72. 1966.

29. COHEN, S. Biology of the Lymphokines, 1st ed., 65. 1979.

30. LEU, R. W. et al. Mechanism of Action of Migration Inhibitory Factor (MIF). J. Exp. Med., 136:589-603. 1972.

31. COHEN, S. The Role of Cell-mediated Immunity in the Production of Inflammatory Responses. Am. J. Path., 88:501-528. 1977.

32. CARPENTER, R. R. et al. Antigen-induced Inhibition of Cell Migration in Agar Gel Plasma Clot and Liquid Media. J. Reticuloendothelial Soc., 5:472. 1968.

33. CLAUSEN, J. E. Leukocyte Migration Agarose Technique, Some Technical Details. Acta Allergol, 28:351-364. 1973.

34. LOLEKHA, S. et al. Macrophage Aggregation in-vitro: A Correlate of Delayed Hypersensitivity. J. Immunol., 104:296-304. 1970.

35. NATHAN, C. F. et al. Alterations of Macrophage Functions by Mediators from Lymphocytes. J. Exp. Med., 133: 1356-1376. 1971.

36. BERGMAN, R. and MORENO-LOPES, J. Lymphocyte Stimulation and Leukocyte Migration Inhibition as Diagnostic Tools for the Detection of Mycobacterium avium Infection in Cattle. Acta. Vet. Scand., 17:528-260. 1976.

37. BENDIXEN, P. H. Application of the Direct Leukocyte Migration Agarose Test in Cattle Naturally Infected with M. paratuberculosis. Am. J. Vet. Res., 38, 8:1161-1162. 1977.

38. MORENO-LOPES, J. Cell-mediated Immunity to Parain-fluenza-3 Virus in Cattle Evaluation of in-vitro and in-vivo Test. Zbi. Vet. Med. B., 24:231-240. 1977.

PAPER 1

LEUKOCYTE MIGRATION INHIBITION STUDIES IN PASTEURELLOSIS

INTRODUCTION

Pasteurellosis in cattle is usually associated with infection by *Pasteurella hemolytica* or *Pasteurella multocida* type 2 (or A) in combination with viral infection of the respiratory tract or devilitation of the host by stress (Collier, 1968; Blood, 1979).

In Canada and the United States, the disease occurs most commonly in beef calves after weaning in the fall of the year and is often the most important disease occurring in cattle which have recently entered a feedlot. Major economic loss results in the feedlot industry with the mortality rate reaching 0.75-1.0% (Blood et al., 1979).

Biological and chemoprophylactic preventive measures have been used extensively in attempts to control *Pasteurellosis* in cattle but these measures have resulted in limited success (Kahrs, 1974). *Pasteurella* bacterins used may be inefficient because they do not contain the serotype most prevalent in the area at the time, or they may not be suitably antigenic and protective. The technology of producing a bacterin which contains the necessary antigens to stimulate the appropriate protective antibodies has not been developed (Lillie, 1974).

Standardized methods of diagnosis are also needed in order to aid in differential diagnosis. There is no simple and reliable test for *Pasteurella* humoral immune reactions

and only limited study of the disease has been done by cell-mediated immune reaction.

The present study was undertaken with the purpose of contributing to our understanding of pathogenesis, diagnosis, and immunity in Pasteurellosis in cattle.

The cell-mediated immune response in cattle infected intratracheally with Pasteurella suspension or inoculated intramuscularly with Pasteurella bacterin were studied in acute and convalescent phases by the leukocyte migration inhibition (LMI) test.

MATERIALS AND METHODS

A group of 6 Guernsey cattle, ages 1-9 months, weighing from 92-361 pounds were selected on June 20, 1979. Calves were placed in two 11 X 17 ft. pens, fed ground grain mixture, alfalfa hay, prairie hay and water. The pen temperature was maintained constant 23 C throughout the study. Body temperature was measured daily during 0700-0900 hours by rectal thermometer. Heart and respiratory rates and lung sounds were recorded daily by auscultation and any abnormal clinical signs were noted.

On July 13, 1979, after 3 weeks of observation, four calves (#1, #2, #3, #5) were inoculated intratracheally with Pasteurella hemolytica biotype A, serotype 1, strain No. 236 suspension in 10 ml of sterile distilled water. This strain was originally obtained from sick cattle with clinical signs of pneumonic Pasteurellosis. The bacteria was cultured 24 hours on blood agar plates and harvested. Calves were inoculated intratracheally with dilutions of 10^{-3} through 10^{-6} according to the following schedule: calf #1, 15 X 10^{5} organisms; calf #2, 15 X 10^{4} organisms; calf #3, 15 X 10^{3} organisms; calf #5, 15 X 10^{2} organisms.

Intratracheal inoculation was accomplished in the following manner; the ventral half of the neck was clipped surgically and washed with an iodine soap solution and alcohol. A needle (14 gauge, 3.7 cm) was inserted into

the trachea approximately 15 cm ventral to the larynx in a standing position with the head extended upward. A canine urinary catheter (polypropylene 55 cm, 3.5 French size) was inserted through the needle for approximately 30-40 cm into the trachea.

Blood samples were obtained from the jugular vein for the LMI test and complete blood counts (CBC) at 3 or 4 day intervals for 3 weeks.

Four calves (#1, #2, #3, #5) and one control calf (#6) were re-inoculated with a suspension of the same strain of Pasteurella in 10 ml of phosphate buffer saline solution (PBS) intratracheally on Sep. 7, 1979, 56 days following the initial inoculation and blood samples were taken again for the LMI test and CBC at 3 or 4 day intervals for 3 weeks. For this inoculation, the Pasteurella was grown in one liter of tryptose broth for 24 hours. The culture was sedimented at 16,000 G for 30 minutes and the pellet of bacteria resuspended in 20 ml PBS. Calves were inoculated intratracheally according to the following schedule: calf #1, 28×10^4 organisms; calf #2, 28×10^3 organisms; calf #3, 28×10^2 organisms; calf #5, 28×10^1 organisms; calf #6, 28×10^0 organisms.

Blood samples were obtained for the LMI test and CBC at 3 or 4 day intervals for 3 weeks.

A second group of 4 calves, ages 2-4 months, weighing from 86-164 pounds, was selected on Oct. 26, 1979. After 2 weeks of observation, the calves were inoculated

intramuscularly with Pasteurella hemolytica bacterin on Nov. 15, 1979. Two calves (#8, #12) were vaccinated with 1 ml of aqueous bacterin into the right coccygral muscle and the other two calves (#9, #11) were vaccinated with 2 ml of incomplete Freund's adjuvant-bacterin, 1 ml into the right and 1 ml into the left coccygeal muscle. To prepare the bacterin, Pasteurella was cultured for 72 hours in a liter of tryptose broth, sedimented at 16,000 G for 30 minutes and resuspended in 15 ml of distilled water with 0.1% formalin. The suspension was incubated at 37 C for 24 hours and held at 4 C until used. This aqueous bacterin contained 8.43×10^9 organisms per ml which was estimated spectrophotometrically with a Spectronic 20[a] at 510 nanometers and referring to a standard line prepared with a suspension of Pasteurella in which the number of organisms had been determined by plating on blood agar. The incomplete Freund's adjuvant-bacterin combination was then made by homogenizing equal volume of above aqueous bacterin with an equal volume of incomplete Freund's adjuvant.

Blood samples for LMI test and CBC were taken at 7 days interval for 5 weeks.

The four vaccinated calves were challenged intratracheally with the same strain of live Pasteurella hemolytica organisms, 100,000 organisms in 10 ml of PBS on Dec. 13, 1979

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[a]Bausch and Lomb, Rochester, N.Y.

and 900,000 organisms in 10 ml of tryptose broth on Dec. 19, 1979.

Blood samples for the LMI test, CBC and blood chemistry analysis were taken at 7-day intervals for 4 weeks.

The technique for the LMI test described by Clausen (1971), Moreno-Lopes and Bendixen (1977) was modified in the following manner:

Preparation of agar medium: 2 X RPMI[b] in 0.05 M TRIS-HCl with penicillin 120 unit per ml, streptomycin 120 ug per ml, 1 ml of 3% glutamine solution per 100 ml, PH 7.4 was warmed to 47 C and mixed with an equal volume of sterile 2% purified agar[c] in 0.05 M TRIS-HCl, PH 7.4 at 47 C. Seven ml of the above preparation were placed in a 15 X 60 mm tissue culture dish[d] and stored at 4 C. Agar plates were cut with 4 equidistant wells made with a sterile 5 mm diameter cork corer.

Leukocyte separation: Blood (20 ml) was collected in a tube with heparin (10 IU/ml). The blood was divided equally into two siliconized 40 ml polycarbonate centrifuge tubes and 20 ml of sterile distilled water was added to each. After shaking the tubes gently for 45 seconds, 10 ml of 2.7% NaCl in PBS was added to restore isotonicity. The mixture was

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[b]Roswell Park Memorial Institute Medium 1640.

[c]Difco Lab., Detroit, Michigan,

[d]Falcon Plastics, 1950 Williams Drive, Oxnard, Cal.,

centrifuged for 15 minutes at 150 G. The supernatant was removed by aspiration and the pellet of leukocytes was washed with 10 ml of Hanks buffered saline solution with heparin (50 IU/ml), and sedimented at 225 G for 10 minutes. If there were any visible deposits of red cells remaining at this stage, the pellet was recycled as before. To the clean pellet of leukocytes, one ml of RPMI 1640 was added and the cells resuspended by pipetting with a siliconized Pasteur pipet.

Leukocyte Migration Inhibition test: The cell suspension was incubated for 1 hour at 37 C in a siliconized glass tube with antigen suspension for test cells or with RPMI 1640 in 0.05 M TRIS HCl for control cells. After this pre-incubation, cell suspensions were centrifuged for 5 minutes at 150 G and three-fourths of the supernatant removed and the cell pellet resuspended in the remaining supernatant. The cell mixtures were each placed into 4 wells in an agar plate and incubated for 24 hours at 37 C in a humidified incubator. The cells were then fixed with 7.5% glutaraldehyde in distilled water for 20 minutes and the agar removed with a spatula and the plate rinsed gently with tap water. The cells were stained with Giemsa for 30 minutes and the migration of cells were measured with an ocular micro-meter using substage objective microscope. The degree of migration inhibition was expressed as a Percentage Migration Inhibition (% MI) for each calf

according to the following formula (Remold 1970; 1979).

Percentage Migration Inhibition (% MI).

$$= \left(1 - \frac{\text{average migration of test cells}}{\text{average migration of control cells}}\right) \times 100$$

The migration was measured from the near edge of the well to the perimeter of the area covered by migrating cells.

Preparation antigen: Pasteurella was cultured for 48 hours in tryptose broth and sedimented at 16,000 G for 30 minutes. The pellet of bacteria was resuspended in PBS to an opacity of approximate tube 3 by the MacFarland scale. The suspension was then autoclaved at 15 pounds pressure for 15 minutes sedimented at 12,000 G for 10 minutes and the supernatant collected for use as a heat soluble antigen. To prepare sonicated soluble antigen, the Pasteurella was cultured 48 hours in tryptose broth, sedimented at 16,000 G for 30 minutes, resuspended in PBS, frozen and thawed 3 times, sonicated for 1 minute for each ml of suspension with a microtip at output control setting 3 of a Sonifier Cell Disruptor Model 185[e], centrifuged for 30 minutes at 16,000 G, the supernatant collected and filtered through an 02 micron membrane filter. Protein content of both antigen was estimated by biuret test with optical density read in a Spectronic 20 spectrophotometer at 545 namometer.

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[e]Heat Systems Ultrasonics, Inc., Plainview, L.I., N.Y.,

Antigen toxicity test: The remaining cell suspension not used to fill the wells of the agar plates was incubated along with the test plates and stained with trypan blue to determine viability at the time the test was read after 24 hours incubation.

RESULTS

Four of the 10 calves (#1, #2, #3, #5) were inoculated intratracheally with Pasteurella hemolytica twice at 56 day interval and 2 calves (#4, #6) were used as controls. Calf #6 was inoculated with Pasteurella at the time of the second inoculation of the 4 calves. The other 4 calves (#8, #9, #11, #12) were vaccinated with Pasteurella hemolytica bacterin and challenged intratracheally.

Clinical Signs

All calves exposed to initial intratracheal inoculation of Pasteurella exhibited clinical signs of Pasteurellosis. Acute febrile reactions (40-41 C) with rapid shallow respirations (60-90/min.), tachycardia (120-150/min.) and moderate depression developed in all calves beginning 24 hours post inoculation. One calf (#5) had a serous conjunctival discharge. Increased harsh vesicular lung sounds and dry inspiratory bronchial tones were auscultated over the anterior and ventral one-third of the lung by 48 hours post inoculation in all calves. An occasional cough was present in the calves (#1, #3).

After clinical signs of Pasteurellosis were confirmed, antibiotic treatment was initiated 24 hour post inoculation to save the life of calves for further study. The calves responded quickly to antibiotic therapy. Body temperature returned to normal in 24 hours (calves #1, #2), in 48 hours

(calf #5), and in 72 hours (calf #3). Calves became less depressed, walking more in the pen. Moist lung sounds were auscultated over the entire lung fields for 3 days in one calf (#3) while slight moist rales were heard in the anterior ventral portion of the lungs for 2 days in two calves (#1, #5). The calves had slightly decreased appetites the first day post-inoculation, but following antibiotic therapy, the appetite returned to normal.

The second intratracheal inoculation of live Pasteurella did not cause clinical signs of Pasteurellosis. The control calf (#6) inoculated intratracheally with Pasteurella for the first time developed signs of Pasteurellosis: depression, fever (39.7 C), harsh inspiratory vesicular lung sound on 9th day post inoculation. Antibiotic therapy was initiated the following day and continued for 7 days before the calf made a complete recovery.

Only one of the next 4 calves vaccinated with Pasteurella bacterin had an elevated temperature (39.7 C), but was normal without therapy after 24 hours.

When the calves were challenged with Pasteurella hemolytica intratracheally on days 27 and 34 post-vaccination, none developed clinical signs of Pasteurellosis.

Clinical Pathology

The first 4 calves exposed to the initial intratracheal inoculation of Pasteurella organisms developed elevated

leukocyte counts and elevated fibrinogen values (Figure 3, 5). The elevation in leukocyte counts was attributed primarily to neutrophils and lymphocytes and values returned to preinoculation levels in 3-5 days. The fibrinogen values were elevated in all calves and gradually returned to preinoculation levels in 7 days. The packed cell volume, hemoglobin, erythrocyte counts and total protein values were not changed.

