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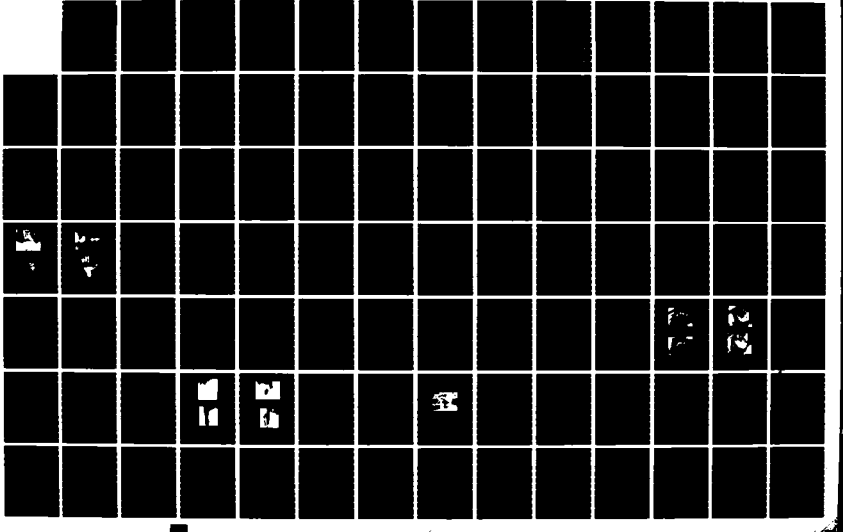

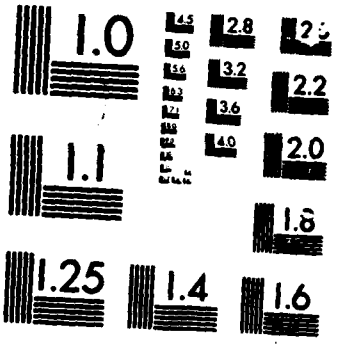
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The Evaluation of Jet Injection for Use in Veterinary Medicine

Howard Wayne Whitford

May 1976

Supported by

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Fort Detrick, Frederick, Md 21701-5012

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A. Ammended Final Scientific Report

B. Final Inventory of Government property remaining

C. Final Patent Report.

A. Addendum to Final Report

Contract No.: DADA 17-71-C-1087

Contractor: Texas Agricultural Experiment Station of Texas A&M University

Principle Investigator: S. McConnell

Report Date: July 1, 1977 - May 30, 1978

Project Title: "Continued Evaluation of Jet Injector Apparatus Equipped for Use in Veterinary Medicine."

This contract was extended from 1 July 1977 through 31 December 1977 without additional funds to permit a continuation of the study on the administration of rabies vaccine to dogs. A report in the scientific literature indicating that the intramuscular route of inoculation was at least 100 times more effective in producing a satisfactory immune response precipitated the study.

The rabies vaccine study is now complete. Testing for this segment of the study was performed by two independent laboratories using different test methods. The mouse protection test (MPT) was conducted by Dr. Howard J. Koonse, Fort Dodge Laboratories, Fort Dodge, Iowa, and the Rabies Fluorescent Focus Inhibition Test (RFFIT) by Dr. George M. Baer, Center for Disease Control, Lawrenceville, Georgia.

The accumulated data shows that vaccine application with the jet-injector elicited immune responses equal to or better than that obtained with needle and syringe. These results are summarized in Table 1 and Table 2.

Table 1. Serologic Response--RFFIT.

Method of Administration	Day Post Vaccination 28	360
Needle - Syringe	8/10 (1:250)*	6/7 (1:10)
Jet - Injector	9/10 (1:250)	7/9 (1:14)

*Number of responders/total tested with an antibody titer at dilution specified.

Table 2. Serologic Response - MPT

Method of Administration	Day Post Vaccination 28	180
Needle - Syringe	3/10 (1:100)	4/9 (1:5)
	3/10 (1:50)	5/9 (<1:5)
	4/10 (1:10)	
Jet-Injector	4/11 (1:200)	7/9 (1:6)
	1/11 (1:50)	2/9 (<1:5)
	6/11 (1:8)	

The technical assessment of the jet injection equipment has been completed. Based on this information, the necessary documents to establish a military requirement for the development of this item as military equipment have been produced. A "Joint Working Group" representing the various agencies of the Department of the Army involved in the evaluation and assessment of the study convened on 27 July 1977 to review the status of the jet injection apparatus. Subsequent to this meeting, Department of the Army "in-house" product testing commenced.

A final scientific report was forwarded to Headquarters, Department of the Army, Surgeon General--Research and Development Command, Washington, D. C., in February 1978. This consisted of two bound copies of a completed dissertation by Dr. H. W. Whitford entitled "The Evaluation of Jet Injection for Use in Veterinary Medicine". Additional copies of this dissertation were forwarded to Mr. Aaron Ismach, Chief, Engineering Division, U. S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Maryland.

Summary of Accomplishments

1. The study demonstrated that the jet-injector equipment presently in use by the U. S. Department of Defense for immunizing personnel can be readily adapted for use in all species of animals held under intensified management conditions. We have shown the present engineering design of the equipment and all component parts to be ideally suited to meet this prime objective.
2. One Doctor of Philosophy degree and one Master of Science degree evolved from studies conducted under this contract.
3. Two scientific manuscripts, three presentations at scientific meetings and numerous field demonstrations at national and international levels evolved from this study.
4. The equipment was shown to be useful for administering biologics, pharmaceuticals, antibiotics, and nutritional supplements. In addition, the potential for tuberculosis testing of livestock via intradermal injection was demonstrated.
5. Selected advantages of this equipment are (a) potential for eliminating infectious agent spreading via contaminated needles, (b) elimination of sterile abscess losses in livestock and other animal species and (c) speed, economy and effectiveness.
6. Negative Aspect of Study--the inability to price and deliver equipment to interested parties. There is an urgent need for a well planned and financed promotional endeavor to expedite the use of this equipment in disease control and eradication programs.

THE EVALUATION OF JET INJECTION FOR USE IN VETERINARY MEDICINE

A Dissertation

by

HOWARD WAYNE WHITFORD

Submitted to the Graduate College of
Texas A&M University
in partial fulfillment of the requirement for the degree of

DOCTOR OF PHILOSOPHY

May 1976

Major Subject: Veterinary Microbiology

ERRATA

Page 151, paragraph 2, line 12 should read "about 16% of expected delivery."

Page 151, paragraph 2, line 14 should read "injector was accurate to within 5% of the calculated dose for 1 and 2 ml delivery."

Page 161, line 17 should read "infection, malignancy, and iron deficiency.[50] Since none"

Page 162, paragraph 1, line 3 should read "where a discernible pattern could be ascertained."

Page 162, paragraph 2, line 12 should read "an initial lymphopenia with a concurrent neutrophilia"

Page 167, paragraph 1, line 7 should read "VEE developed virus titers ranging from $10^{3.7}$ to $10^{4.8}$.

THE EVALUATION OF JET INJECTION FOR USE IN VETERINARY MEDICINE

A Dissertation

by

HOWARD WAYNE WHITFORD

Approved as to style and content by:

(Chairman of Committee)

(Head of Department)

(Member)

(Member)

(Member)

(Member)

(Member)

May 1976

ABSTRACT

The Evaluation of Jet Injection for Use in Veterinary Medicine (May, 1976)

Howard Wayne Whitford, DVM,

Texas A&M University

Directed by: Dr. Stewart McConnell

The purpose of this study was to evaluate the jet injection technique for use in veterinary medicine, and attempt to show that jet injection was equal to, or superior to conventional needle and syringe injections.

The dose delivery characteristics of the prototype jet injector were determined and statistically evaluated. Jet injected dye penetration studies were conducted in several species of domestic mammals, also in chickens and fish. The jet injector with modifications designed for use in animals was tested and found to be superior to the original equipment as designed for use in humans. Several adjunct studies i.e. intra-articular injections, nerve block anesthesia, permanent identification, and adjuvant injections were made and evaluated as to efficacy.

Several experiments were conducted to compare jet injection with needle and syringe injection. Radiopaque dye, modified live virus vaccine, and virulent VEE virus were the inoculums used. Radiographic studies in dogs

highlighted the similarities and differences of jet injected vs needle and syringe injected radiopaque dye. Modified live virus injections were used to compare the antibody response between jet injected and needle and syringe injected calves and dogs. Virulent VEE virus studies in dogs, calves, pigs, sheep, and goats were done to compare clinical response, hematological response, viremia patterns, and antibody response to the different modes of injection. All studies showed that there were no significant differences between the methods of injection. The role of these domestic animals as possible reservoirs or amplifiers of VEE epidemics was also investigated. Only dogs and young pigs were found to be good potential sources for vector infection based on magnitude of viremia obtained.

Additionally, field studies in dogs and calves were done to evaluate the use of jet injection for immunizing large numbers of animals. Mechanically the jet injector was shown to function well under field conditions. Serologically, dogs had excellent seroconversion rates to VEE vaccine whereas calves had a low seroconversion rate to IBR vaccination.