The second intratracheal inoculation of live Pasteurella organism caused all calves including control calf (#6) to have elevated leukocyte counts. The fibrinogen value remained normal in all calves except for the control calf which had an elevated fibrinogen value.

Vaccinated calves had an elevation in absolute neutrophil numbers (Figure 4), but the lymphocyte counts did not show a significant change. Fibrinogen values remained normal in the calves except for calf #12 which had an elevated fibrinogen value of 800 mg % for 3 days and this was accompanied by body temperature elevation (Figure 6).

Upon challenge with live Pasteurella organism, vaccinated calves did not demonstrate a change in leukocyte count or fibrinogen values. The mean value of packed cell volume, hemoglobin level, red blood cell counts and blood chemistry profiles did not show significant changes.

Leukocyte Migration Inhibition Test

Calves inoculated intratracheally: The sequential cell-mediated immunologic responses of calves infected and

challenged intratracheally with Pasteurella hemolytica are shown by average migrations of control cells and test cells (Table 1), and MIF activity is expressed as the % MI of these cells (Table 2, Figure 1).

By 13 days after the appearance of clinical signs of pneumonic Pasteurellosis, a peak value of the % MI of cells from infected 4 calves was seen (mean ± SE % MI 72.90 ± 23.61). At day 17 and 56 post inoculation, the mean value of % MIs of the cells were 56.20 ± 15.02 and 42.10 ± 16.32, respectively. These % MIs of the leukocytes of infected calves represented significant inhibition of migration when stimulated with the heat soluble antigen at a concentration of 30 µg of protein per ml. The leukocytes migrated from the wells in a circular pattern and the mean migration of the control cells from the infected calves were: 1.80 ± 0.58 mm in calf #1, 1.72 ± 0.56 mm in calf #2, 1.78 ± 0.48 mm in calf #3, 1.65 ± 0.32 mm in calf #5 and 1.67 ± 0.05 mm in calf #6.

At 4 days post challenge, the % MI of the cells from the 4 infected calves was the highest (92.90 ± 7.62) among the test. At days 7, 11, 14, 18 post challenge, the mean % MI of the cells were 67.90 ± 15.48, 62.10 ± 10.93, 56.60 ± 6.71 and 48.10 ± 10.20, respectively.

Calves vaccinated intramuscularly: The average migration of control and test cells from the calves vaccinated intramuscularly with Pasteurella hemolytica bacterin are shown in Table 3 and % MIs of these cells are shown in

Table 4 and Figure 2.

At 14 days post vaccination, the % MIs were 24.10 ± 3.19 with heat soluble antigen, 30 ug of protein per ml, and 26.00 ± 2.95 with sonicated soluble antigen, 30 ug of protein per ml. At day 21 and 28 post vaccination, the mean value of % MIs of the cells were 25.20 ± 1.10 and 27.00 ± 5.32 with heat soluble antigen, 25.50 ± 2.92 and 31.35 ± 6.42 with sonicated soluble antigen.

At days 7, 14, 21 post challenge, the mean % MIs of the cells were 32.30 ± 14.57, 32.40 ± 5.12 and 39.40 ± 13.47 with heat soluble antigen and 28.70 ± 7.66, 30.30 ± 3.49 and 47.40 ± 11.66 with sonicated soluble antigen, respectively.

There was no significant differences between the % MI of the cells with heat soluble antigen and the % MI of the cells with sonicated soluble antigen.

The mean percentage of viability of cells read after 24 hours incubation at 37 C were 87.42 ± 8.44 with RPMI 1640, 86.57 ± 9.12 with heat soluble antigen, 30 µg of protein per ml, and 84.85 ± 10.13 with sonicated soluble antigen, 30 ug of protein per ml.

DISCUSSION

Clinical Signs

The initial intratracheal exposure to Pasteurella hemolytica serotype 1 without significant environmental stress factors produced clinical signs of Pasturellosis by 24 hour post inoculation in all four calves.

The pathogenic process of Pasteurellosis consists of a build up of bacterial population in the lung parenchyma of the host (Biberstein and Thompson, 1965). It appears that the lung provides an ideal medium for Pasteurella hemolytica to grow rapidly from a small inoculum to toxic concentrations (Wessman and Hilker, 1968). The growth curve of Pasteurella hemolytica demonstrates a sharp log phase beginning at 3 hours of incubation and reaching a peak at 8 to 12 hours (Wessman, 1966). This peak is followed by a phase of rapid decline accompanied by lysis of cells and release of endotoxin (Keiss and Collier, 1964). If the growth of massive number of Pasteurella hemolytica in vivo is accompanied by a similar rapid die off and lysis of cells, it would be logical to assume a similar release of massive quantities of endotoxin into the lung.

The dyspnea and hypoxia can be attributed not only to the loss of functional lung parenchyma but also to endotoxicity (Keiss and Collier, 1964). The pyrogenic effect of Pasteurella is due to the action on the hypothalamus,

resulting in slower symtemic circulation, pulmonary hypertension and tachycardia (Reece and Wahlstrom, 1973).

Clinical Pathology

Elevated leukocyte count within a few days post inoculation is attributed to an influx of neutrophils from the bone marrow stores, and it is preceeded by a shift of neutrophils from the circulating pool into the inflammatory lesion. An immune response to Pasteurella hemolytica bacterin does not cause significant increase in the number of circulating lymphocytes.

The elevation of fibrinogen value which paralleled body temperature is anticipated in inflammatory processes of the lung.

Calves either recovered from artificial infection or vaccination did not exhibit clinical signs after intratracheal challenge with live Pasteurella hemolytica.

Leukocyte Migration Inhibition Test

Clausen (1971) introduced the in-vitro Leukocyte Migration Inhibition technique for diagnosis of Tuberculosis in man. More recently, Bergman (1976) used this technique for the detection of Mycobacterium avium infection in cattle and Bendixen (1977) applied the direct Leukocyte Migration Agarose technique to cattle naturally infected with Mycobacterium paratuberculosis. Moreno-Lopes (1977) performed LMI test on cattle infected with Parainfluenza-3. Present

results indicate that LMI technique can be successfully used to evaluate cell mediated immune responses in cattle infected with Pasteurella hemolytica or vaccinated with Pasteurella hemolytica bacterin.

The heat soluble antigen and sonicated soluble antigen (at concentration of 30 ug protein per ml in each) used was not toxic to leukocytes compared to RPMI 1640 after 24 hours incubation at 37 C. And there was no significant difference between the % MI of leukocytes with heat soluble antigen and the % MI of leukocytes with sonicated soluble antigen (Figure 2).

The fact that leukocytes from normal uninfected calves were neither inhibited nor enhanced in motility in the presence of specific antigen ruled out non-specific motility of leukocytes.

Because of the occurence of both MIF and Migration Activation factor (Aastrov and Anthony, 1976) in the same supernatant, difficulty occurs in using crude lymphokine preparations with 48 hour incubation periods (Cohen, 1979). The leukocytes in the whole migration area consisted of both granulcytes and mononuclear leukocytes (Clausen, 1973).

In both group of calves infected or vaccinated, initial significant % MI of leukocytes were exhibited by 2 weeks post inoculations (mean $\pm$ SE % MI 72.90 $\pm$ 23.61 in infected calves and 24.10 $\pm$ 3.19 in vaccinated calves). The % MI of 20% or more is considered a positive indication of MIF (Remold, 1970, 1979; and Moreno-Lopes, 1977). The

observation that leukocytes from cattle infected with Pasteurella hemolytica had significant higher % MIs than the % MIs of leukocytes from vaccinated calves suggest a linear relationship with MIF concentration and, therefore, correlates with the degree of delayed hypersensitivity (Rocklin, 1974).

Result of the present investigation demonstrates that the LMI technique is suitable for evaluating cell-mediated immunity in bovine species infected with Pasteurella hemolytica or vaccinated with Pasteurella hemolytica bacterin.

SUMMARY

The search for a suitable in-vitro correlation of cell-mediated immune reactions in cattle has recently been intensified. Since Clausen (1971) introduced the in-vitro Leukocyte Migration Inhibition (LMI) technique for diagnosis of Tuberculosis, the Leukocyte Migration Agarose test was applied to detect cell-mediated immunity in Mycobacterium avium infection in cattle (1976). Bendixen (1977) performed the Leukocyte Migration Agarose test on cattle infected with Paratuberculosis. Moreno-Lopes (1977) performed Leukocyte Migration Inhibition (LMI) test on cattle infected with Parainfluenza-3 virus. The present study indicates that the LMI technique can be successfully used to evaluate cell-mediated immune responses in cattle infected with Pasteurella hemolytica or vaccinated with Pasteurella hemolytica bacterin.

All cattles exposed to initial intratracheal inoculation of Pasteurella developed clinical signs of Pasteurellosis in 24 hours; an acute febrile reaction, rapid and shallow respiration and tachycardia. Infected cattle and vaccinated cattle were challenged intratracheally with Pasteurella hemolytica and did not exhibit clinical signs or change in the clinical pathologic parameters.

In both groups of cattle, infected or vaccinated, initial significant Percentage Migration Inhibition (% MI) of leukocytes were exhibited by 2 weeks post inoculations

(mean ± SE % MI 72.90 ± 23.61 in infected calves and 24.10 ± 3.19 in vaccinated calves). A migration inhibition over 20% is considered as positive indication of the presence of Migration Inhibition Factor (MIF).

The observation that leukocytes from cattle infected with Pasteurella hemolytica had significantly higher % MI than the % MI of leukocytes from the vaccinated calves suggest a linear relationship with MIF concentration and, therefore, correlates with the degree of delayed hypersensitivity.

REFERENCES

1. AASTROV, J. G. and ANTHONY, H. M. Australian Journal of Experimental Biology and Medical Science, 54:527. 1976.

2. BENDIXEN, P. H. American Journal of Veterinary Research, 38, 8:1161. 1977.

3. BERGMAN, R. and MORENO-LOPES. ACTA Veterinaria Scandinavica, 17:528. 1976.

4. BLOOD, D. C., HENDERSON, J, A. and RADOSTITS. Veterinary Medicine, a testbook of the disease of cattle, sheep, pigs and horses, 5th ed., 488, Lea & Febiger, Philadelphia, Pa., U. S. A.

5. CLAUSEN, J. E. ACTA Allergol, 26:56. 1971.

6. COHEN, S. E. and PECK, E. Biology of the Lymphokine, 2nd ed., 85, Academic Press, New York, U. S. A. 1979.

7. COLLIER, J. R. Journal of the American Veterinary Medical Association, 152, 6:824. 1968.

8. KAHRS, U. L. Canadian Veterinary Journal, 15, 9:252. 1974.

9. KEISS, R. E. and COLLIER, J. R. American Journal of Veterinary Research, 25:935. 1964.

10. LILLIE, L. E. Canadian Veterinary Journal, 15, 9:233. 1974.

11. MORENO-LOPES. Zbi Veterinary Medicine B., 24:231. 1977.

12. REMOLD, H. G. et al. Journal of Immunology, 122, 5:1920. 1979.

13. REMOLD, H. G. et al. Cell Immunology, 1:133. 1970.

14. ROCKLIN, E. R. Journal of Immunology, 112:1461. 1974.

15. WESSMAN, G. E. Applied Microbiology, 14:597. 1966.

16. WILLIAM, O. R. and WAHLSTROM, J. D. American Journal of Veterinary Research, 34:765. 1973.

Table 1. Leukocyte Migration Inhibition Test of Calves Infected Intratracheally with Pasteurella hemolytica: Sequential Average Migration of Control and Test Cells.

Day Blood Sample Collected	Average Migration mm									
	Calf #1		Calf #2		Calf #3		Calf #5		Calf #6	
	Cont.	Test	Cont.	Test	Cont.	Test	Cont.	Test	Cont.	Test
Preinoculation										
1	1.73	1.71	1.43	1.35	1.53	1.48	1.81	1.66		
3	0.68	0.66	0.57	0.54	0.66	0.63	0.73	0.71		
11**	1.77	1.74	1.66	1.46	1.53	1.36	1.78	1.70		
Post-inoculation										
3	1.87	1.81	1.69	1.67	1.71	1.53	1.75	1.62		
6	2.99	2.83	1.76	1.54	1.49	1.20	1.86	1.74		
10	1.86	1.63	2.87	2.39	1.91	1.77	1.74	1.47		
13	1.77	0.23	1.76	0.31	1.67	0.29	1.65	1.03		
17	1.70	0.95	1.48	0.42	2.81	0.94	1.81	1.04		
56***	2.75	0.92	2.46	1.65	1.86	1.23	1.91	1.24	(1.67	1.56)
Post-challenge										
4	1.81	0.10	1.87	0.05	1.91	0.04	1.09	0.20	(1.59	1.54)
7	1.74	0.60	2.07	0.19	2.44	1.09	1.75	0.70	(1.71	1.59)
11	1.88	0.65	1.79	0.44	1.76	0.88	1.74	0.74	(1.64	1.18)
14	0.87	0.39	0.91	0.31	1.70	0.85	1.77	0.79	(1.74	1.13)
18	1.81	1.17	1.85	0.98	1.78	0.71	1.78	0.89	(1.69	1.21)

*Control calf #6 was inoculated first time with 4 calves at day 56 post inoculation.

**Calves were inoculated with Past. hemolytica after blood samples were collected on day 11 preinoculation, July 13, 1979.

***Calves were reinoculated with Past. hemolytica after blood samples were collected on day 56 post inoculation, Sep. 7, 1979.

Table 2. Leukocyte Migration Inhibition Test of Calves Infected Intratracheally with Pasteurella hemolytica: Sequential Percentage Migration Inhibitions.

Day Blood Sample Collected	Percentage Migration Inhibition (% MI) Calf #1	Calf #2	Calf #3	Calf #5	Calf #6	Mean* ± SE**
Preinoculation						
1	1.2	5.6	3.3	8.3		4.6 ± 3.05
3	3.0	5.3	4.6	2.8		3.9 ± 1.22
11+	1.7	12.1	11.2	4.5		7.3 ± 5.05
Post-inoculation						
3	3.3	1.2	10.6	7.5		5.6 ± 4.21
6	5.4	12.5	19.5	6.5		10.9 ± 6.48
10	12.4	16.8	7.4	15.6		13.0 ± 4.20
13	87.1	82.4	84.5	37.6		72.9 ± 23.61
17	44.2	71.7	66.6	42.6		56.2 ± 15.02
56++	66.6	33.0	33.9	35.1	(6.6)***	42.1 ± 16.32
Post-challenge						
4	94.5	97.4	98.0	81.7	(3.2)	92.9 ± 7.62
7	65.6	90.9	55.4	60.0	(7.1)	67.9 ± 15.84
11	65.6	75.5	50.0	57.5	(28.1)	62.1 ± 10.93
14	55.2	66.0	50.0	55.4	(35.1)	56.6 ± 6.71
18	35.4	47.1	60.2	50.0	(18.5)	48.1 ± 10.20

*Mean % MI of leukocytes from 4 calves.