ACKNOWLEDGEMENTS

This study was made possible through grants from the U. S. Army Research and Development contracts in cooperation with the Department of Veterinary Microbiology, College of Veterinary Medicine, Texas A&M University, College Station, Texas.

The author wishes to thank Dr. Stewart McConnell, whose support and encouragement over the past 10 years are largely responsible for this and continuing studies related to jet injection in veterinary medicine.

To my committee members: Drs. R. J. Hidalgo, C. F. Hall, C. W. Boyd, R. S. Halliwell, and graduate college representative, Dr. L. M. Pike, I extend grateful appreciation for their time, effort, and constructive criticisms in helping to prepare this dissertation.

Appreciation is also extended to Dr. L. C. Grumbles, Head, Department of Veterinary Microbiology, College of Veterinary Medicine, Texas A&M University, for his continuing support of this project and his personal interest in the author's graduate training program.

The author wishes to extend special thanks to Dr. Stan Harris and to his wife Billie whose assistance, friendship, and encouragement are greatly appreciated.

For their help in furnishing animals and collecting serum samples the author thanks Drs. John Coleman,

Winslow Sheldon, Dan Hightower, Wayne Moore, and Tom Galvin.

In appreciation for technical assistance the author wishes to thank Mrs. Kim Bennett, Mrs. Lynda Kidd, Mrs. Marilyn Magourik, Mrs. Georgia Cummings, and Mr. Chuck Gasaway.

To my family, Peggy, Jeffrey, and Darci, special recognition and appreciation for their continued support, encouragement and self-sacrifice.

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INTRODUCTION

The prevention and control of disease in animal populations is a primary task of the veterinarian. Infectious diseases have best been prevented by parenteral immunizations against the specific disease causing agents. Disease control often involves parenteral administration of therapeutic drugs. As the demand for food animal protein increases and as pet animal populations increase, technical advances into more advantageous methods of drug administration should be explored.

In 1853, Alexander Wood perfected a hollow needle and adapted it to an improved Ferguson syringe. Since that time parenteral administration of drugs and vaccines has been traditionally administered into living tissue by means of the needle and syringe[51]. Historically, this method of drug administration has proven to be very effective. However, as a tool for mass inoculation of a population, the needle and syringe has several distinct disadvantages. The major disadvantage is the cost of the system in terms of money, time, labor and equipment. An excerpt from a paper by Hingson[30] illustrated this point:

The citations of the following pages follow the style of the American Journal of Veterinary Research.

> In Cleveland, Ohio-a city of one million population-a team of 75 people working around the clock on eight-hour shifts was required to process, clean, match, replace broken and missing parts, sterilize and package equipment during the eight week period. More than 300,000 persons were inoculated. More than 30,000 syringes were required at a cost of $2.00 to $3.50 each. During this program, almost 6,000 syringes and 12,000 needles were either broken or lost for a net loss of more than $20,000; a considerably larger initial capital investment added substantially to this figure.

The time and labor required to clean, sterilize and package needles and syringes has largely been eliminated with the increased use of pre-packaged, sterile disposable units. Time and effort are still involved in unpackaging the units, filling syringes, disinfecting injection sites, inserting the needle, aspirating for blood, administering the injection, and disposing of the used unit, in addition to the increased equipment cost. There is also a pain factor associated with administration of parenterals with the needle and syringe.

In human medicine, many of the problems associated with mass injections administered by the conventional method have been solved by the advent of a needleless, multiple dose delivery device--the jet injector.

The objectives of this study were to evaluate the applications of the technique of jet injection for use in veterinary medicine by 1) determining the physical

characteristics of and possible modifications needed for use in domestic animals, 2) comparison of the biological interactions involved between jet and needle and syringe injected material and recipient, and 3) the practical applications of the jet injection.

LITERATURE REVIEW

Basically, the jet injector operates on the principle of forcing a liquid through a small orifice with sufficient pressure to cause penetration of the skin with subsequent deposition of the liquid in the underlying tissues. Interestingly, the concept of jet injection post-dates the invention of the needle and syringe by only 13 years. In 1866, a French physician by the name of Galante developed and demonstrated a method of parenteral injection called "aquapuncture".[7] The technique involved the use of a syringe and a platinum plate through which a minute orifice had been drilled. The operator was able to exert considerable pressure on the liquid in the syringe which in turn was forced through the hole in the plate and into the tissues of the recipient against whom the plate was held.[32] M. Guerart invented a special instrument for the introduction of a number of fine streams of water simultaneously. This technique was described in Bartholow's Manual of Hypodermic Medication, dated 1879, and was termed "douches filiformes".[4] An excerpt from the book describes the therapy:

> The immediate effect of the sudden introduction of a fine jet of water was a sense of burning, which lasts a few minutes, a feeling of distention and warmth lasting longer, for about the point of puncture considerable swelling takes place, presenting the appearance of a wheal of urticaria. The immediate effect of the introduction of

> cold water is to cool the nerve filaments; of hot water to raise their temperature, and the distention of the parts stretches the nerve filaments. Undoubtedly, therapeutical effects are produced by such impression.

Although other reports relative to needleless injections appeared in the literature in the late 1800's and early 1900's,[32] it was not until the mid-1930's that the forerunner of modern day jet injection was invented.

Arnold Sutermeister, a mechanical engineer, first noticed the effects of accidental injections of diesel oil into individuals exposed to small breaks in high pressure lines. Subsequently, in 1933, he and a surgery instructor, Dr. John F. Roberts, corroborated to develop a workable jet injection device. They experimentally injected methylene blue dye into a variety of cadavers and living animals. However, they abandoned their studies due to inaccuracies in the dose regulating mechanisms in the instrument.[27,32]

In 1936, another engineer, Marshall Lockhart, designed and patented an instrument for jet injection,[a] which he subsequently sold to three medical supply companies (E. R. Squibb, Becton Dickinson, Co., and R. P. Scherer Corp.). Further development and testing of the instrument by F. H. J. Figge of the Scherer

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[a]M. L. Lockhart, U. S. Patent Application #69, 119, March 16, 1936.

Corp. resulted in a report by Figge to the American Association of Anatomists in April, 1947.[3,21] This instrument was initially called a "Sub-Q-Jet", however in a report by Hingson and Hughes[33] which followed Figge's initial report, the name of the instrument was changed to the "Hypospray" jet injector. The Hypospray was an instrument about the size of a two-celled flashlight. The power source consisted of a spring which was cocked by rotating a sleeve and could deliver pressures up to 250 kg/sq cm [3500 lbs/sq in (psi)]. This high pressure then forced the injectable liquid through a capillary hole 75 microns (u) (0.003 inch) in diameter. The material to be injected was contained in a prefilled, rubber stoppered, bullet shaped "metapule" (a metal ampule) with the orifice at one end. The metapule was clamped into the Hypospray and when the spring was released, it activated a plunger which depressed the rubber stopper in the metapule thereby forcing the material out of the orifice.[33]

Between 1947 and 1955 several papers appeared concerning the Hypospray jet injector. The majority of these papers reported improvements or new data supporting its use. In 1948, Figge and Barnett[20] reported that they could successfully inject aqueous solutions, colloidal suspensions, oil solutions, emulsions, and metallic mercury.

They also investigated the penetration and dispersion pattern of injected radiopaque material. They determined the rate of absorption of jet injected material, compared the results to the rate of absorption of needle and syringe injected material, and found the difference to be negligible. They found that an injector equipped with a spring rated at 60 kg/sq cm (125 psi) would exert a pressure of 274 kg/sq cm (3900 psi) in a metapule with a diameter of 0.513 cm (0.2 inch). However, the pressure of a stream of liquid as it left an orifice diameter of 75 u (0.003 inch) would only exert a pressure of 11 gm (0.024 lbs) on the tissues. This rather small amount of pressure minimized trauma to the underlying tissues of the recipient. Additionally, Figge and Barnett showed that, depending on the skin thickness at the site of injection, materials could be injected intramuscularly (IM) into tissues covered by thin skin and subcutaneously (SC) into tissues covered by thicker skin. When he deliberately tried to inject material intravenously (IV), he found that the probability of IV penetration was less than that expected by needle and syringe injection.

Further developments in jet injection reported circa 1948 included use of the Hypospray jet injector for administration of penicillin to treat patients with

gonorrhea[31] and administration of procaine to patients with leprosy for relief of pain caused by the disease.[34] Both procedures were shown to be equal to the needle and syringe in effectiveness. Another report dealt with the administration of penicillin and streptomycin via jet injector compared to administration by needle and syringe.[35] Penicillin was not as rapidly absorbed when administered by jet injection and this was attributed to the fact that part of the inoculum was deposited SC. The toxicity or undesirable side effects seen with administration of antibotics included induration, small hematomas, and small subcutaneous nodules, all in relation to the injection site. Additionally, since aqueous solutions of penicillin and streptomycin were used, stability of these antibiotics stored in ampules was relatively short-lived.

The progress of jet injection was summarized in a 1948 paper by Hughes et al.[36] This report also included the first use of jet injection for administration of immunogens (diphtheria and tetanus toxoids). The authors found that toxin neutralizing antibody levels following toxoid administration via jet injection were comparable to the levels obtained using a syringe and needle for administration. Also, they found that a suspension of bacteria was still viable after it had been fired through the jet injector. This indicated that the instrument

could be used to administer live attenuated bacterial vaccines without diminishing the bacterial titer.

In 1949, typhoid bacterin was administered by jet injection, and when compared to needle and syringe administration, was found to give a slightly higher agglutinin titer.[5]

Other techniques of interest which supported the use of the Hypospray jet injector included jet injection of radioisotopes[6,17] and intra-articular injection of cortisone.[62] Jet injected radioisotopes were found to disappear more uniformly from the tissues than did needle and syringe injected material. Also, the jet injected radioisotopes tended to diffuse into the tissues whereas needle and syringe injected radioisotopes pooled in the tissues. When cortisone was injected intra-articularly into patients suffering from rheumatoid arthritis, 66% reported relief of pain with no complications.

Two papers were published which did not completely support the use of the Hypospray jet injection in human medicine. In 1949, Brown[11] deliberately attempted to introduce jet injected inoculum into the veins of anesthetized dogs. He concluded that the probability of accidental intravenous injection was small unless the injection was made over a major vessel. Later, in 1954,

a paper appeared by Coon et al[14] which was decidedly anti-jet injection. The authors used an experimental prototype jet injector called a Velodermic syringe which was supplied by Becton, Dickinson and Co. They injected shaved rabbits and made the following conclusions: 1) Compared to needle and syringe, the jet injected material was absorbed equally. 2) Extensive tissue damage in the form of hemorrhage, muscle necrosis, nerve injury, inflammation, and foreign body reactions was seen in animals receiving inoculum by jet injection. 3) The jet injector was unsafe and should not be used in a clinical situation. This position was readily attacked by Warren et al[58] who stated:

> These authors (Coon et al) only experimented on the rabbit, whose cutaneous tissues are less resistant to penetration than those of a man, and high incidence of jet lacerations, 9 of 32 injections, has no parallel in any reported human studies and is probably due to twitching or tremors of the rabbit skin.

Prior to 1955, however, information concerning the Hypospray jet injector indicated that it had certain inherent advantages over the needle and syringe. These included: 1) elimination of sterilizing needles and syringes, 2) elimination of the chance of transmission of viral agents such as hepatitis virus from an infected individual to a susceptible one due to subsequent injections, 3) reduced injection time, and 4) from a

strictly human standpoint, a decreased pain factor and lack of psychological aversion as was experienced with needle injections.[20,32,33] The Hypospray jet injector was not without its disadvantages which included: 1) initial high cost of the instrument and high cost of the pre-filled metapules, 2) maximum dosage of only 1 cc, 3) the necessity to develop different metallic containers for different active drugs, 4) the possible injury to tissues due to high pressure jet injections, 5) the necessity of using less viscous materials for certain medicaments so that the solution would pass freely through the metapule orifice, and 6) the mechanical difficulty of maintaining a more technically complicated instrument in operation.[32]

The most significant breakthrough for jet injection occurred in 1955 with the development by the Army Medical Graduate School of an automatic, multiple-dose jet injector.[58] This machine differed significantly from the old Hypospray and offered several additional advantages. Although both systems utilized a spring as the power source, the multiple dose injector was cocked by a hydraulic pump, and the number of doses that could be delivered in sequence was limited only by the capacity of the inoculum container. Since this eliminated the use of pre-filled single dose containers, considerable time and cost was saved. With only minor modifications in

design, the automatic multiple dose injector described by Warren et al [58] is the same device that is currently in use for mass inoculation of human populations.

Recognizing the fact that jet injection has been tried successfully in a diversity of ways, such as in pediatric medicine[37] and in dental anesthesia,[52] probably the most beneficial use of the jet injector in human medicine has been the mass immunization of persons against a variety of bacterial and viral diseases. Some of the bacterial vaccines which have been successfully used in human populations are typhoid-paratyphoid and diphtheria-tetanus toxoid,[8] BCG tuberculosis vaccine,[23,55] cholera, and bubonic plague bacterins.[30] Another report deals with the experimental use of an anthrax spore vaccine which was jet injected into guinea pigs.[49] The viral vaccines which have been shown to be effective when administered by jet injection are polio,[29,40] influenza,[2,15] and smallpox.[1,18,43,45,47] Myer et al successfully jet injected a combination of modified live measles, smallpox, and yellow fever viruses into a population of Volta children.[42]

Other uses for jet injection which have been investigated and found to be feasible in human medicine are skin testing and hormone therapy. Skin test antigens which have been used are tuberculin,[25] coccidioidin,[22]

and histoplasmin.[44] Ismach[25] designed a special intradermal (ID) nozzle which deposited the injected material directly into the dermis and was used for intradermal skin testing. In patients suffering from diabetes mellitus, insulin therapy has been shown to be more desirable when given by jet injection because of the reduced pain and ease of administration.[59] Experimentally, rhesus monkeys have been given insulin therapy by jet injection with equal effectiveness when compared to needle and syringe administered insulin.[13] The jet injection of medroxyprogesterone acetate into rats has been reported by Welty et al[60] in their attempt to find an animal model for the study of contraception in women.

Compared to the conventional needle and syringe administration of parenteral drugs, certain advantages and disadvantages concerning jet injection became evident. Advantages are best summarized by Hingson et al[30] as follows: 1) Since it has been shown that blood contamination was essentially negative (<15 gammas of human serum) on the jet nozzle following 762 injections,[8] transmission of blood borne diseases such as malaria and hepatitis were very unlikely when serial jet injections were given to a group of individuals. 2) The inoculum entered the skin through a micro-orifice much smaller than a needle tract and thus contamination

by skin bacteria was reduced, plus the probability that such bacteria would be pulverized by the impact of the inoculum. 3) Sterilization was not required between inoculations. 4) Jet injections were relatively safe, and accidental intravenous injections were extremely unlikely.[11] 5) The inoculum was not exposed to the external environment or to the operator and contamination of the inoculum or the instrument was impossible when the machine was properly assembled. 6) Time, labor and equipment costs were reduced because there were no syringes to unpack or fill, no needles to be kept sharp or sterile, no need to aspirate prior to injection, and no used syringes to discard. Also, time of actual inoculation was reduced to less than one second and it was reported that as many as 1,400 military personnel had been vaccinated in one hour.[8] 7) Pain was reduced because the jet stream was so fine that fewer pain fibers were stimulated than with the finest needle. Trauma to the skin was reduced to a minimum. 8) Dose wastage was reduced; the delivery was within 4-5% of the dose.

Disadvantages, cited in the same report[30] are: 1) There was about 10% incidence of minimal bleeding; in an additional 12-15% of the patients a tiny drop of blood appeared at the site of puncture. In only 3% of the patients did a fine trickle of blood develop. These

instances were easily controlled by application of cotton compresses or adhesive bandages. 2) Ecchymotic hemorrhages due to capillary damage occurred occasionally at 24 to 36 hours following injection. 3) Movement by the recipient resulted in a superficial intradermal cut or zigzag ecchymosis. 4) An occasional patient experienced considerable momentary pain from the injection which was thought to be due to directly hitting a nerve fibril with the jet stream. However, this occurred less frequently than with the needle and syringe.

One report appeared in the literature which implicated jet injection as the cause of a fatal staphylococcal pneumonia in a child who received a routine measles vaccination by jet injector.[38] However, it was determined at autopsy that the child was hypogammaglobulinemic and this may have accounted for the resultant overwhelming infection.

The preponderance of information concerning jet injection has shown that it is a valuable tool in human medicine, especially in the field of disease control, prevention, and therapy involving large populations. A search of the medical literature has yielded very little published information of the application of the technique to veterinary medicine[9] and aquatic medicine[24] other than for research purposes as related to uses in human

medicine.[13,49,60] Harris[b] used the jet injector to inject dogs and calves with virulent VEE virus. The author[61] has previously reported some preliminary findings in the use of jet injections in animals. McKercher has vaccinated swine with foot-and-mouth disease vaccine using the jet injector.[c] McConnell used the jet injector to administer Venezuelan equine encaphalitis vaccine to equines in South America during the spread of the epizootic in the late 1960's.[61]

The administration of parenteral materials to domestic animals by jet injector could become an invaluable tool in at least three general areas at the present time. 1) As society's demand for meat protein increases, mass production methods will become increasingly necessary in order to meet the demand. This is already true with poultry and egg production and is rapidly becoming the method of choice for beef production and to a lesser extent for lamb and fish production. Mass production requires large numbers of animals to be concentrated in a defined area and fed concentrated rations. This

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[b]Harris, S. K.: The Role of Domestic Animals in the Epidemiology of Venezuelan Equine Encephalomyelitis. Masters Thesis, Texas A&M University, College Station, Tx. 1973.