**Standard error.

***% MIs of leukocytes from control calf #6 in the parenthesis is not included in mean ± SE value.

+Calves were inoculated with Past. hemolytica after blood samples were collected on day 11 preinoculation, July 13, 1979.

++Calves were reinoculated with Past. hemolytica after blood samples were collected on day 56 post inoculation, Sep. 7, 1979.

Table 3. Leukocyte Migration Inhibition Test of Calves Vaccinated Intramuscularly with *Pasteurella hemolytica* Bacterin and Challenged: Sequential Average Migration of Control and Test Cells with Heat Soluble and Sonicated Soluble Antigen.

Day Blood Sample Collected	Average Migration mm											
	Calf #8			Calf #9			Calf #11			Calf #12		
	Cont.	H.S.*	S.S.**	Cont.	H.S.	S.S.	Cont.	H.S.	S.S.	Cont.	H.S.	S.S.
Post Vaccination***												
0	1.32	1.28	1.28	1.31	1.23	1.23	1.18	1.11	1.09	1.25	1.17	1.12
7	1.23	1.21	0.97	1.34	1.31	1.08	1.16	0.98	0.97	1.24	1.21	1.08
14	1.27	0.92	0.94	1.25	0.96	0.97	1.21	0.89	0.87	1.30	1.04	0.91
21	1.16	0.87	0.85	1.31	0.95	0.87	1.16	0.97	0.89	1.31	0.99	0.93
28	1.28	0.92	0.81	1.18	0.84	0.76	1.21	0.94	0.84	1.15	0.74	0.89
Post-challenge+												
7 & 13	1.40	1.02	0.96	1.46	1.07	1.10	1.31	1.02	1.03	1.26	0.58	0.78
14 & 20	1.18	0.83	0.86	1.21	0.78	0.86	1.21	0.88	0.84	1.19	0.74	0.77
21 & 27	1.24	0.88	0.77	1.27	0.88	0.79	1.28	0.78	0.51	1.28	0.53	0.57
Control Cells Mean ± SE++ Migration	1.26 ± 0.07			1.29 ± 0.08			1.21 ± 0.05			1.24 ± 0.05		

*Heat soluble antigen 30 ug of protein per ml.

**Sonicated soluble antigen 30 ug of protein per ml.

***Calves (#8 & 12) were vaccinated with aqueous bacterin and calves (#9 & #11) were vaccinated with incomplete Freund adjuvant bacterin after blood samples were collected Nov. 11, 1979.

+Calves were challenged intratracheally on day 28 post-vaccination, Dec. 13, 1979 after blood samples were collected and challenged again on day 34 post vaccination, Dec. 19, 1979.

++Standard error.

Table 4. Leukocyte Migration Inhibition Test of Calves Vaccinated Intramuscularly with Pasteurella hemolytica Bacterin and Challenged: Sequential Percentage Migration Inhibitions of Leukocytes from 4 Calves with Heat Soluble Antigen and Sonicated Soluble Antigen.

Day Blood Sample Collected	Percentage Migration Inhibition (% MI)									
	Calf #8		Calf #9		Calf #11		Calf #12		Mean ± SE***	
	H.S.*	S.S.**	H.S.	S.S.	H.S.	S.S.	H.S.	S.S.	H.S.	S.S.
Post-vaccination										
0+	3.1	3.1	6.2	6.2	5.1	7.7	6.4	5.6	5.2 ± 1.51	5.6 ± 1.91
7	1.7	21.4	1.5	19.3	14.7	15.6	1.7	12.1	4.9 ± 6.53	17.1 ± 4.16
14	26.8	25.2	23.2	22.4	26.5	27.3	20.0	29.3	24.1 ± 3.19	26.0 ± 2.95
21	25.0	26.8	26.8	23.0	25.0	23.3	24.2	29.1	25.2 ± 1.10	25.5 ± 2.92
28	28.2	36.8	24.6	35.6	21.5	30.6	33.9	22.7	27.0 ± 5.32	31.3 ± 6.42
Post-challenge++										
7 & 13	27.2	31.5	26.1	24.7	22.2	20.7	54.0	38.1	32.3 ± 14.57	28.7 ± 7.6
14 & 20	28.9	27.2	35.6	29.0	27.3	29.8	37.9	35.3	32.4 ± 5.12	30.3 ± 3.4
21 & 27	29.3	37.1	30.8	37.8	39.1	59.4	58.6	55.1	39.4 ± 13.47	47.4 ± 11.6

*Heat soluble antigen 30 ug of protein per ml.

**Sonicated soluble antigen 30 ug of protein per ml.

***Standard error.

+Calves (#8 & #12) were vaccinated with aqueous bacterin and calves (#9 & #11) were vaccinated with incomplete Freund adjuvant bacterin after blood samples were collected on Nov. 11, 1979.

++Calves were challenged intratracheally on day 28 post-vaccination, Dec. 13, 1979, after blood samples were collected and challenged again on day 34 post-vaccination, Dec. 19, 1979.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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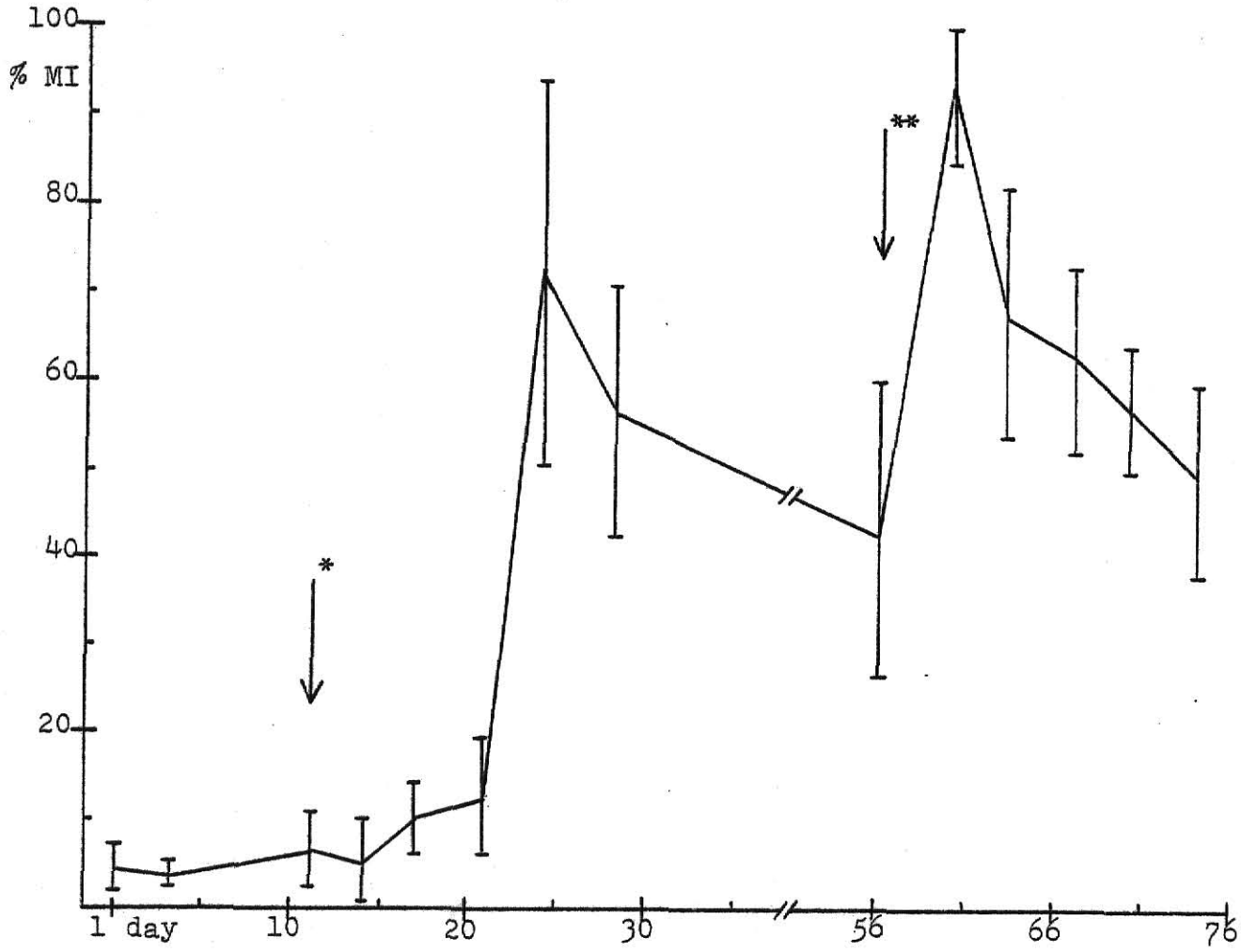
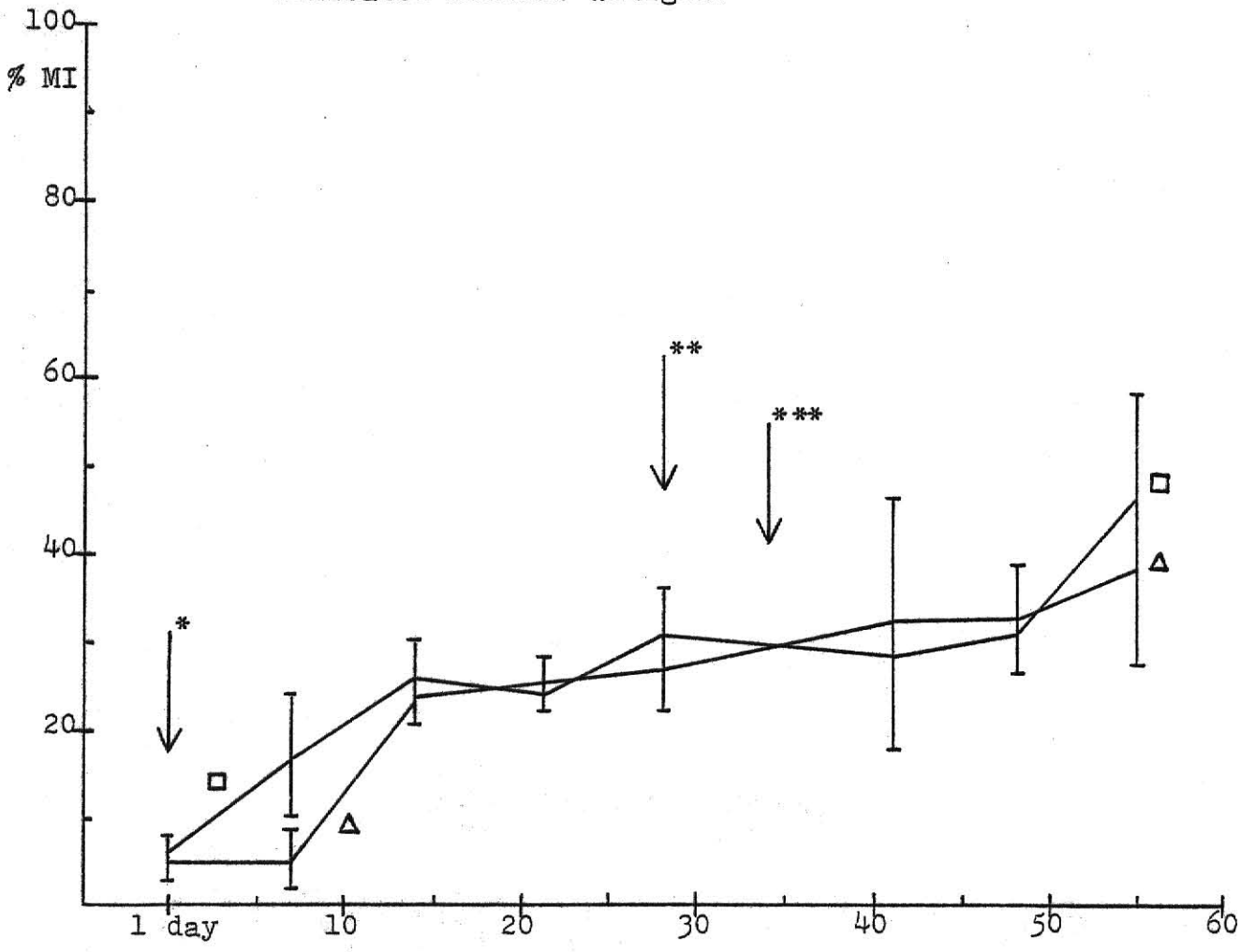
Figure 1. Leukocyte Migration Inhibition Test of Calves Infected Intratracheally with Pasteurella hemolytica and Challenged: Mean ± SE Percentage Migration Inhibitions of Leukocytes from 4 Calves.



*Calves were inoculated on July 13, 1979.

**Calves were inoculated again with live Pasteurella hemolytica on day 56 post-inoculation, Dec. 7, 1979.

Figure 2. Leukocyte Migration Inhibition Test of Calves Vaccinated Intramuscularly with Pasteurella hemolytica Bacterin and Challenged: Mean ± SE Percentage Migration Inhibitions of Leukocytes from 4 Calves with Heat Soluble Antigen and Sonicated Soluble Antigen.



□ Mean ± SE % MIs of leukocytes with heat soluble antigen 30 ug of protein per ml.