[c]Ismach, A., Chief, Engineering Div., U.S. Army Bioengineering Research and Development Laboratory, Ft. Detrick, Md.: Personal communication, 1971.

concentration creates inherent health problems associated with overcrowding, stress, and population dynamics, thus creating a demand for highly sophisticated vaccination and disease control programs. 2) Mass vaccination of animals in the face of an epizootic disease outbreak is necessary to quickly establish an immune population and help control the spread of the disease. The 1971 epizootic of Venezuelan equine encephalitis in the equine population in Texas is an example of an epizootic disease which was controlled by mass immunization of the equine population. 3) Routine immunizations of pet populations to control zoonotic diseases such as rabies could be carried out more efficiently by using jet injection.[9]

MATERIALS AND METHODS

Physical Characteristics of the Jet Injector

Description of the Jet Injector Prototype

The Ped-O-Jet, Model POJ,[d] jet injector used throughout the study. This machine is a foot operated, hydraulically cocked, multiple dose, hypodermic jet inoculator (Fig 1 and 2). Basic equipment with a variety of components included: 1) Nozzles--human design (uncrowned) and nozzles designed for use in animals (crowned) (Fig 3). 2) Orifice diameters[e] of 125u (0.005 inch), 150u (0.006 inch), 175u (0.007 inch), 200u (0.008 inch), 225u (0.009 inch) in the uncrowned nozzles; 125u, 225u, 250u (0.01 inch), 275u (0.011 inch), and 300u (0.012 inch) in the animal adapted crowned nozzles. 3) Intensifier (Fig 4). 4) Three spring sizes rated at 17.6 kg/sq cm (250 psi), 25 kg/sq cm (350 psi), and 42.2 kg/sq cm (600 psi) which interchange in the intensifier. 5) One ml and 2 ml capacity barrels (Fig 4).

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[d]Manufactured by Scientific Equipment Mfg Co (SEMCO) under Z&W Mfg Co and A. Ismach patents.

[e]For purposes of brevity, orifice diameters will be henceforth referred to as #5 (0.005 inch=125u), #6 (0.006 inch=150u), #7 (0.007 inch=175u), #8 (0.008 inch=200u), #9 (0.009 inch=225u), #10 (0.01 inch=250u), #11 (0.011 inch=275u), and #12 (0.012 inch=300u).

Fig 1--The jet injector in its carrying case.

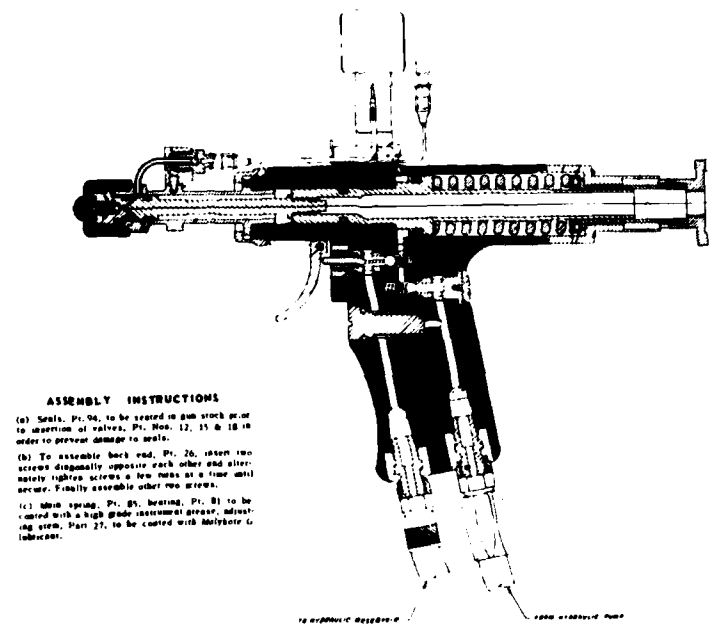

Fig 2--Schematic of the jet injector gun showing the mechanical aspects of operation.

Fig 3--Comparison of crowned nozzles for animal injections and uncrowned nozzles for human injections.

Fig 4--Various components of the jet injector showing the two dosage barrels, the intensifier, inoculum holder, and feed needle.

Animals

A variety of animal species were used in this phase of the study and represented the following: canine, bovine, equine, porcine, ovine, caprine, galline, and piscine randomly selected from available sources.

Dogs. A total of 7 dogs were used in the study. The dogs were mixed breeds and sexes ranging in weight from 4.5 kg (10 lbs) to 20 kg (45 lbs).

Cattle. A total of 3 calves were injected. Two were Angus calves weighing approximately 202 kg (450 lbs) and 270 kg (600 lbs); the other was a crossbred calf weighing approximately 90 kg (200 lbs).

Horses and mules. Three horses were used; 1 was a Shetland weighing approximately 180 kg (400 lbs), the other 2 were adult quarter horses weighing approximately 450 kg (1000 lbs). The mule was an adult male weighing approximately 560 kg (1250 lbs).

Pigs. Two 67 kg (150 lbs) Duroc boars were injected.

Sheep. Three adult sheep; 2 Southdown and 1 Ramboullet weighing between 27 kg (60 lbs) and 54 kg (120 lbs) were used.

Goats. Two adult Angora goats were injected. They weighed approximately 22 kg (50 lbs).

Chickens. A total of eight 6 to 7 week old cockerels were injected.

Fish. Five catfish were injected. Three were young

fry approximately 15 cm (6 inches) long and 2 were approximately 0.3 kg (0.75 lbs) and 30 cm (12 inches) long.

Inoculums

Sterile glass distilled water was used to determine dose delivery characteristics of the jet injector.

Methylene blue dye (1% or 0.1%) was used for penetration depth and dispersion of inoculum studies. Preparation of a stock solution of the dye was made by adding 1 gm of powdered methylene blue[f] to 100 ml of sterile water. Working solutions were made by adding 1 part stock solution to 9 parts of sterile water.

Diodrast,[g] a radiopaque dye, was used for radiographic studies.

India ink[h] diluted 1:10 with sterile water was used for permanent identification studies in the horse.

An adjuvant-virus mixture for injection into a sheep was prepared as follows: a water-in-mineral oil adjuvant described by Herbert[26] was mixed with tobacco mosaic

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[f]Methylene Blue, Matheson Coleman and Bell Chemical Co., Norwood, Ohio and East Rutherford, NJ

[g]Diodrast, Brand of Iodopyracet, Winthrop Laboratories, New York, NY

[h]Pelikan Black Waterproof Drawing Ink, Gunther Wagner, Germany.

virus (TMV)[i] by adding 1 part adjuvant to 1 part antigen.

Procedure

Jet injector operation. The procedure for operation of the jet injector was outlined in the instruction manual provided by the manufacturer and followed throughout the study.

Dose delivery characteristics. The quantity of diluent delivered to each setting of the jet injector, i.e. 0.5, 1, or 2 ml, was measured using an analytical balance.[j] Each dose fired into the pre-tared beaker was carefully weighed and recorded. A standardized system consisting of sterile distilled water, a sterile 30 ml vaccine bottle with a rubber stopper and a 100 ml beaker containing gauze or absorbent paper was used throughout the study. Adjustments to the dosage setting at the rear of the injector gun were made until the desired dosage was consistently within acceptable limits. Final tests were conducted in replicate series of 10 consecutive injections for each dosage delivered. The resultant data were recorded and statistically evaluated according to mean and range for each dosage.

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[i] Supplied by Dr. R. S. Halliwell, Professor, Plant Sciences Department, Texas A&M University, College Station, Tx. Dilution end point 1:1024.

[j] Mettler Instrument Corporation. Mettler Analytical Balance, Model #HGT Digital, Highstown, NJ

Penetration characteristics of dye in various species of animals. Methylene blue dye was injected into animals of each species represented in the study. Several injections were made into each animal at different locations. By interchanging various components adapted for the jet injector and varying the sites of injection, ease of penetration of the dye was established. Following the injections, depth of penetration and localization of the inoculum were established by dissecting the site of injection. At least one animal from each species tested was euthanized and examined by the dissection method.

In a second study, non-terminal animals (two calves and one pig) were injected with methylene blue dye at various sites and the results were tabulated in terms of (1) ease of penetration of the dye and (2) amount of residual dye remaining on the external skin surface following injection. In the calves extracellular dye was measured by blotting the site with absorbent paper toweling and measuring the diameter of the spot formed on the paper.

Radiographic comparison of jet injection vs. needle and syringe injection. Two dogs were given radiopaque dye injections by jet injection and needle and syringe injection to compare by radiographic methods, penetration and localization characteristics of both modes of injection. The procedure involved the jet injection of either 1 ml

or 2 ml amounts of Diodrast, a radiopaque dye. Adjacent to the jet injected site, a similar amount of the dye was injected with needle and syringe, and the animal radiographed as soon as possible after injection.

Adjunct studies. Epidural caudal nerve block anesthesia: Methylene blue dye was injected between the 1st and 2nd coccygeal vertebra in the horse and goat.[48] Following injection, the area was dissected and localization of the dye was determined.

Intra-articular injections: Injections were made over the carpal joint and hock joint of the horse and goat. The animals were euthanized and the joints examined for presence of dye.

Skin testing: The caudal fold of the cow was injected with 0.2 ml methylene blue dye using the ID nozzle. The area was then examined for ID localization of dye.

Permanent identification in horses: India ink was injected into the mucosal surface of the upper lip of a horse (adult gelding). The amount of inoculum was 0.2 ml and the ID nozzle was used.