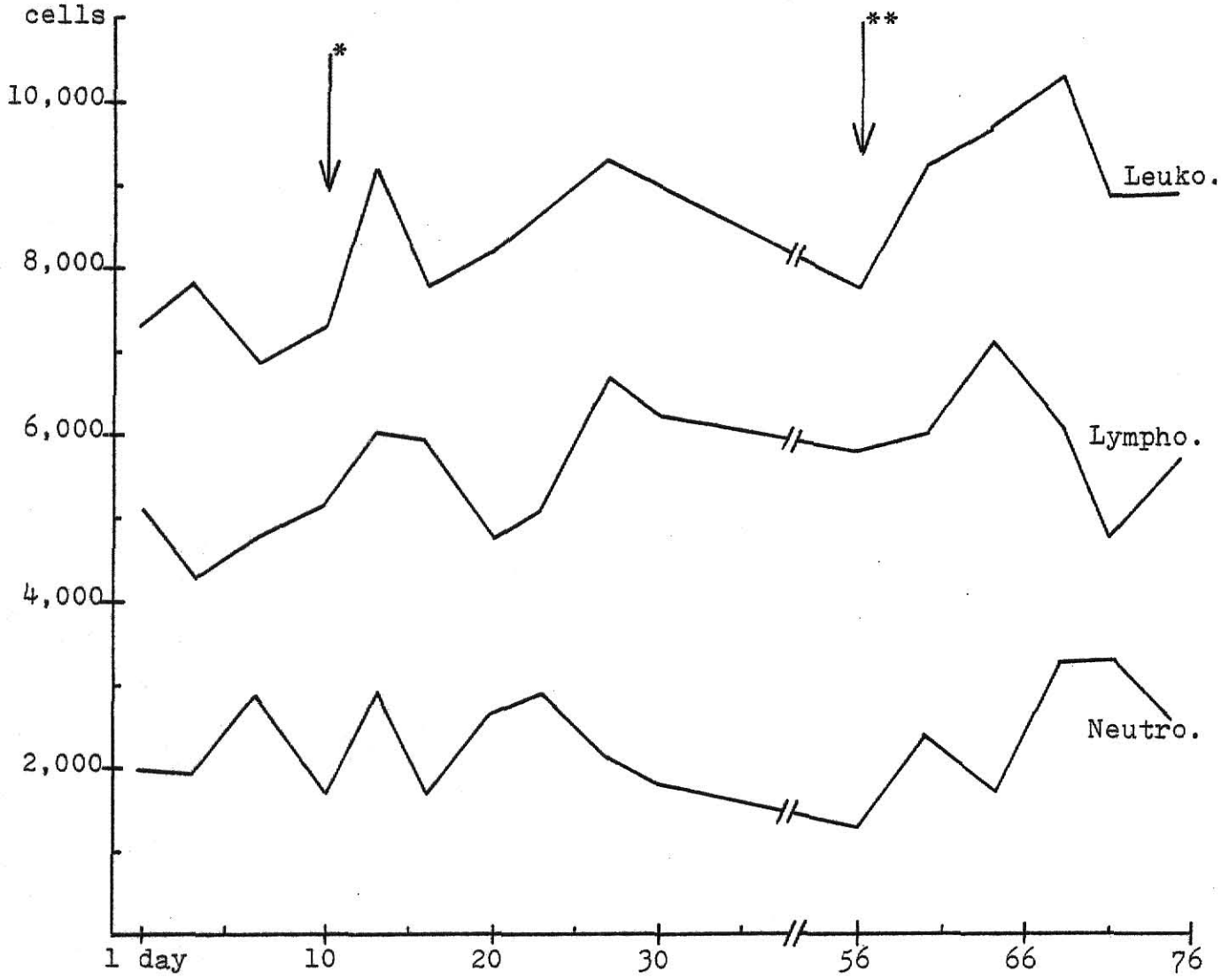
△ Mean ± SE % MIs of leukocytes with sonicated soluble antigen 30 ug of protein per ml.

*Calves were vaccinated on Nov. 11, 1979.

**Calves were challenged intratracheally on Dec. 13, 1979.

***Calves were challenged intratracheally on Dec. 19, 1979.

Figure 3. Total Leukocytes, Lymphocytes, Neutrophils of Calves Infected Intratracheally with Pasteurella hemolytica and Challenged.



*Calves were inoculated on July 13, 1979.

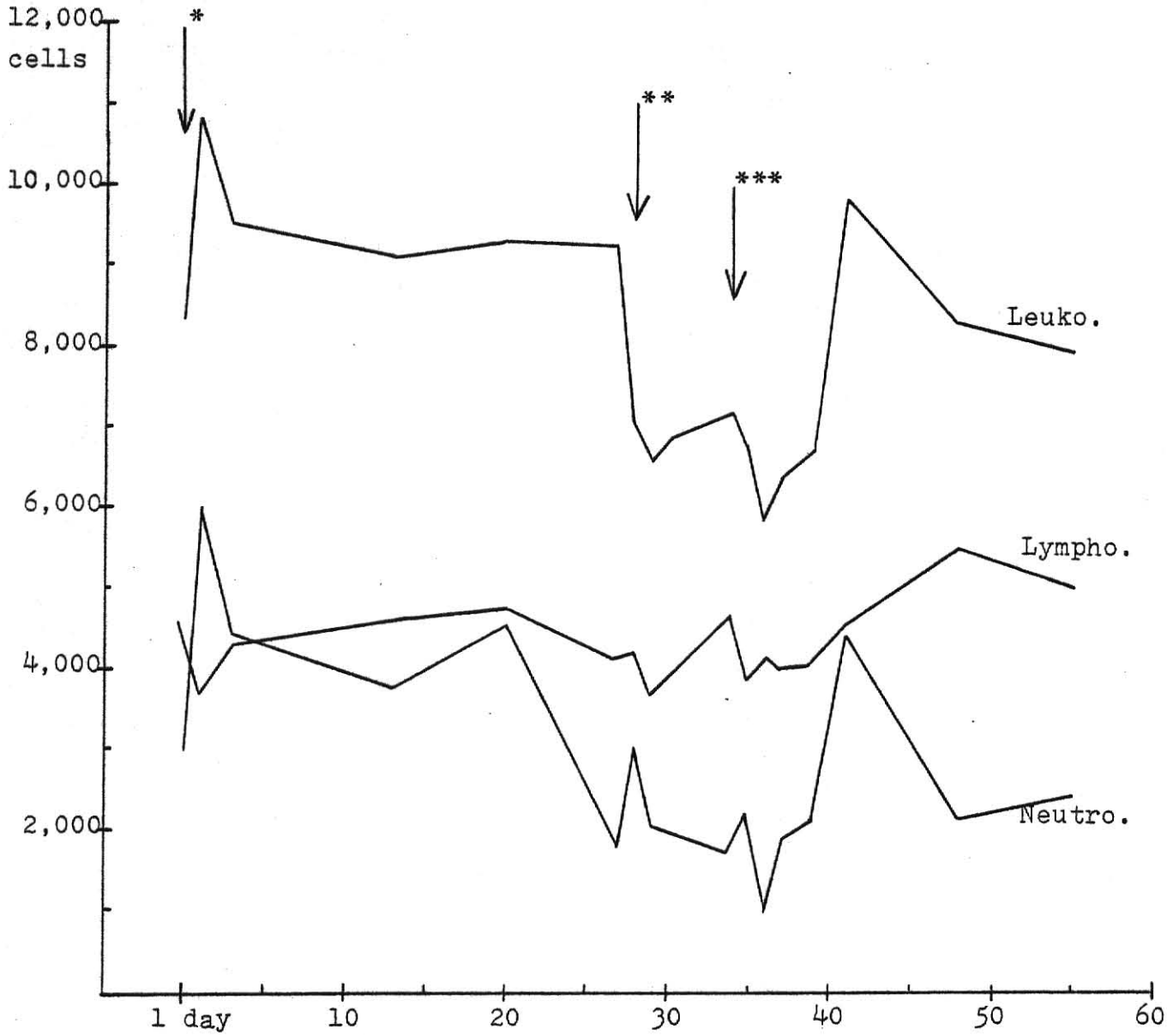
**Calves were inoculated again with live Pasteurella hemolytica on day 56 post-inoculation, Dec. 7, 1979.

Leuko. Leukocytes.

Lympho. Lymphocytes.

Neutro. Neutrophils.

Figure 4. Total Leukocytes, Lymphocytes, Neutrophils of Calves Vaccinated Intramuscularly with Pasteurella hemolytica Bacterin and Challenged: Sequential Average Cell Counts Per Micro-liter from 4 Calves.



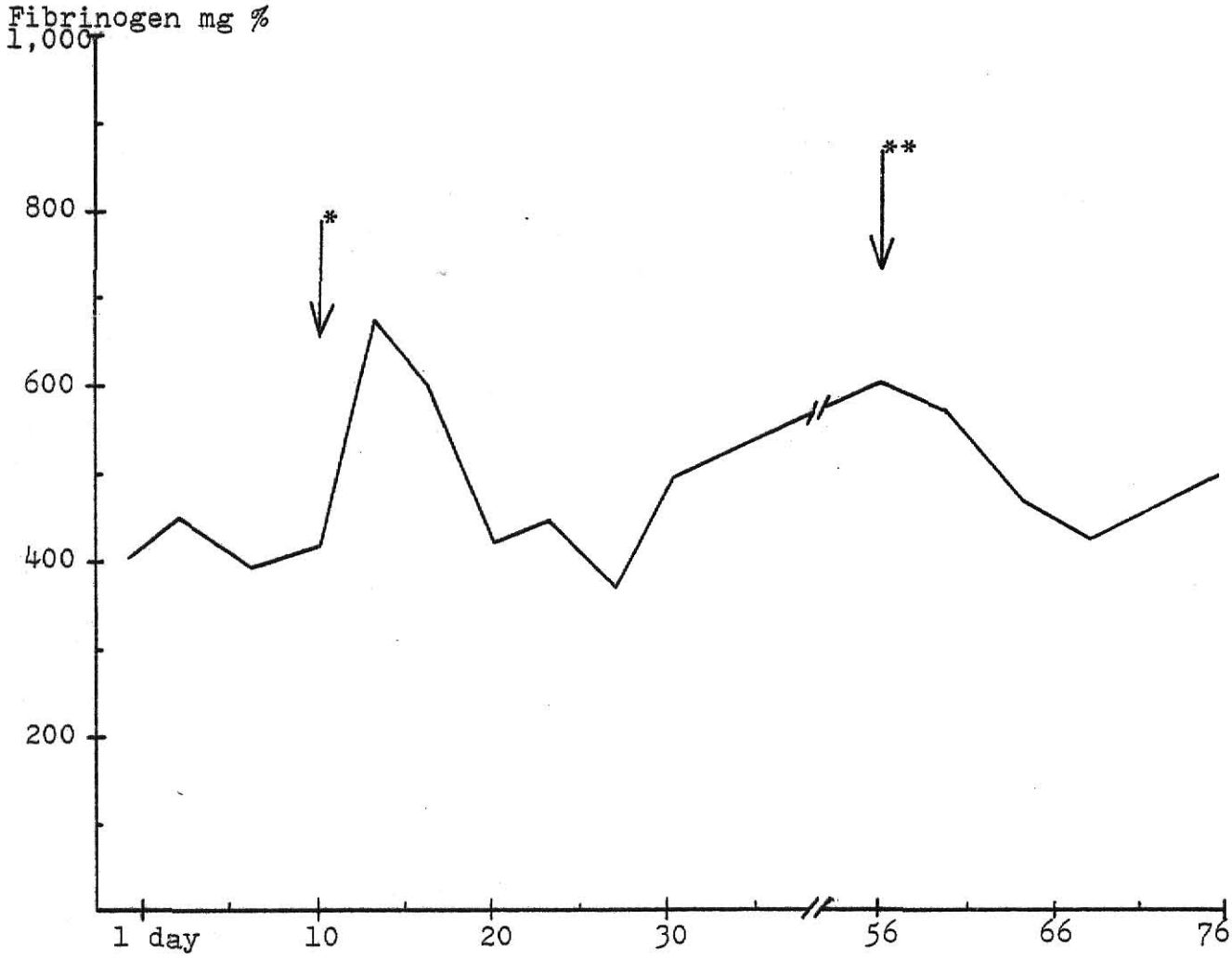
*Calves were vaccinated on Nov. 11, 1979.

**Calves were challenged intratracheally on Dec. 13, 1979.

***Calves were challenged intratracheally on Dec. 19, 1979.

Leuko. Leukocytes; Lympho. Lymphocytes; Neutro. Neutrophils.

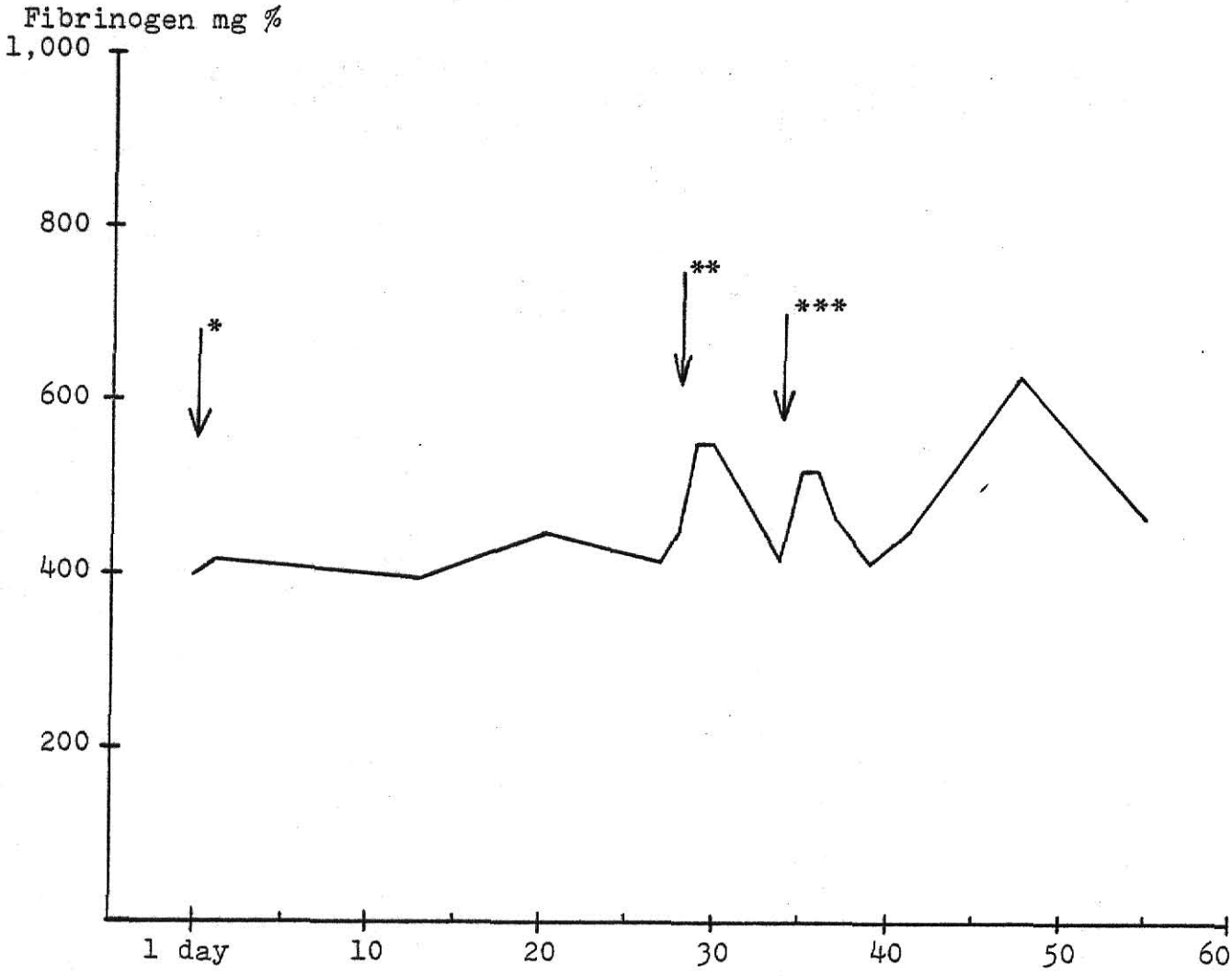
Figure 5. Fibrinogen Values of Calves Infected Intratracheally with Pasteurella hemolytica and Challenged: Sequential Average Fibrinogen Value (mg %) from 4 Calves.



*Calves were inoculated on July 13, 1979.

**Calves were inoculated again with live Pasteurella hemolytica on day 56 post-inoculation, Dec. 7, 1979.

Figure 6. Fibrinogen Value of Calves Vaccinated Intra-Muscularly with Pasteurella hemolytica Bacterin and Challenged: Sequential Average Fibrinogen Value (mg %) from 4 Calves.



*Calves were vaccinated on Nov. 11, 1979.

**Calves were challenged intratracheally on Dec. 13, 1979.

***Calves were challenged intratracheally on Dec. 19, 1979.

APPENDIX

LIST OF TABLES

Table 1. Summary of Calves Used in Leukocyte Migration Inhibition Test in Pasteurellosis: Ten Calves were Selected from Conventionally Raised Normal Guernsey Breed.

Calf No.	Wt. (lbs.)	Age (mon.)	Sex	Experiments
1	396	9.0	M	infected on July 13, 1979 challenged on Sep. 7, 1979
2	333	8.5	M	infected on July 13, 1979 challenged on Sep. 7, 1979
3	99	2.0	M	infected on July 13, 1979 challenged on Sep. 7, 1979
4	79	2.0	F	control calf
5	94	1.0	M	infected on July 13, 1979 challenged on Sep. 7, 1979
6	92	2.0	M	infected on Sep. 7, 1979
8	125	3.0	M	vaccinated on Nov. 15, 1979 challenged on Dec. 13, 19, 1979
9	164	4.0	M	vaccinated on Nov. 15, 1979 challenged on Dec. 13, 19, 1979
11	120	3.0	M	vaccinated on Nov. 15, 1979 challenged on Dec. 13, 19, 1979
12	130	2.5	M	vaccinated on Nov. 15, 1979 challenged on Dec. 13, 19, 1979

a. Calves were infected intratracheally with Pasteurella hemolytica serotype 1.

b. Calves were challenged intratracheally with Pasteurella hemolytica.

c. Calves were vaccinated intramuscularly with Pasteurella hemolytica bacterin.

d. Calves were weighed on day of receiving.

Table 2. White Blood Cells and Fibrinogen Profiles of Calf #1 Infected with Pasteurella hemolytica and Challenged.

Day Blood Collected	W.B.C.	Band	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil	Fibrinogen
Preinoculation								
1	9,400	-	3,102	5,452	564	188	94	300
3	9,600	-	3,168	5,472	864	96	-	400
7	10,300	-	2,987	6,644	566	103	-	300
13	9,500	-	2,850	6,365	-	285	-	400
Post-inoculation								
3	10,900	-	3,870	6,540	436	54	-	700
6	8,400	-	2,604	5,544	84	168	-	500
10	10,900	-	3,924	6,213	436	109	218	400
13	9,800	-	3,038	6,272	392	98	-	600
17	10,300	-	2,472	7,519	309	-	-	400
20	10,600	-	2,756	6,042	1,696	106	-	500
56	9,600	-	1,330	7,885	95	190	-	800
Post-challenge								
4	10,600	106	2,968	6,837	159	530	-	600
7	11,900	-	1,607	9,163	-	1,130	-	400
11	12,600	-	4,914	6,300	378	1,008	-	500
14	7,700	-	3,234	3,773	693	-	-	500
18	11,700	-	3,042	7,898	292	468	-	400

a. Calf was inoculated intratracheally with Pasteurella hemolytica 1,500,000 organisms on day 13 preinoculation, July 13, 1979 and challenged intratracheally with Pasteurella hemolytica 280,000 organisms on day 56 post inoculation, Sep. 7, 1979.

b. Calf was treated with antibiotic for 2 days from July 14, 1979.

Table 3. White Blood Cells and Fibrinogen Profiles of Calf #2 Infected with *Pasteurella hemolytica* and challenged.

Day Blood Collected	W.B.C.	Band	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil	Fibrinogen
Preinoculation								
1	7,000	-	1,400	5,180	210	210	-	300
3	7,800	-	2,262	4,914	234	234	156	400
7	8,000	-	2,080	5,280	240	240	160	300
13	8,400	-	2,436	5,544	84	336	-	400
Post-inoculation								
3	9,500	-	3,230	6,080	95	95	-	500
6	8,600	-	1,806	6,450	172	172	-	600
10	8,900	-	3,249	5,117	357	89	89	400
13	7,500	-	2,025	4,500	300	525	150	300
17	9,700	-	2,522	6,499	97	582	-	300
20	9,700	-	2,716	6,402	-	485	97	500
56	6,400	-	320	5,312	128	576	64	700
Post-challenge								
4	6,400	-	1,488	6,464	418	791	139	500
7	9,300	-	1,140	7,125	95	1,140	-	300
11	11,500	-	2,300	7,360	575	1,265	-	400
14	11,600	-	4,060	5,568	116	1,856	-	400
18	10,600	-	3,551	6,254	106	689	-	500

a. Calf was inoculated intratracheally with *Pasteurella hemolytica* 150,000 organisms on day 13 preinoculation, July 13, 1979 and challenged intratracheally with *Pasteurella hemolytica* 28,000 organisms on day 56 post inoculation, Sep. 7, 1979.

b. Calf was treated with antibiotic for 2 days from July 14, 1979.

Table 4. White Blood Cells and Fibrinogen Profiles of Calf #3 Infected with Pasteurella hemolytica and challenged.

Day Blood Collected	W.B.C.	Band	Neutro-phil	Lympho-cyte	Mono-cyte	Eosino-phil	Baso-phil	Fibrinogen
Preinoculation								
1	7,000	-	2,030	4,550	350	70	-	600
3	7,200	-	3,096	3,024	936	72	72	500
7	8,600	-	4,558	3,698	344	-	-	600
13	6,300	-	1,575	4,599	126	-	-	500
Post-inoculation								
3	9,400	-	3,290	6,110	-	-	-	900
6	6,500	-	1,150	5,070	325	-	-	800
10	5,600	-	2,464	2,912	224	-	-	500
13	9,900	-	3,168	6,534	99	99	-	500
17	9,000	-	1,260	7,020	540	90	-	400
20	8,000	-	880	6,800	160	80	80	500
56	8,400	-	2,604	5,208	420	168	-	600
Post-challenge								
4	8,800	-	3,080	5,368	352	-	-	600
7	8,800	-	2,024	6,776	-	-	-	600
11	9,600	-	3,696	5,280	528	48	48	300
14	7,800	-	2,652	5,070	-	78	-	500
18	7,400	-	1,776	5,328	296	-	-	600

a. Calf was inoculated intratracheally with Pasteurella hemolytica 15,000 organisms on day 13 preinoculation, July 13, 1979 and challenged intratracheally with Pasteurella hemolytica 2,800 organisms on day 56 post inoculation, Sep. 7, 1979.

b. Calf was treated with antibiotic for 6 days from July 14, 1979.

Table 5. White Blood Cells and Fibrinogen Profiles of Calf #5 Infected with Pasteurella hemolytica and Challenged.

Day Blood Collected	W.B.C.	Band	Neutro-phil	Lympho-cyte	Mono-cyte	Eosino-phil	Baso-phil	Fibrinogen
Preinoculation								
1	5,700	114	1,539	3,591	285	171	-	400
3	7,200	-	2,016	4,176	1,008	-	-	500
7	5,900	-	1,888	3,894	118	-	-	400
13	5,000	-	900	4,050	50	-	-	400
Post-inoculation								
3	7,000	-	1,680	5,320	-	-	-	600
6	7,700	-	1,386	6,237	-	-	-	500
10	7,400	-	2,072	5,032	222	74	-	400
13	7,800	-	3,354	3,354	780	234	78	400
17	8,300	-	2,324	5,727	249	-	-	400
20	7,600	-	1,748	5,852	-	-	-	500
56	7,000	-	1,368	5,040	144	648	-	500
Post-challenge								
4	8,400	-	2,184	5,544	84	504	84	600
7	8,700	-	2,001	5,742	-	957	-	600
11	7,800	-	2,184	5,460	156	-	-	500
14	8,300	-	3,403	4,814	83	-	-	400
18	5,900	-	2,183	3,540	177	-	-	500

a. Calf was inoculated intratracheally with Pasteurella hemolytica 1,500 organisms on day 13 preinoculation, July 13, 1979 and challenged intratracheally with Pasteurella hemolytica 280 organisms on day 56 post inoculation, Sep. 14, 1979.

b. Calf was treated with antibiotic for 5 days from July 14, 1979.

Table 6. White Blood Cells and Fibrinogen Profiles of Calf #6 Infected with Pasteurella hemolytica.

Day Blood Collected	W.B.C.	Band	Neutro-phil	Lympho-cyte	Mono-cyte	Eosino-phil	Baso-phil	Fibrinogen
Post-inoculation								
1	7,400	-	1,702	5,698	-	-	-	400
4	8,600	-	2,752	4,815	430	258	344	600
7	7,400	-	1,776	5,476	-	-	-	600
11	7,100	-	2,130	4,686	142	71	71	400
14	6,200	-	1,922	3,782	124	124	248	400
18	5,700	-	1,140	4,275	-	285	-	500

a. Calf was inoculated with Pasteurella hemolytica 1,500 organisms on Sep. 17, 1979.

b. Calf was treated with antibiotic for 7 days from Sep. 17, 1979.

Table 7. White Blood Cells and Fibrinogen Profile of Calf #8 Vaccinated Intramuscularly with Pasteurella hemolytica Bacterin and Challenged.

Day Blood Collected	W.B.C.	Band	Neutro-phil	Lympho-cyte	Mono-cyte	Eosino-phil	Baso-phil	Fibrinogen
Post-vaccination								
1	8,600	-	3,784	3,870	774	86	86	700
2	9,100	182	4,095	4,004	819	-	-	600
4	10,500	210	5,880	4,095	315	-	-	500
14	8,900	-	4,628	3,738	267	89	89	200
21	9,000	-	4,680	3,150	630	270	270	400
28	7,000	-	2,380	3,640	630	140	140	400
Post-challenge								
1	7,100	-	1,775	4,473	497	71	71	400
2	6,300	-	1,827	3,654	693	63	63	400
3	5,800	116	2,378	2,900	290	-	-	500
5	6,700	-	2,211	4,020	335	-	-	400
6 & 0	7,700	-	3,543	3,619	539	-	-	400
7 & 1	5,500	-	1,705	3,135	550	-	-	400
8 & 2	7,000	-	2,240	4,550	70	70	70	500
10 & 4	7,500	-	3,000	3,975	525	-	-	400
12 & 6	7,600	-	4,256	3,040	305	0	0	500
19 & 13	7,500	-	2,035	4,800	525	-	-	900
26 & 20	7,700	-	3,850	3,696	154	-	-	700

a. Calf was vaccinated with Pasteurella hemolytica aqueous bacterin on Nov. 15, 1979.

b. Calf was challenged intratracheally with Pasteurella hemolytica 100,000 organisms on day 28 post vaccination, Dec. 13, 1979 and 900,000 organisms on Dec. 19, 1979.

Table 8. White Blood Cells and Fibrinogen Profile of Calf #9 Vaccinated Intramuscularly with Pasteurella hemolytica Bacterin and Challenged.

Day Blood Collected	W.B.C.	Band	Neutro-phil	Lympho-cyte	Mono-cyte	Eosino-phil	Baso-phil	Fibrinogen
Post-vaccination								
1	9,900	-	3,861	4,851	990	99	99	500
2	12,900	129	5,934	5,160	903	387	387	600
4	12,400	124	7,068	4,092	992	-	124	400
14	9,440	-	3,290	5,499	470	94	47	400
21	10,300	-	4,223	4,841	1,130	103	-	400
28	7,400	148	2,220	4,218	740	74	-	300
Post-challenge								
1	7,600	76	2,432	4,484	532	76	-	300
2	6,880	-	1,700	4,692	272	68	68	300
3	7,900	79	1,975	5,135	474	-	-	300
5	8,600	-	3,096	4,988	430	86	-	200
6 & 0	7,400	-	2,738	4,144	370	148	-	300
7 & 1	5,000	-	850	4,100	-	50	-	500
8 * 2	5,700	-	1,710	3,705	228	57	-	300
10 & 4	5,600	-	953	4,200	336	56	-	300
12 & 6	8,400	-	1,680	5,460	1,176	84	-	300
19 & 13	9,000	-	2,475	5,850	495	-	180	300
26 & 20	8,800	-	2,728	5,632	440	-	-	300

a. Calf was vaccinated with Pasteurella hemolytica incomplete Freund adjuvant bacterin on Nov. 15, 1979.

b. Calf was challenged intratracheally with Pasteurella hemolytica 100,000 organisms on day 28 post vaccination, Dec. 13, 1979 and 900,000 organisms on Dec. 19, 1979.

Table 9. White Blood Cells and Fibrinogen Profile of Calf #11 Vaccinated Intramuscularly with Pasteurella hemolytica Bacterin and Challenged.

Day Blood Collected	W.B.C.	Band	Neutro-phil	Lympho-cyte	Mono-cyte	Eosino-phil	Baso-phil	Fibrinogen
Post-vaccination								
1	8,700	-	2,610	5,481	609	-	-	300
2	11,200	-	6,048	4,256	986	-	-	400
4	9,900	-	3,267	5,841	495	99	198	400
14	12,700	-	5,207	6,731	508	190	64	400
21	10,700	-	2,563	7,276	856	-	-	400
28	7,000	-	1,470	4,760	630	-	140	400
Post-challenge								
1	8,500	-	3,485	4,335	510	170	-	500
2	7,300	146	2,409	3,869	730	73	73	800
3	9,200	276	2,576	5,520	736	92	-	700
5	7,400	-	740	6,216	148	222	74	500
6 & 0	6,500	-	1,105	4,810	455	130	-	600
7 & 1	7,200	-	1,728	5,256	72	144	-	600
8 & 2	5,600	-	1,624	3,584	336	56	-	600
10 & 4	8,400	-	2,604	4,956	756	84	-	500
12 & 6	6,900	-	1,173	5,750	414	-	138	600
19 & 13	11,300	-	3,616	7,119	339	226	-	200
26 & 20	11,000	-	2,750	8,030	110	110	-	300

a. Calf was vaccinated with Pasteurella hemolytica incomplete Freund adjuvant bacterin on Nov. 15, 1979.

b. Calf was challenged intratracheally with Pasteurella hemolytica 100,000 organisms on day 28 post vaccination, Dec. 13, 1979 and 900,000 organisms on Dec. 19, 1979.