Adjuvants: A sheep was injected once every other week for 8 weeks with 10 ml of TMV:water-in-oil adjuvant mixture. The injections were made in 1 ml amounts using the crowned #9 nozzle with intensifier. Multiple injections were given in the musculature at different

sites on the animal (hip, neck, shoulder) until the inoculum had been completed injected. On weeks when adjuvant-virus mixtures were not given, a single injection of 0.1 ml of virus in aqueous diluent was given ID with a 1 cc syringe and 25 ga needle.

Laboratory Studies Comparing Jet Injection with Needle and Syringe Injection using Vaccine and Virulent Virus Models

Vaccine Virus Studies

Dogs. Sixteen adult beagle dogs[k] were divided into 2 groups of 8 dogs each. One group was given modified live virus Venezuelan Equine Encephalomyelitis (VEE) vaccine[1] via jet injector equipped with 1 ml dose barrel, #9 crowned nozzle and intensifier. The other group received 1 ml VEE vaccine by needle and syringe. Blood samples were drawn and serum collected immediately prior to, and 35 days after vaccination. Additionally, whole blood was drawn on days 2 through 5 post vaccination.

Antibody determinations for serum neutralizing (SN) and hemagglutination-inhibition (HI) antibodies were performed as follows:

1) SN antibody determination was made in a monkey

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[k]Supplied by Dr. T. H. Galvin, Veterinary Parasitology Department, College of Veterinary Medicine, Texas A&M University, College Station, Tx.

[1]Jen-Sal Encephalovac, Jensen Salsbery Laboratories, Kansas City, Mo.

kidney (VERO) continuous cell line[m] prepared in the following manner:

Stock cell cultures were grown in 32 oz prescription bottles by adding 30 ml suspension of cells containing about 1.5 X 10^6 cells per ml of growth medium. Growth medium consisted of 2% fetal bovine serum (FBS), 3% calf serum (CS), 1% non-essential amino acids (NEAA), 1% penicillin/streptomycin[n] (p/s), and 0.5% Hepes buffer in commercially prepared minimal essential medium (MEM). After 3 days growth at 37°C or when a confluent cell sheet was attained, cells were sub-cultured. Medium was poured off the cells, 5 ml trypsin solution was added, cells monolayers were washed, and the excess trypsin decanted. Five to seven ml of fresh trypsin were added and allowed to stand until cells began to detach. The excess trypsin was poured off, the cells washed from the glass by flushing with 20 to 30 ml growth medium using a needle and syringe. Cells were drawn into the syringe and forced out several times to allow for complete dispersion of cells. Medium was added to the cell-medium mixture to make a 3:1 split ratio. Usually 2 or 3 seed bottles (32 oz) were

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[m]Supplied by Dr. Stewart McConnell, Department of Veterinary Microbiology, Texas A&M University, College Station, Tx.

[n]Stock solution contained 100,000 units penicillin and 0.5 gm streptomycin per ml.

harvested at once giving 180 to 270 ml of cells in suspension. After preparing stock cultures in 32 oz bottles by adding 30 ml to each bottle, the remainder of the cell suspension was used to seed test tubes[o] by adding 2 ml of cell suspension to each tube. Cells in the tubes became confluent in 3 to 4 days and were suitable for use in determination of SN antibodies.

The procedure for determination of VEE SN antibody was as follows: Pre- and post-vaccination serum samples were diluted 1:5 with phosphate buffered saline (PBS) containing 1% p/s. Equal volumes of 1:5 serum was mixed with 10^2 median cell culture infectious doses ($CCID_{50}$) of VEE vaccine virus[p] and incubated 1 hour at 37 C. After decanting the fluid media from 5 tubes of VERO monkey kidney cell monolayers, 0.2 ml of serum virus mixture was added and adsorbed for 1 hour at 37 C. The cells were then fed with 2 ml of maintenance media consisting of MEM, 2% CS, 1% p/s, 1% NEAA, and 0.5% Hepes buffer. Cells were examined daily for 7 days for evidence of cytopathic effect (CPE). Serum was determined to be positive for neutralizing antibody if CPE was prevented or delayed during the 7 day examination period.

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[o]Kimax 16 ml x 150 mm screw top glass tubes, Kimble Products, Division of Owens Illinois, Toledo, Oh.

[p]Prepared by passing VEE vaccine (Encephalovac, Jen-Sal) once through VERO cells to increase virus titer to approximately 10^8 $CCID_{50}$.

2) The procedure for measuring the presence of HI antibody was a microtiter technique described by Casals and Clarke.[12] HI titers in pre-injection serum was compared to the HI titer in the 35 day serum. A rise in HI titer of twofold or greater was considered significant.

Attempts to isolate vaccine virus from blood samples drawn on days 2 through 5 post vaccination were done by the following method: Equal volumes of PBS containing 1% p/s were added to the clotted blood samples and agitated to break up the clots. After decanting the fluid medium from tubed monolayers of VERO monkey kidney cells, 0.2 ml of the blood sample was then added to each of 4 tubes and allowed to adsorb for 1 hour at 37 C. The cells were then fed with maintenance medium and incubated at 35 C. Cells were observed daily for 7 days for evidence of CPE.

Cattle. Thirty-seven mixed breed bull calves weighing 136 to 227 kg (300 to 500 lbs) and located at McGregor, Texas were divided into 3 groups and given IBR vaccine[q] as follows: Ten calves received 1 ml of vaccine reconstituted to ½ volume (2 ml usual dose) via jet injector equipped with 1 ml dose barrel, #9 crowned nozzle, and intensifier.

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[q]Bovine rhinotracheitis vaccine, modified live virus, bovine tissue culture origin, vacuum dried, Franklin Biological Laboratories, Amarillo, Tx.

Ten calves received 2 ml of IBR vaccine reconstituted to full volume via needle and syringe. Seventeen calves received 2 ml of IBR vaccine via jet injector equipped with 2 ml dose barrel, #9 crowned nozzle, and intensifier. Animals were bled and serum collected immediately prior to and 35 days after vaccination. Two weeks after vaccination the animals were transported to Bushland, Texas and placed in an experimental feedlot for the duration of the study.

Detection of SN antibody was done in a continuous bovine kidney cell line.[r] Stock cell cultures were grown in 32 oz prescription bottles by adding 1 to 1.5 x 10^6 cells suspended in 30 ml growth medium consisting of 5% CS, 1% p/s in MEM. Cells were harvested and distributed into tubes as previously described for VERO cells. Pre- and post-injection serums were screened for the presence of SN antibody according to a procedure described by Bitsch.[10] Each serum sample was inoculated into 5 tubes. Tubes were examined for 7 days for the presence or absence of CPE.

Virulent virus studies

A study was conducted to determine the possible

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[r]Heteroploid continuous bovine kidney cells established by Dr. Robert F. Kennedy, Columbia, Mo.

deleterious effects of jet injection on a live virulent antigen by comparing several biological parameters in 5 species of domestic animals which received virus by jet injection and by needle and syringe. Dogs, calves, pigs, sheep and goats were the domestic animals used in the study.

Dr. S. K. Harris and the author worked jointly on this study in which Dr. Harris reported on the role of dogs and calves in the epidemiology of virulent VEE virus.[b] The investigators shared the data collected from the work done in dogs and calves since the same animals, inoculum, and parameters were used in both the epidemiological investigation and the jet injector study.

The following protocol was used for all species tested:

Animals were confined in the test area (an enclosed isolation room) for 3 to 5 days before experimental data were recorded. The experimental procedure is given in Table 1. All animals were monitored for 7 days prior to virus exposure to establish a baseline for each parameter tested. On day 0 animals were divided into two groups. One-half of the animals received inoculum via jet injector equipped with either the #9 or #10 orifice crowned nozzle, 1 ml dose barrel, and intensifier. The other half of the animals received inoculum via a 3 ml syringe equipped with a 20 gauge, 1½ inch needle.

TABLE 1--Outline of Experimental Procedure for Virulent Virus Studies in Domestic Animals.

FUNCTION	DAY						
	-7 thru -1	0	1 thru 7	8 thru 13	14	15 thru 20	21
Temperature 8AM & 8PM	+	+	+	+	+	+	+
Blood Sample*	+	+	+		+	+	+
Virus Challenge*		+					
Necropsy					+**		+

*Collection of blood samples corresponded to time of challenge (8AM or 8PM).

**Two animals were necropsied on day 14, the remaining animals were necropsied on day 21.

Animals. All animals used were prebled before purchase and tested for evidence of VEE, western equine encephalitis (WEE) and eastern equine encephalitis (EEE) by the HI test. Table 2 gives a summary of the animals used.

Inoculum. Each animal received either 10^6 or 10^2 (8PM dogs and goats) $CCID_{50}$ per ml of the Texas strain of VEE virus.[s] The virus pool was made in VERO cells, standardized as to titer (10^8 $CCID_{50}$ per ml), and maintained at -70 C until needed. The virus was diluted in Hanks balanced salt solution (HBSS) so that 1 ml volume contained the desired amount of virus. Following injection of the inoculum, an aliquot was frozen at -70 C as soon as possible and back-titrated at a later time.