Table 10. White Blood Cells and Fibrinogen Profile of Calf #12 Vaccinated Intramuscularly with Pasteurella hemolytica Bacterin and Challenged.

Day Blood Collected	W.B.C.	Band	Neutro-phil	Lympho-cyte	Mono-cyte	Eosino-phil	Baso-phil	Fibrinogen
Post-vaccination								
1	6,100	-	1,830	3,660	427	122	61	500
2	10,400	-	8,112	1,144	1,040	-	104	500
4	5,400	-	1,512	3,510	270	-	108	800
14	5,400	-	2,268	2,754	162	54	162	-
21	7,300	-	3,066	3,504	703	-	-	600
28	5,500	-	990	4,125	330	55	-	600
Post-challenge								
1	5,300	53	795	3,710	424	106	212	600
2	5,900	118	2,360	2,773	590	59	-	700
3	4,600	46	996	2,392	598	230	92	700
5	6,000	-	1,320	3,900	540	60	180	600
6 & 0	5,400	-	1,458	2,862	1,026	54	-	800
7 & 1	5,500	-	825	4,345	330	-	-	600
8 & 2	7,000	-	2,240	4,340	420	-	-	500
10 & 4	5,400	-	1,998	3,078	324	-	-	500
12 & 6	16,800	-	11,256	4,032	1,344	-	168	-
19 & 13	5,600	-	784	4,368	392	56	-	1,100
26 & 20	4,500	-	895	3,330	270	45	-	600

a. Calf was vaccinated with Pasteurella hemolytica aqueous bacterin on Nov. 15, 1979.

b. Calf was challenged intratracheally with Pasteurella hemolytica 100,000 organisms on day 28 post vaccination, Dec. 13, 1979 and 900,000 organisms on Dec. 19, 1979.

Table 11. Leukocyte Viability Test with RPMI 1640, Heat Soluble Antigen and Sonicated Antigen at 37 C for 24 Hours; Percentage Viability of Leukocytes from 4 Calves Vaccinated with Pasteurella hemolytica Bacterin and Challenged.

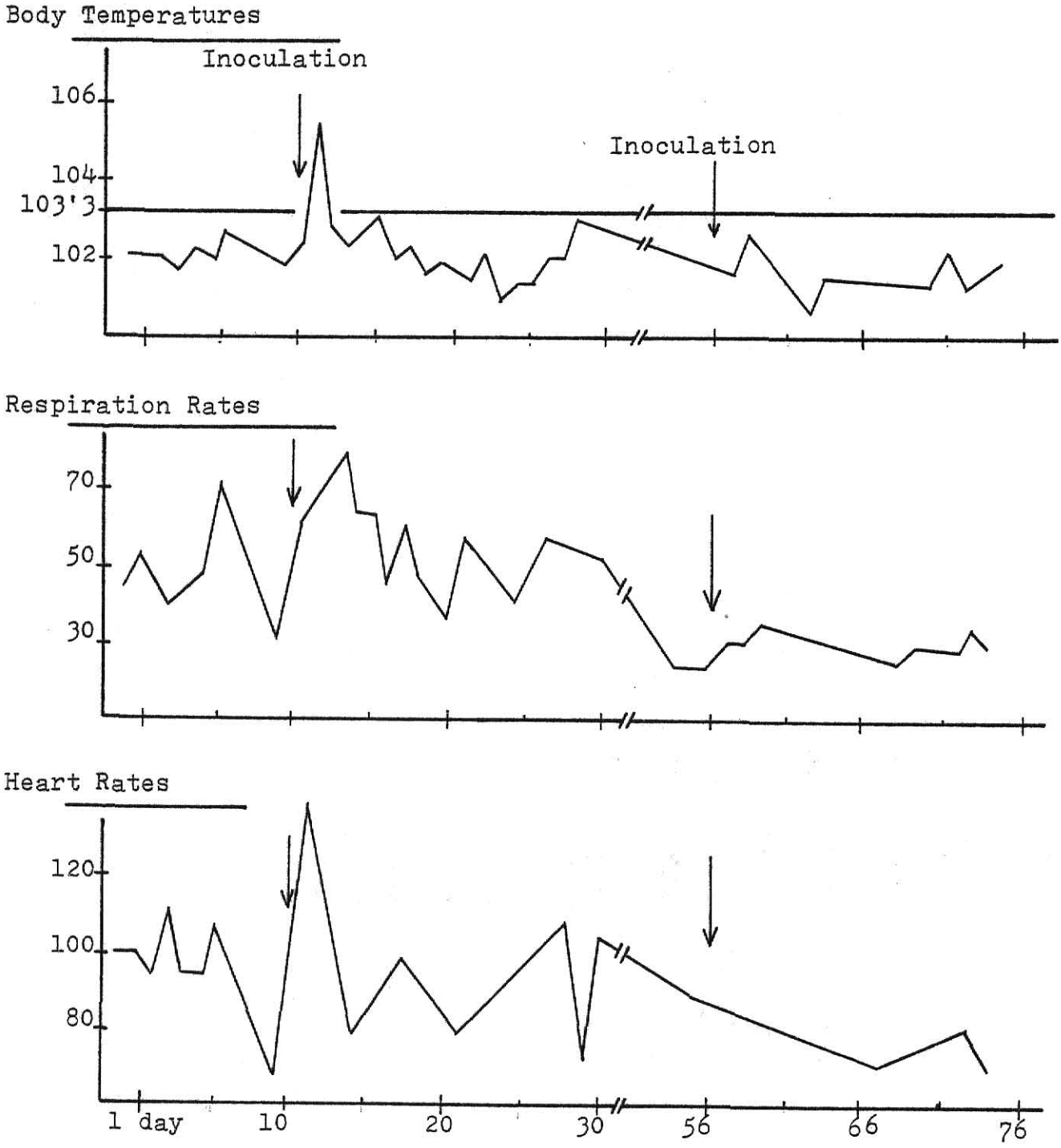
Day Blood Collected	Leukocyte Viability Percentage RPMI 1640	Heat Soluble Antigen	Sonicated Soluble Antigen
Post-vaccination			
0	95	94	92
7	87	88	84
14	91	90	91
28	73	70	66
Post-challenge			
12 & 6	79	78	77
19 & 13	92	93	90
26 & 20	95	93	94
Mean ± SE	87.42 ± 8.44	86.57 ± 9.12	84.85 ± 10.13

a. Calf was vaccinated with Pasteurella hemolytica on Nov. 15, 1979.

b. Calf was challenged intratracheally with Pasteurella hemolytica 100,000 organisms on day 28 post vaccination, Dec. 13, 1979 and 900,000 organisms on Dec. 19, 1979.

LIST OF FIGURES

Figure 1. Calf #1, Clinical Parameters.

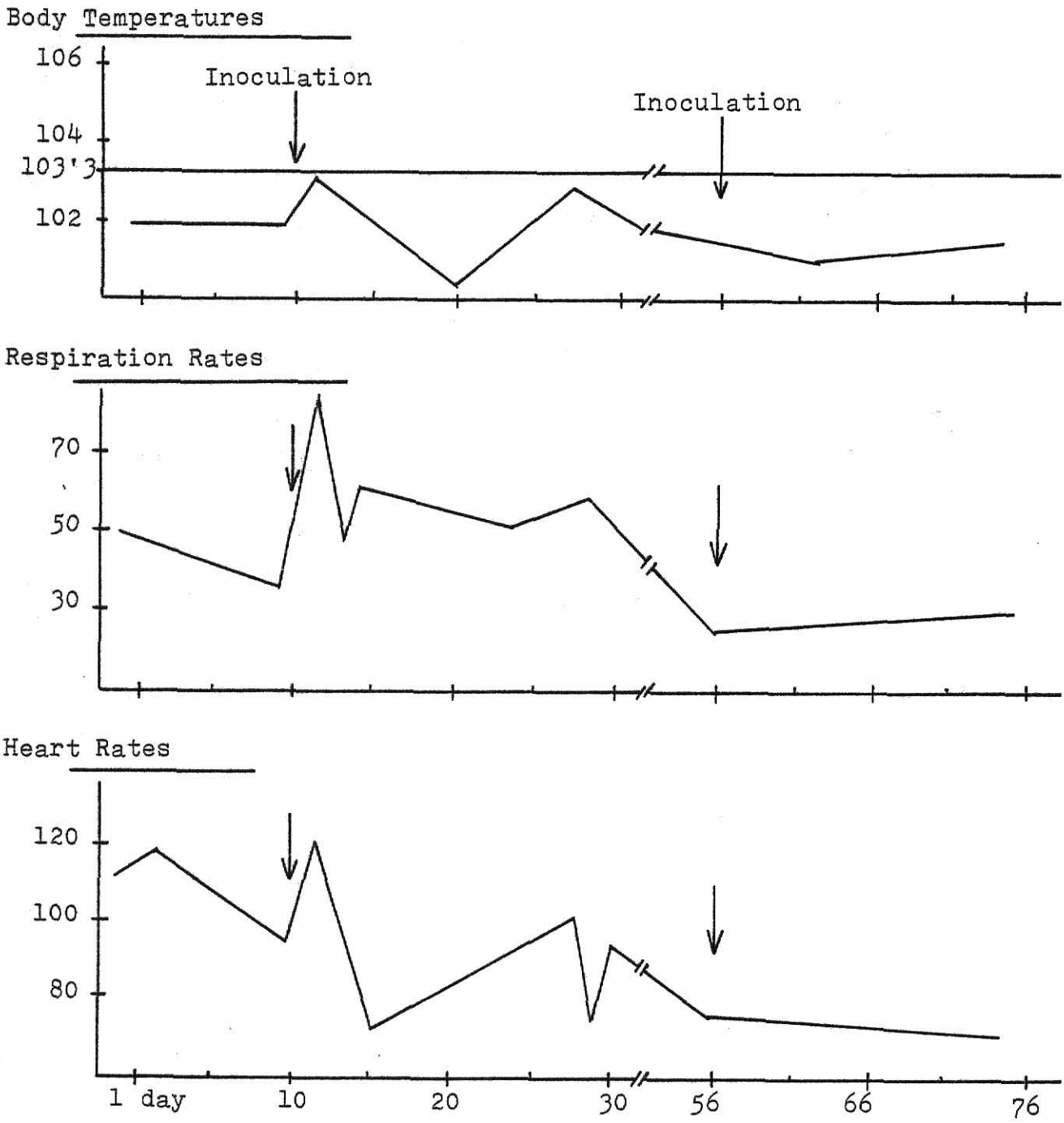


1. Calf was inoculated intratracheally with Pasteurella hemolytica 1,500,000 organisms on July 13 and 280,000 organisms on Sep. 7, 1979.

2. Calf was treated with antibiotic for 2 days from July 14, 1979.

3. Body temperature, respiration and heart rates were measured daily at 0700-0900 and at 1600-1800 hours.

Figure 2. Calf #2, Clinical Parameters.

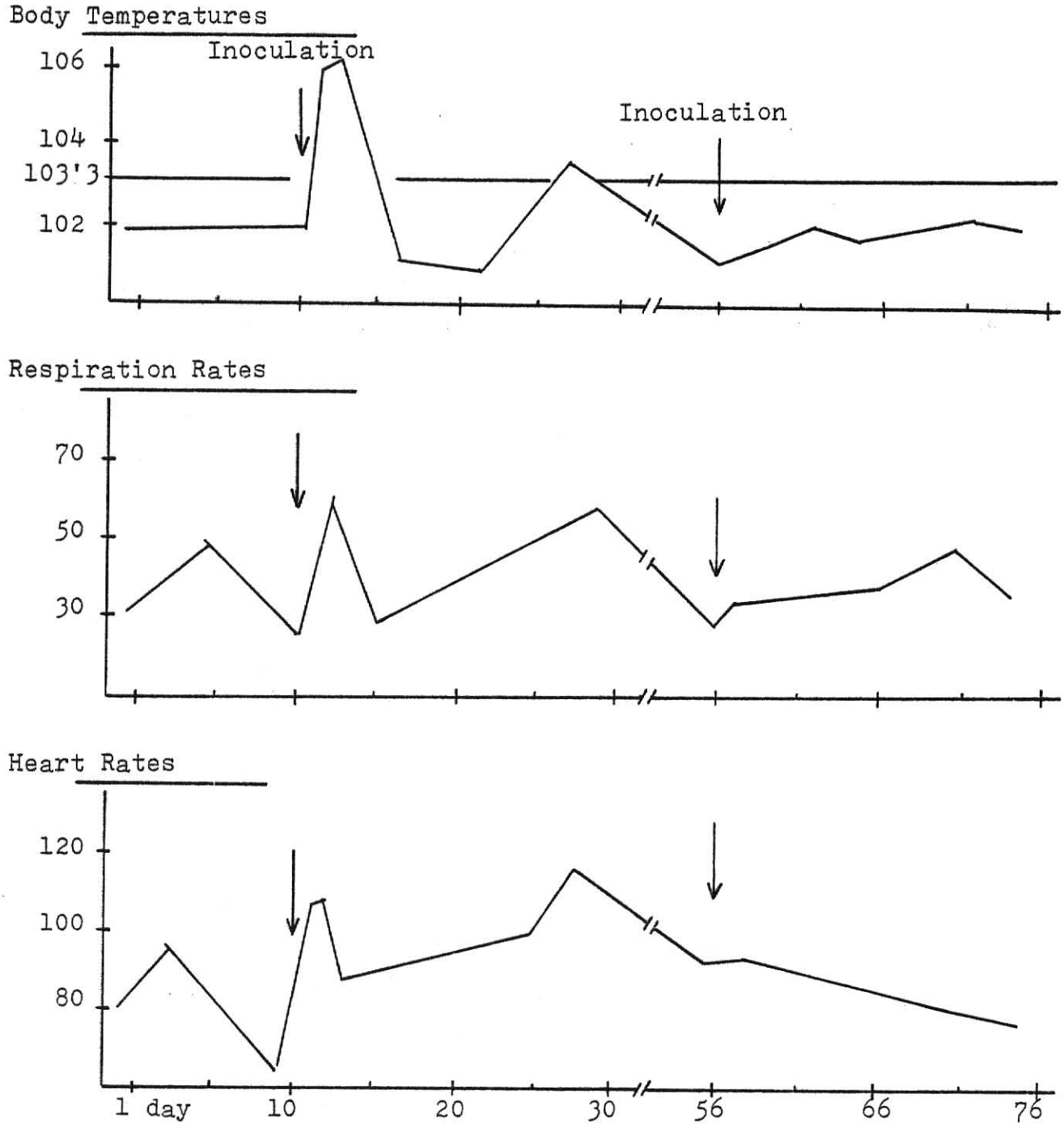


1. Calf was inoculated intratracheally with Pasteurella hemolytica 150,000 organisms on July 13, 1979 and 28,000 organisms on Sep. 7, 1979.

2. Calf was treated with antibiotic for 2 days from July 14, 1979.

3. Body temperature, respiration and heart rates were measured daily at 0700-0900 and at 1600-1800 hours.

Figure 3. Calf #3, Clinical Parameters.

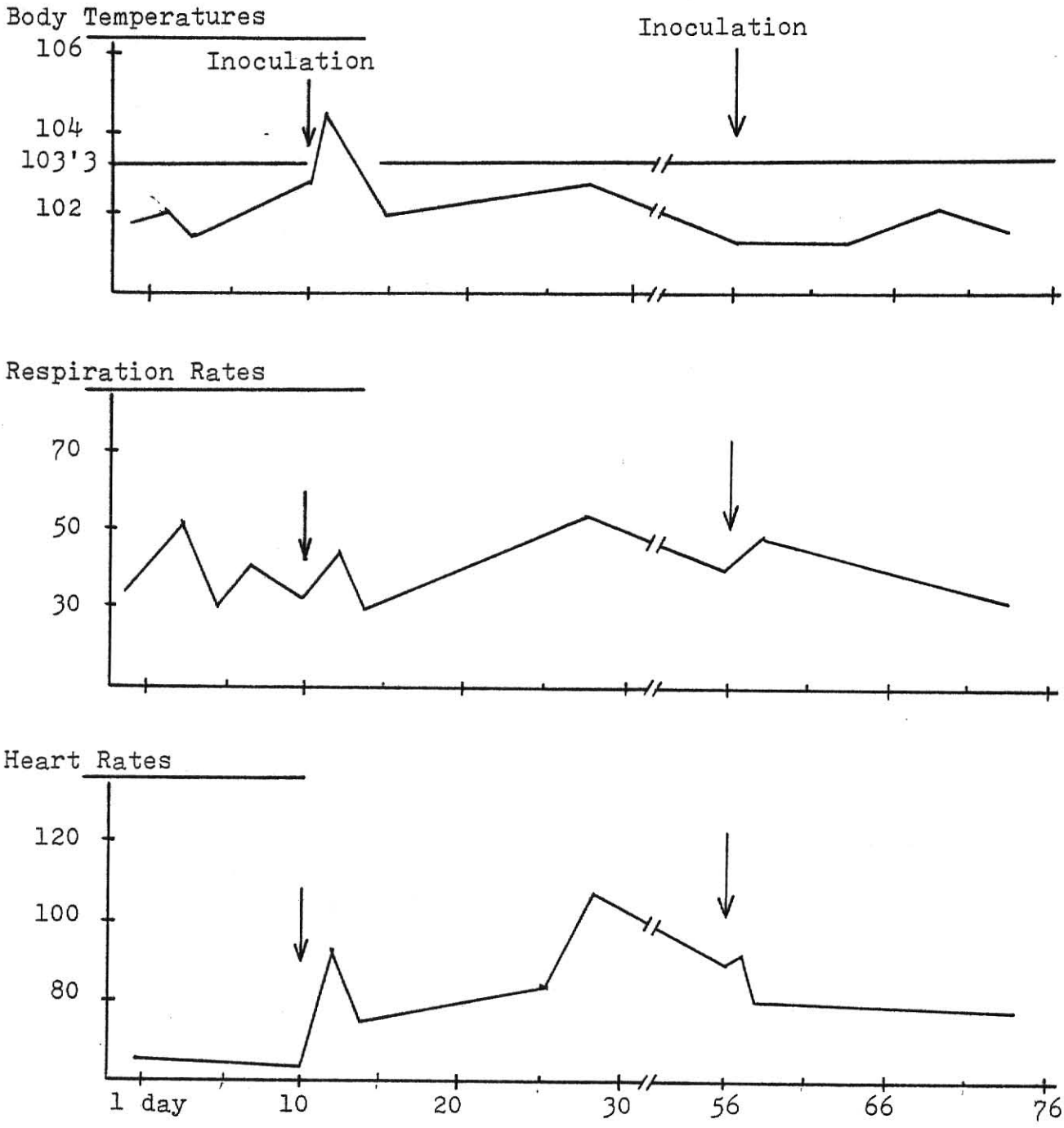


1. Calf was inoculated intratracheally with Pasteurella hemolytica 15,000 organisms on July 13, 1979 and 2,800 organisms on Sep. 7, 1979.

2. Calf was treated with antibiotic for 6 days from July 14, 1979.

3. Body temperature, respiration and heart rates were measured daily at 0700-0900 and at 1600-1800 hours.

Figure 4. Calf #5, Clinical Parameters.

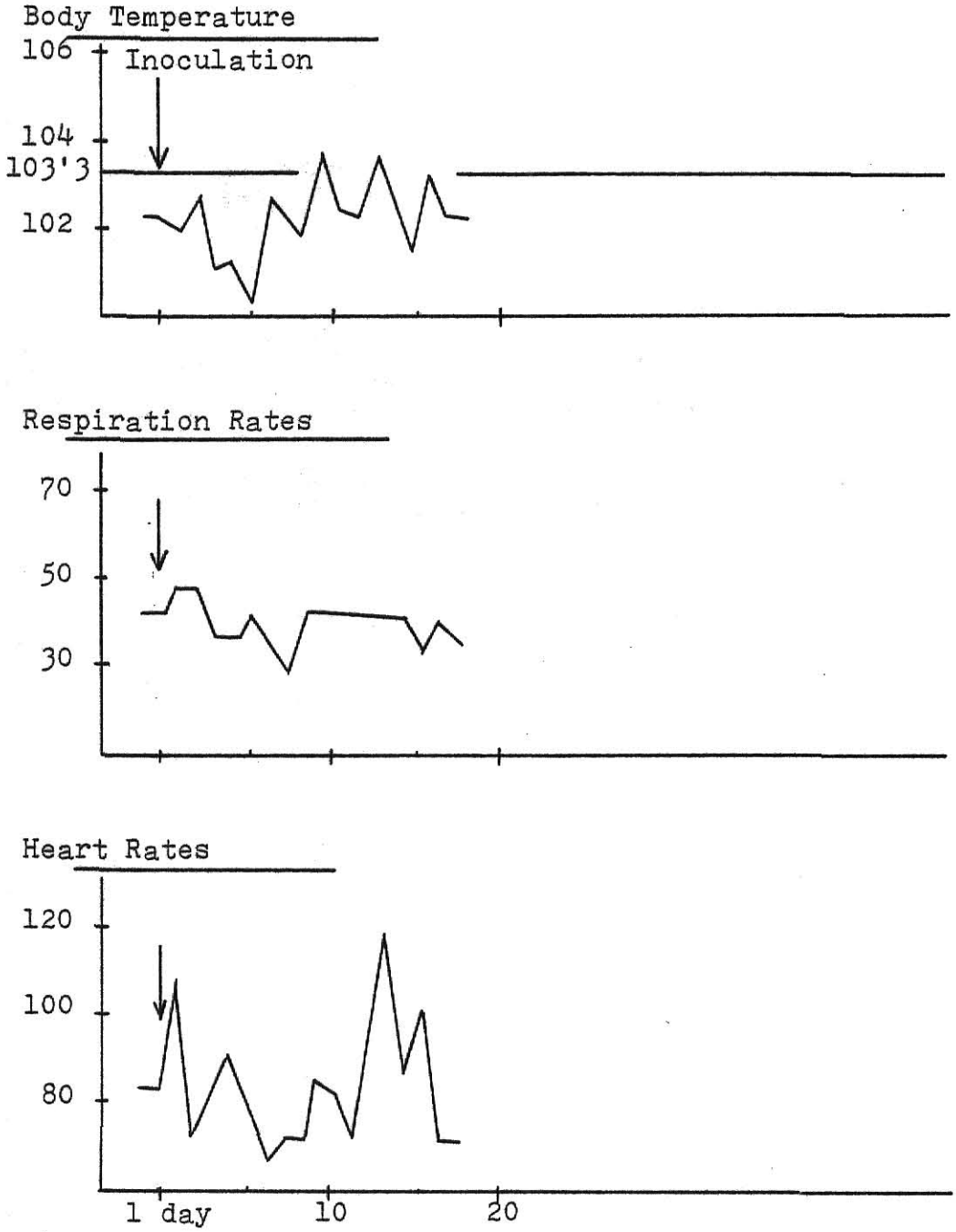


1. Calf was inoculated intratracheally with Pasteurella hemolytica 1,500 organisms on July 13, 1979 and 280 organisms on Sep. 7, 1979.

2. Calf was treated with antibiotic for 5 days from July 14, 1979.

3. Body temperature, respiration and heart rates were measured daily at 0700-0900 and at 1600-1800 hours.

Figure 5. Calf #6, Clinical Parameters.