Parameters tested. As follows:

Clinical examinations. Following a 2 to 3 day acclimatization period, baseline values were gathered until day 0. Following virus challenge, animals were observed for an additional 21 days. Both visual observations as to attitude, appetite, excretory functions and rectal temperatures were recorded twice daily at

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[s]Isolated by Dr. Konrad Eugster, Texas Veterinary Medical Diagnostic Laboratory, College Station, Tx. from a horse killed during the 1971 VEE epidemic.

TABLE 2--Summary of Domestic Animals Used for Virulent VEE Virus Studies.

Animal	Number	Breed	Age	Source
Dogs	12	Beagle	6 mo.	LRE*
Cattle	4	Charolais	10 mo.	Bushland, Tex.**
Cattle	4	Hereford	8-10 mo.	Tunis, Tex.+
Swine	6	Duroc	3½ mo.	TAMU‡
Swine	6	Duroc	2 mo.	TAMU‡
Sheep	8	Rambouillet	6-10 mo.	San Angelo, Tex.§
Goats	12	Spanish Cross	6-10 mo.	San Angelo, Tex.§

*Purchased from Laboratory Resource Enterprises, Kalamazoo, Mi.

**Purchased from Texas Agricultural Experiment Station, Bushland, Tx.

+Purchased from Mr. Ed Mikeska, Tunis, Tx.

‡Supplied by Dr. R. W. Moore, Department of Veterinary Microbiology, College of Veterinary Medicine, Texas A&M University, College Station, Tx., from a hysterectomy derived, pathogen free herd.

§Purchased from the Texas Agricultural Experiment Station, San Angelo, Tx.

approximately 8AM and 8PM.

Hematological examinations. From blood samples collected daily at day -7 through day 7 and day 14 through day 21, the following hematological tests were performed: 1) Total and differential white blood cell (WBC's) counts from which absolute numbers of lymphocytes, polymorphonuclear leucocytes (neutrophiles), monocytes, eosinophiles and basophiles were calculated. 2) Platelet (thrombocyte) counts. 3) Packed cell volume (PCV). 4) Blood urea nitrogen (BUN). Procedures for conducting these tests were as follows:

1) Total WBC and platelet counts. For total counts, whole unclotted blood was collected in Unopette disposable pipettes #5855,[t] and immediately placed in the diluent container. A diluted sample was placed onto a Hausser[u] hemocytometer and WBC's and platelets counted according to instructions supplied by the manufacturer of the Unopette disposable diluting pipettes.

2) Differential leukocyte counts. The blood smears were prepared at the time of bleeding and allowed to air dry. The slides were then stained using Wampole's Gugol blue stain kit,[v] which utilized a polychromatic stain

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[t] Becton-Dickinson and Co., Rutherford, NJ.

[u] C. A. Hausser & Son, Philadelphia, Pa.

[v] Wampole Laboratories, Stamford, Ct.

for differential staining of WBC's. The counts were then made as time permitted using 930X magnification (oil immersion) and recorded as relative percent of the total WBC's. From the percentage, absolute numbers of lymphocytes, neutrophiles, monocytes, eosinophiles, and basophiles were calculated.

3) PCV. Blood was collected in heparinized micro-hematocrit tubes,[w] plugged with Sealease[w] clay, and placed in an Adams Autocrit[w] and centrifuged at approximately 14,500 x g for 5 minutes. The percentage of cells was read directly on a built-in revolving scale in the centrifuge.

4) BUN determination. Ten to 20 ml of whole blood were placed in tubes, allowed to clot, and serum collected. Two-tenths ml of this serum was placed in a vertical position in 10 X 75 mm glass culture tubes into which a Urograph strip[x] was inserted for 30 minutes. The BUN content was then read using a special caliper furnished with the kit. Readings were in mg of urea per 100 ml of blood.

Serological testing. Two milliliter aliquots of serum collected from bleedings on days -7, 0, 7, 14, and 21 were

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[w]Clay-Adams, Parsippany, NJ

[x]General Diagnostics Division, Warner-Chilcott Laboratories, Morris Plains, NJ

tested for HI and SN antibody against VEE antigen. The HI antibody levels were measured by the microtiter technique.[12] Serums which showed the presence of VEE antibodies were also tested for the presence of EEE and WEE antibodies using the respective antigens in the microtiter HI test.

Virological testing. Presence of virus was tested on serum samples collected on days 0 through 7 and on days 14 through 21. The suckling mouse intracerebral (SMIC) inoculation method was used to detect virus in the serum. Serum samples collected during the experiment were inoculated IC into litters of eight 48 to 96 hour old suckling mice.[y] Each suckling mouse received a dosage of 0.025 ml of undiluted serum. If death occurred within 24 hours in more than 2 mice, the serum was diluted 1:2 or 1:3 with diluent (phosphate buffered saline containing 0.75% bovine serum ablumin and 2% p/s) and retested. Those litters having 6 to 8 surviving mice after 24 hours were observed for 7 days and deaths recorded. During the observation period mice which died sporadically, i.e. 1 or 2 in a litter, and mice dying after day 5 were frozen and the brains subsequently

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[y]Charles River Mouse Farms, Inc., Wilmington, Ma. White Swiss Webster females (caesarean originated, barrier sustained) obtained as 13 day timed pregnant dams.

harvested for inoculation into weanling mice by the intraperitoneal route (IP) to detect the presence or absence of VEE virus. Serum samples from which all mice died on days 2 through 5 post inoculation were titered. Titration of serum samples positive for virus was done by making tenfold dilutions of virus infected serum from 10^{-1} through 10^{-5}. These dilutions were injected into litters of 8 suckling mice as previously described. Additionally, the serum sample from which viremia was first detected (usually day 1) and the serum sample collected on the last day of viremia, 1:5 dilutions of virus infected serum were mixed with an equal volume of VEE antiserum and incubated for 1 hour at 37 C along with 10^{-1} dilution of the same virus infected serum. The antiserum-virus mixture was inoculated into a litter of 8 suckling mice. Mice were observed for 7 days and deaths recorded. Virus titers were then calculated by the method of Reed and Muench.[46] Tissues collected at the time of necropsy were prepared in a 20% suspension and screened for the presence of VEE virus by the SMIC method previously described.

Backtitration of challenge inoculum. One-tenth ml quantities of log dilutions of the challenge inoculum were placed into tubes containing VERO cell monolayers from which the fluid medium had been decanted. Five

tubes were inoculated with each dilution. The virus inoculum was allowed to adsorb for one hour at 37 C then cells were fed with a maintenance medium previously described. Cells were observed for 7 days for the presence of CPE. Virus titer was then calculated as $CCID_{50}$ per ml by the method of Reed and Muench.[46]

Necropsy examination. Two animals, 1 from the jet injected group and 1 from the needle and syringe injected group were killed and necropsied on day 14 of the experiment; the remainder were killed and necropsied on the 21st day. Gross pathological examinations of the animals were made during the necropsy. Samples of spleen, lymph node, adrenal gland, spinal cord, and bone marrow were collected and frozen at -70 C for subsequent examination for the presence of residual virus. Samples of the tissues listed plus liver, lungs, brain, and kidney were collected from dogs, cattle and pigs and placed in 10% formalin for histopathological examination.

Field Studies

VEE Vaccination in Dogs

One hundred thirty-one adult beagle dogs[z] were

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[z]Furnished by Dr. Dan Hightower, Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Tx.

injected with modified live virus VEE vaccine.[1] The injections were made IM into the left hip area with the jet injector. The jet injector was equipped with the 1 ml capacity barrel, #9 crowned nozzle, and intensifier. Immediately prior to and 35 days after vaccination the dogs were bled and serum collected for SN and HI antibody determinations. The procedures for VEE antibody determinations were the same as previously described.

IBR Vaccination in Feedlot Cattle

Approximately 200 head of light feeder calves of mixed breeding and sex were vaccinated with IBR-leptospirosis vaccine.[aa] Each calf received 2 ml vaccine via jet injector equipped with the 2 ml dose barrel, #10 crowned nozzle, and intensifier. The site of injection was either the neck or the shoulder area. Twenty-five percent of the calves (50) were bled and serum harvested from samples collected immediately prior to and 35 days post vaccination for SN antibody determination. Serum neutralizing antibody was determined in a continuous BK cell line as previously described.

[aa]IBR-Lepticon, bovine rhinotracheitis vaccine, modified live virus, bovine tissue culture origin, Leptospira pomona bacterin, Anchor Serum Co., Division of Phillips Roxane, St. Joseph, Mo.

RESULTS

Physical Characteristics of the Jet Injector

Dose Delivery Characteristics

Initially, the desired dosage was selected according to the instruction manual by adjusting the dosage mechanism to correspond to the amount shown on the machined dosage gauge. After weighing the amount of inoculum, further adjustments were made. After the final adjustments were made, the exact volume delivered was determined for 0.5 ml, 1 ml, and 2 ml settings by measuring a series of 10 consecutive firings of the jet injector. These data and the statistical evaluation are presented (Table 3).

Depth of Penetration of Jet Injected Inoculum

Since the objective of the jet injection is to deliver an inoculum to a predetermined depth (SC, IM, or ID), extensive studies were performed to determine which components of the jet injector gave the most desirable skin penetration and deepest localization in each animal species. Factors affecting the depth of penetration were skin thickness at the inoculation site, use of the intensifier, orifice diameter, and dose to be injected. The animal species tested were the canine, bovine, equine, ovine, caprine, porcine, galline, and piscine. The

TABLE 3--Measurements of Dose Delivery.