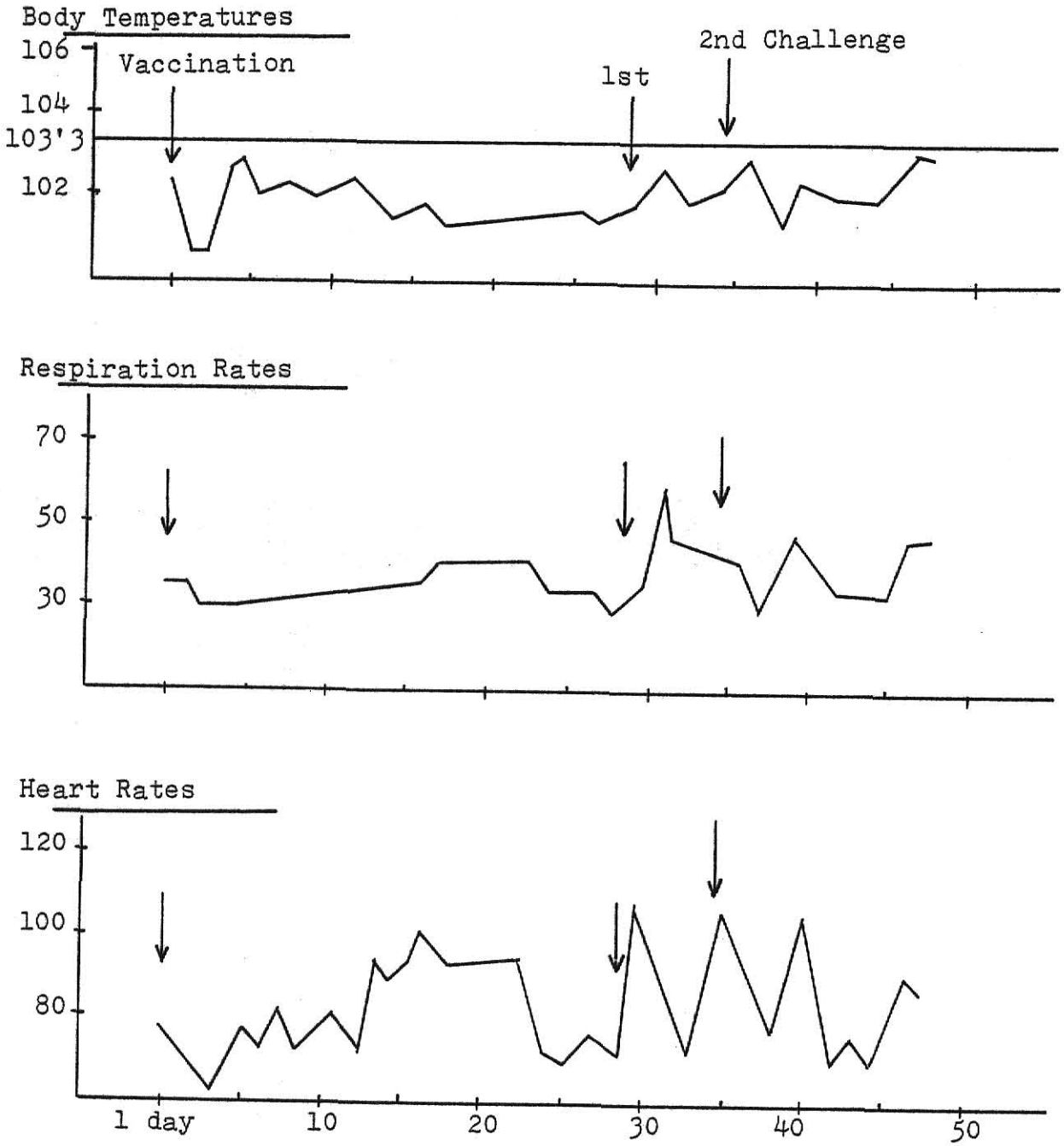


1. Calf was inoculated tratracheally with Pasteurella hemolytica 1,500 organisms on Sep. 7, 1979.

2. Calf was treated with antibiotic for 7 days from Sep. 17, 1979.

3. Body temperature, respiration and heart rates were measured daily at 0700-0900 and at 1600-1800 hours.

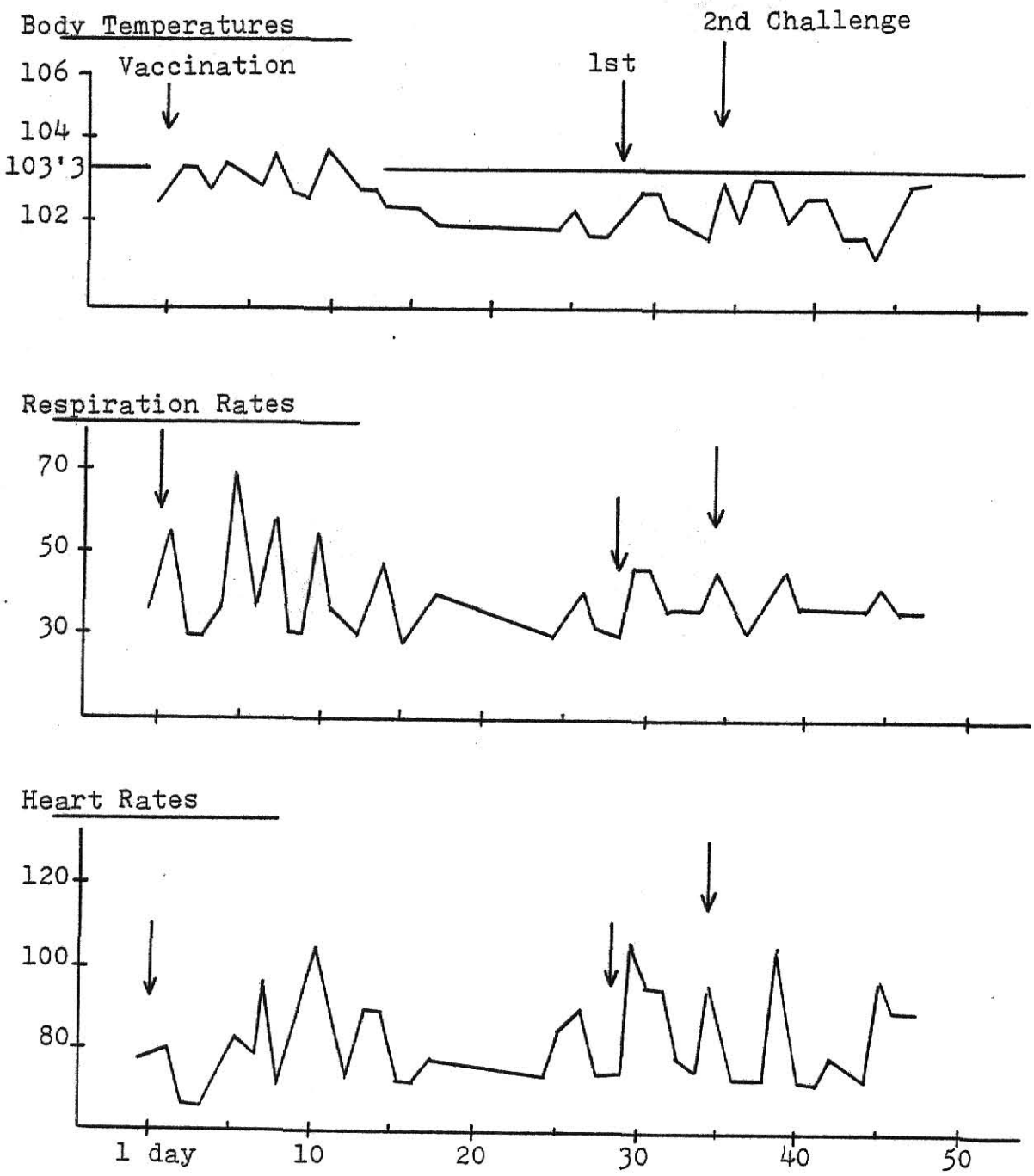
Figure 6. Calf #8, Clinical Parameters.



1. Calf was inoculated intratracheally Pasteurella hemolytica (8.43×10^9) aqueous bacterin IM. on Nov. 15, 1979 and challenged intratracheally with Pasteurella hemolytica 100,000 organisms on Dec. 13, 1979 and 900,000 organisms on Dec. 19, 1979.

2. Body temperature, respiration and heart rates were measured daily at 0700-0900 and at 1600-1800 hours.

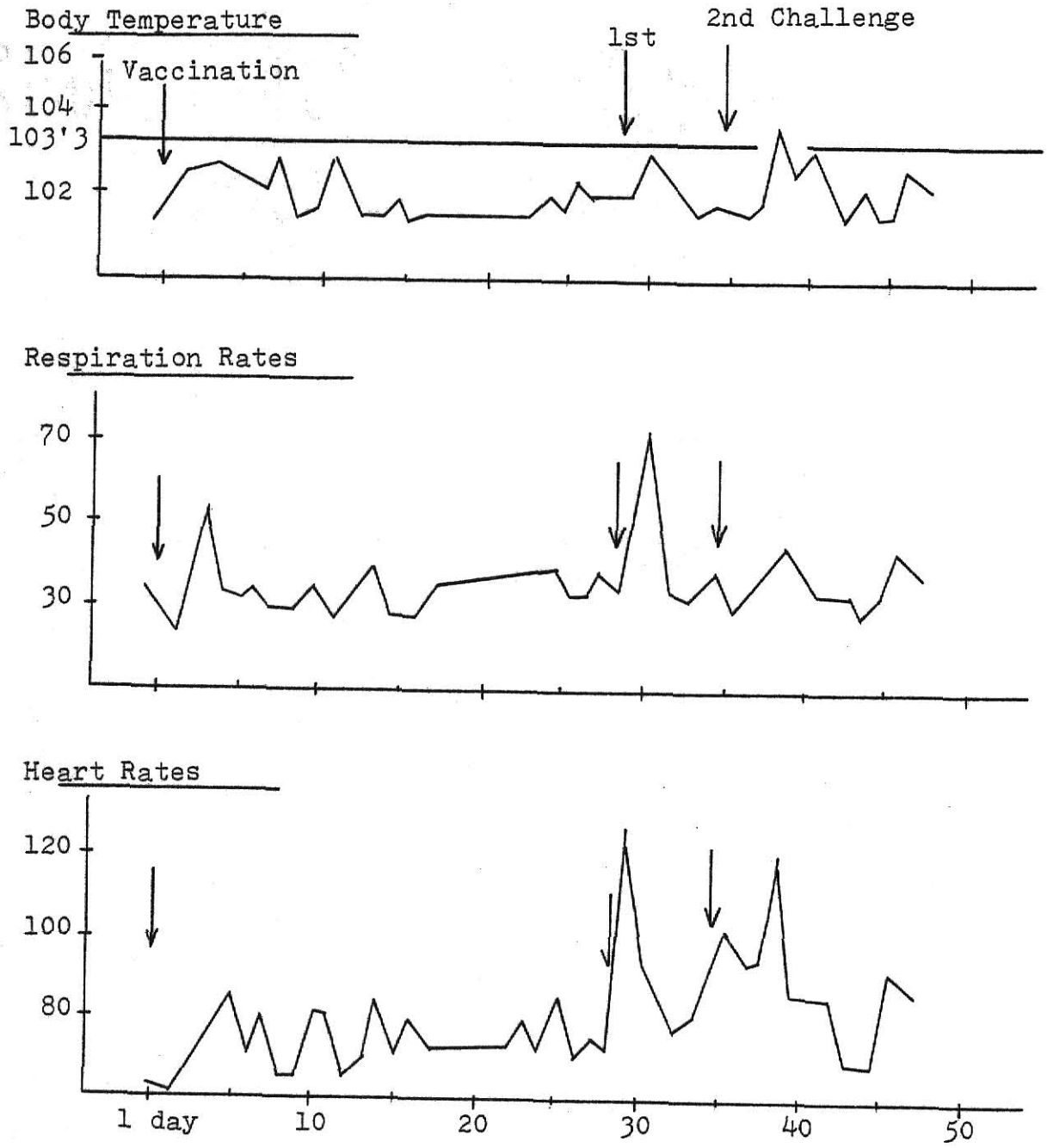
Figure 7. Calf #9, Clinical Parameters.



1. Calf was vaccinated with Pasteurella hemolytica (8.43×10^9) incomplete Freund adjuvant bacterin IM. on Nov. 15, 1979 and challenged with Pasteurella hemolytica 100,000 organisms on Dec. 13, 1979 and 900,000 organisms on Dec. 19, 1979.

2. Body temperature, respiration and heart rates were measured daily at 0700-0900 and at 1600-1800 hours.

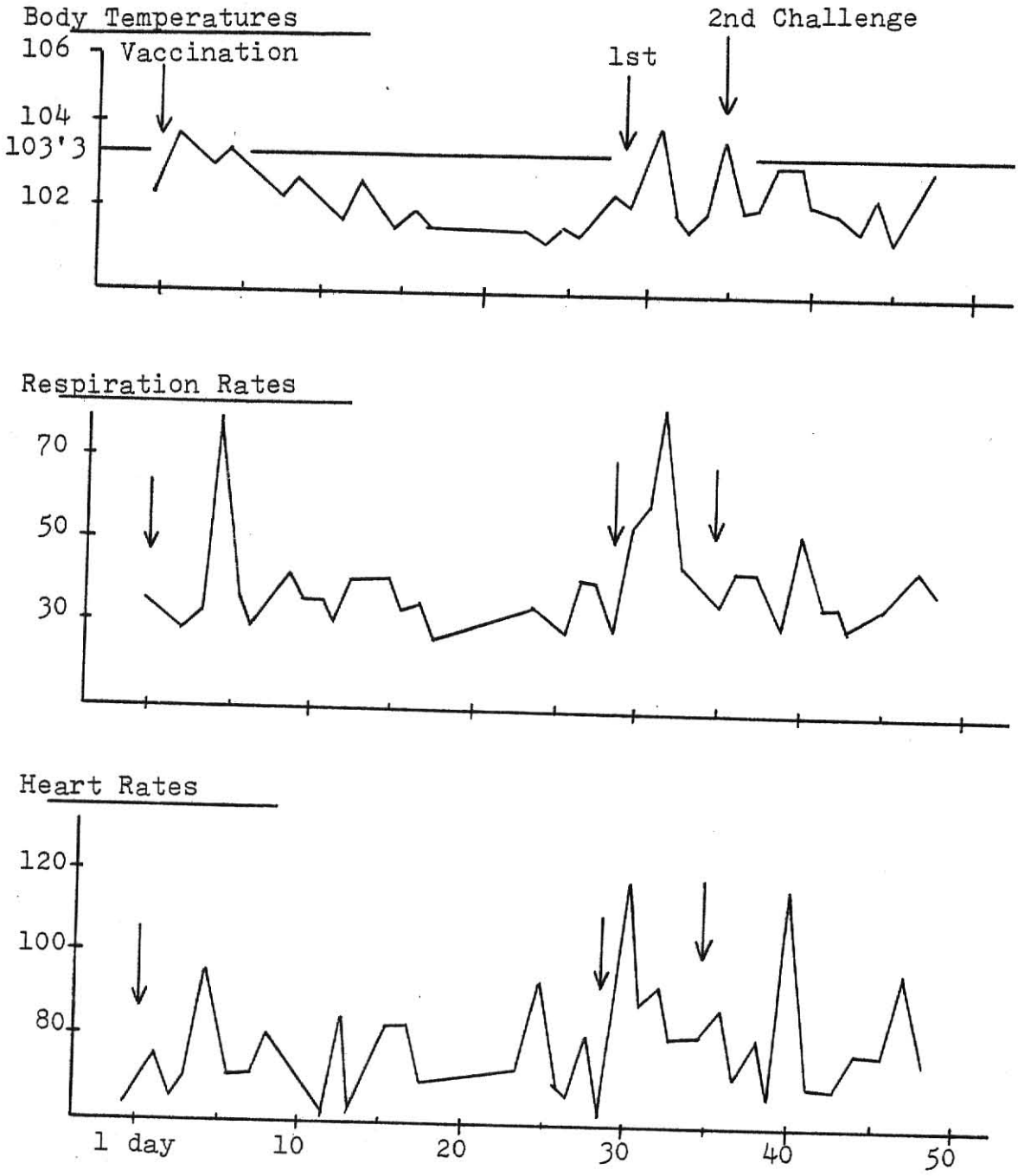
Figure 8. Calf #11, Clinical Parameters.



1. Calf was vaccinated with Pasteurella hemolytica (8.43×10^9) incomplete Freund adjuvant bacterin IM. on Nov. 15, 1979 and challenged with Pasteurella hemolytica 100,000 organisms on Dec. 13, 1979 and 900,000 organisms on Dec. 19, 1979.

2. Body temperature, respiration and heart rates were measured daily at 0700-0900 and at 1600-1800 hours.

Figure 9. Calf #12, Clinical Parameters.



1. Calf was vaccinated with Pasteurella hemolytica (8.43×10^9) aqueous bacterin IM. on Nov. 15, 1979 and challenged intratracheally with Pasteurella hemolytica 100,000 organisms on Dec. 13, 1979 and 900,000 organisms on Dec. 19, 1979.

2. Body temperature, respiration and heart rates were measured daily at 0700-0900 and at 1600-1800 hours.

LEUKOCYTE MIGRATION INHIBITION STUDIES IN PASTEURELLOSIS

by

SUNG HWAN PARK

D.V.M. Seoul Municipal College of Agriculture, Korea, 1969

AN ABSTRACT OF
A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Medicine and Surgery

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1980

Pasteurellosis in cattle is usually associated with infection by Pasteurella hemolytica or Pasteurella multocida in combination with viral infection of the respiratory tract or decreased resistance of the host by stress. This disease most commonly affects young growing cattle from 6 months to 2 years of age and occurs 7-14 days after cattle have arrived in the feedlot following transportation. The disease causes major economic losses to the feedlot industry.

Biological and chemoprophylactic preventive methods have been used extensively in an attempt to control Pasteurellosis in cattle, but have resulted in limited success. The technology of producing a bacterin which contains the antigens necessary to stimulate the appropriate protective antibody has not been developed. Standardized methods for differential diagnosis are needed in order to allow for more intelligent use of biological products.

The present study was undertaken with the purpose of contributing data concerning the pathogenesis, diagnosis and immunity of Pasteurellosis in cattle.

Of the total of 10 calves used, 4 calves were inoculated intratracheally with Pasteurella twice at 56 day intervals and 2 calves were used as controls. Four calves were vaccinated with Pasteurella bacterin and challenged intratracheally.

All cattle exposed to initial intratracheal inoculation of Pasteurella developed clinical signs of Pasteurellosis in 24 hours: acute febrile reaction, rapid, shallow

respirations and tachycardia. Elevation of the leukocyte count was accompanied by elevation of neutrophil count and fibrinogen value.

Both groups of cattle, after recovery from artificial infection or after vaccination, were challenged intratracheally and did not exhibit clinical signs of Pasteurellosis.

The Leukocyte Migration Inhibition technique used to evaluate cell-mediated immune responses in Pasteurellosis was demonstrated to be a successful laboratory procedure for diagnosis. In both groups of cattle, infected or vaccinated, significantly increased migration inhibition of leukocytes was exhibited by 2 weeks post-inoculations. The observation that leukocytes from cattle infected with Pasteurella had significantly higher migration inhibition than the migration inhibition of leukocytes from the vaccinated cattle suggest a linear relationship with Migration Inhibition Factor concentration and, therefore, correlates with the degree of delayed hypersensitivity.