Injection Number	Dosage Setting/Quantity Delivered 0.5 ml	1.0 ml	2.0 ml
1	0.51 gm	1.009 gm	2.07 gm
2	0.50	0.010	2.05
3	0.58	1.020	2.07
4	0.49	0.999	2.14
5	0.48	1.010	1.99
6	0.43	1.014	2.06
7	0.44	1.003	2.06
8	0.50	1.003	2.06
9	0.48	1.007	2.06
10	0.47	1.002	2.05
Mean =	0.488	1.008	2.06
Range =	0.43-0.58=0.15	0.999-1.014= 0.015	1.99-2.14= 0.15
$\frac{\Sigma(\bar{x}-x)^2}{df}$--(Variance)=$\sigma^2$=	0.00170	0.000037	0.0013
Std. Deviation (σ) =	0.0413	0.0061	0.036
95% Confidence Level (2σ)	0.0826	0.0122	0.072
95% of the time dosage will be:	0.488±0.0826 ml	1.008±0.0122ml	2.06±0.072 ml

optimal configurational set-up for maximal depth of penetration was determined for each animal species by varying the different components and injecting at different sites on the live, anesthetized or recently euthanized animal. Skin thickness as well as depth of penetration of inoculum was determined after the animal was euthanized. An illustration of one such study in the canine, comparing nozzle design and orifice diameter with maximal penetration of inoculum is shown (Fig 5,6,7, and 8).

The result of this study and others in the dog and equine clearly indicated that the uncrowned (human design) nozzle was not suitable for animal inoculations. For this reason the uncrowned nozzle was not subjected to further evaluation except in fish injections and ID injections in the horse and cow using the ID nozzle.

The overall results of testing the combinations of available components at several accessible injection sites gave the optimal configurational set-up for maximum depth of penetration at a given injection site on the animal. This was determined for both 1 ml and 2 ml dosages (Tables 4 and 5).

Only the 1 ml dose was tested in the chicken since size of the birds made higher doses impractical.

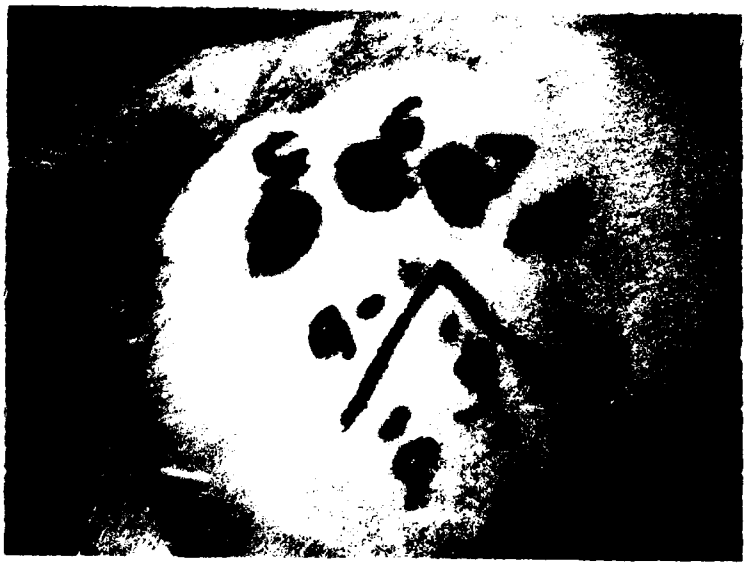


Fig 5--One ml jet injections made with crowned (below line) and uncrowned (above line) nozzles. Numbers 5 through 9 orifice diameters as labeled. Note residual dye on skin with injections using uncrowned numbers 5, 6, & 7.

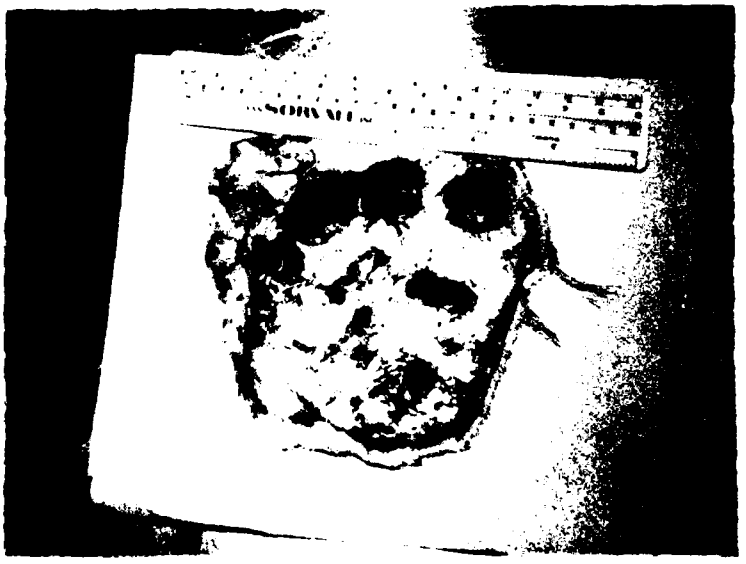


Fig 6--Underside of skin showing intracutaneous deposition of injected dye.

Fig 7--Skin reflected showing deposition of dye in the subcutaneous tissues.

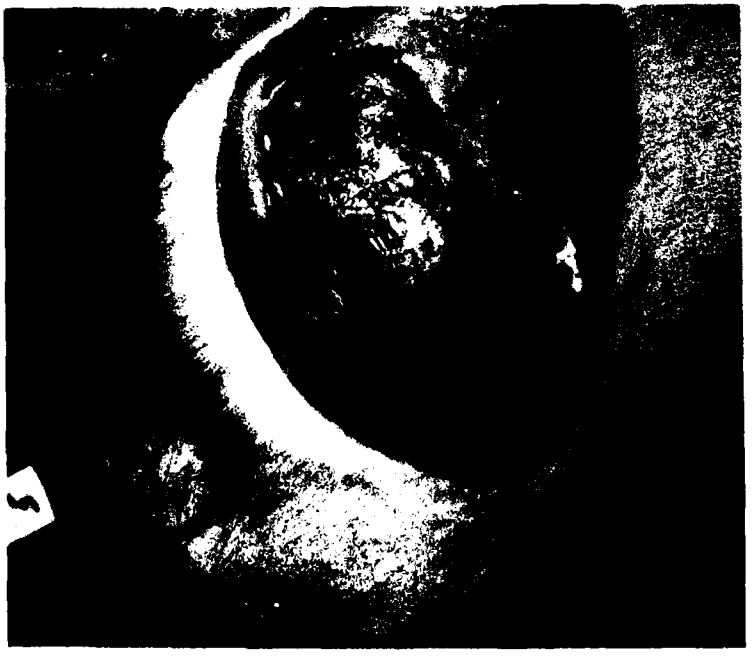


Fig 8--Deep muscle tissue showing dye penetration with only the crowned nozzles number 5 and number 9 and uncrowned number 9 nozzle.

TABLE 4--Optimal Configuration of Jet Injector Components for Maximal Depth of Penetration of 1 ml Dose.

Species	Nozzle Design	Orifice Diameter #	Intensifier	Site
Canine	Crowned	5, 7 & 9*	No	Hip
Bovine	Crowned	9, 10, & 11	Yes	Neck**
Equine	Crowned	9, 10, & 11	Yes	Neck
Ovine	Crowned	5, 7, & 9	No	Hip[+]
Caprine	Crowned	9, 10, & 11	Yes	Hip
Porcine	Crowned	9, 10, & 11	Yes	Behind Ear[‡]
Avian	Crowned	9	No	Leg

*Smaller dogs (<10 lbs.) required smaller orifice diameters.

**The shoulder was a secondary site and the hip a tertiary site.

[+]The wool on unclipped sheep must be parted in order to make a good skin to nozzle contact.

[‡]The skin on other parts of the pig's body was too dense to penetrate.

TABLE 5--Optimal Configuration of Jet Injector Components for Maximal Depth of Penetration for 2 ml Dose.

Species	Nozzle Design	Orifice Diameter #	Intensifier	Site
Canine	Crowned	9, 10, & 11*	Yes	Hip
Bovine	Crowned	9, 10, & 11	Yes	Neck
Equine	Crowned	9, 10, & 11	Yes	Neck
Ovine	Crowned	9	Yes or No	Hip
Caprine	Crowned	9, 10, & 11	Yes	Hip
Porcine	Crowned	9, 10, & 11	Yes	Behind Ear
Avian	NT	NT	NT	NT

*Larger diameter orifices shortens the injection time.

NT - Not tested.

Injections were made in the breast and leg muscles. Injections in the breast resulted in appreciable hemorrhage and in one case, death due to penetration and hemorrhage into the abdominal cavity by the inoculated dye. The leg muscle was a more desirable site of injection in that hemorrhage was minimal and deep intramuscular penetration was obtained.

The piscine species were tested on a limited basis using small catfish. Although injections were successfully accomplished, both the crowned and uncrowned nozzle design proved unsatisfactory. Neither one could be completely immobilized on the fish's skin due to the production of a copious amount of slime.

Other components were tested in the animal species and were found to be less satisfactory than those listed in Tables 4 and 5. For instance, in the equine it was noted that deeper penetration was accomplished with the intensifier attached when it was equipped with the spring rated at 42.2 kg/sq cm (600 psi) than with the smaller spring rated at 25 kg/sq cm (350 psi). As a result, only the 42.2 kg/sq cm (600 psi) rated spring was used in subsequent testing of the intensifier. In another series of tests in the bovine it was shown that the #12 orifice diameter did not give as effective penetration through the skin as the #9, #10 or #11 orifice diameters.

As a result larger orifice diameters were not tested.

Radiographic Comparison of Jet Injection vs Needle and Syringe Injection

One milliliter and 2 ml doses of a radiopaque dye were injected into live dogs by jet injector and needle and syringe. The different methods of injection were made in the same general area of the hip and radiographs were taken as soon as possible after injections. From these radiographs a comparison of depth of penetration and localization pattern between needle and syringe injection and jet injection could be made. The 1 ml needle and syringe injected dye appeared to be more concentrated whereas the jet injected dye was more dispersed in the tissues (Fig 9 and 10). The dispersion pattern of the 2 ml dose was very similar by either method of injection except that the dye injected by the jet injector tended to "tail out" toward the point of contact of the injector on the skin (Fig 11 and 12).

Results of Adjunct Studies

Caudal nerve block. This procedure was attempted in the horse and the goat. The horse had a very thick skin covering the coccygeal vertebrae (approximately 7 mm in a Shetland pony). When the jet injector was equipped with

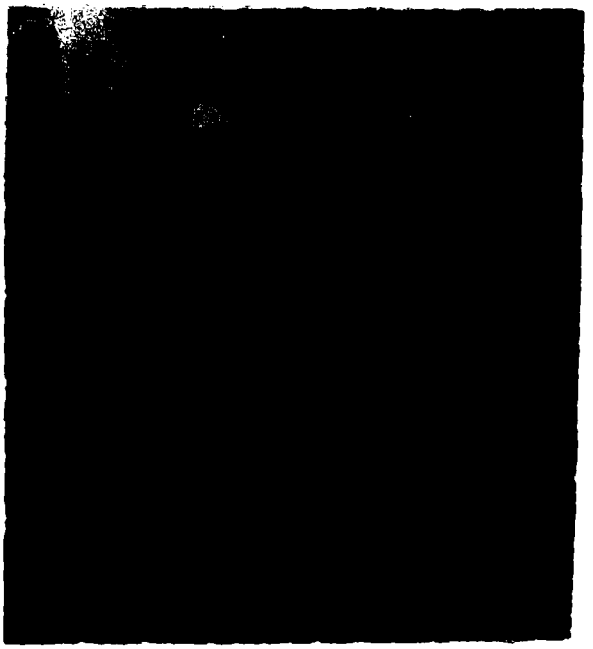


Fig 9--Lateral view of 1 ml Diodrast injected into a 25 lb canine. N=20 ga 1" needle. J=jet injector with #11 crowned nozzle. F=femur.

Fig 10--Anteroventral view of 1 ml Diodrast injected into a 25 lb canine. Note that dye tends to follow fascial planes. N=20 ga 1" needle. J=jet injector with #11 crowned nozzle. F=femur.

Fig 11--Lateral view of 2 ml Diodrast injected into a 40 lb canine. Note "tailing pattern" with jet injected inoculum. N=20 ga 1" needle. J=jet injector with #11 crowned nozzle. F=femur.

Fig 12--Anteroventral view of 2 ml Diodrast injected into a 40 lb canine. N=20 ga 1" needle. J=jet injector with #11 crowned nozzle. F=femur.

the 2 ml dose barrel, the #9 crowned nozzle and intensifier, the deepest penetration accomplished was to the subcutaneous tissue in the horse.

In the goat, the set-up consisted of the 1 ml dose barrel, the #5 crowned nozzle and intensifier. Although excellent skin penetration was accomplished, only the subcutaneous tissue was stained with the dye.

Intra-articular injection. In the equine, attempts were made to introduce injections of dye into the hock and carpal joints with the jet injector. The site of injection for the hock was on the medial side of the joint on either side of the saphenous vein. For the carpal joint, the site of the injection was between the radial and the intermediate carpi on the anteromedial aspect of the joint. Dye penetration in both joint locations was into the subcutaneous tissue only with no evidence of penetration into the joint capsule.

In the goat carpal joint, skin penetration was largely unsuccessful due to the thickened callous tissue covering the carpus of the goat. In the hock joint, skin penetration was no problem, but the dye did not penetrate the joint capsule.

Permanent identification in the equine. An adult horse was injected with India ink solution, 0.2 ml in the

mucosa of the upper lip using the uncrowned ID nozzle with #5 orifice diameter (Fig 13). Three of the injections were made in a horizontal pattern, appeared as circular marks 1 to 1.5 cm in diameter and were still evident at the time the animal died between 4 and 5 weeks after injection.

Skin testing in the bovine. Several attempts were made to accomplish ID injection into the caudal fold of the bovine. Injections of 0.2 ml made with the uncrowned ID nozzle failed to penetrate the skin of the caudal fold and leaked out around the nozzle where it made contact with the skin.

Adjuvanted virus inoculation. The feasibility of injecting an adjuvanted preparation with the jet injector was explored in a sheep. Tobacco mosaic virus in a water-in-oil adjuvant was readily injected into the sheep every other week for a period of 8 weeks. On the ninth week the serum was harvested and TMV specific antibody was detected by agar gel immunodiffusion.

Laboratory Studies Comparing Jet Injection with Needle and Syringe Injection using Vaccine and Virulent Virus Models

Vaccine Virus Studies

Dogs. A group of 16 dogs were vaccinated with VEE virus vaccine. Eight were vaccinated with the jet

Fig 13--Intradermal jet injection of dye for permanent marking in the equine, 21 days post-injection.

injector and 8 with needle and syringe. The presence of antibody was determined in day 0 and day 35 post vaccination serum samples by both SN and HI methods. Attempts to isolate vaccine virus from whole blood collected on days 2 through 5 post vaccination were unsuccessful. Table 6 gives a summary of the serological results. All 16 dogs showed SN antibody in the serums collected on day 35. One of 8 jet injected and 2 of 8 needle and syringe injected dogs showed no HI antibody titer in the day 35 serums, giving a seroconversion rate of 87.5% and 75% respectively.

TABLE 6--Serological Response of Dogs to VEE Vaccination

Serological Test	Mode of Injection	# Serums Positive / # Serums Tested	Seroconversion Rate
SN	Jet	8/8*	100
SN	N&S	8/8	100
HI	Jet	7/8	87.5
HI	N&S	6/8	75

*Screened at 1:5 dilution
N&S - needle and syringe

Cattle. Table 7 gives a summary of the SN antibody response of the calves in this study. Two groups of cattle consisting of 10 per group were vaccinated in a comparative study. One group received 1 ml of IBR

vaccine (full dose reconstituted to ½ volume). Four of these animals showed SN antibody at day 35 and 6 did not, for a seroconversion rate of 40%. The other group received 2 ml IBR vaccine via needle and syringe. Four of the 8 seroconverted for a 50% seroconversion rate. Two of the needle and syringe injected group showed pre-existing antibody in the day 0 serum and were not considered vaccine positives. A third group of 17 calves was vaccinated with 2 ml of IBR vaccine with the jet injector. Two calves showed pre-existing antibody, an additional 3 developed detectable SN antibody titers and 12 failed to respond, for a seroconversion rate of 20%.

TABLE 7--Serum Neutralization Response of 37 Calves to IBR Vaccine given by Jet Injector and Needle and Syringe

Method	Dose	#SN Pos.	#SN Neg.	Pre-existing Antibody	% Sero-conversion
Jet	1 ml	4*	6	0	40
N&S	2 ml	4	4	2	50
Jet	2 ml	3	12	2	20

*Serum screened at 4 parts serum to 1 part virus.
N&S - needle and syringe.

Virulent Virus Studies

The responses of a number of animal species to inoculation with virulent VEE virus by both jet injector

and conventional needle and syringe injections were measured and comparisons between both modes of injection made. Parameters of response measured were clinical signs, temperature response, hematological changes (PCV, platelets, WBC, lymphocytes, and neutrophiles), viremia and serological response, and necropsy findings. Harris[b] previously reported the effects of VEE virus when given to dogs and calves, and compared the differences between diurnal and nocturnal injection times. He used the same parameters of response, the same animals and the same virus inoculum that is covered in this report. The data have been rearranged and presented to compare jet injection with needle and syringe injection. In contrast to Harris' report which presented temperature and hematological responses as "typical" and "representative" the data in this report are presented as median values so that trends for each group of animals might be established and data could be compared to those previously published.[56]

Dogs. Diurnal group. Dogs were alert and clinically normal during the 7 day baseline period. Subsequent to injection with virulent virus, all animals responded with signs of clinical illness. Harris[b] has previously documented these signs. There was no detectable differences between jet injected and needle and syringe injected dogs.

Temperature response (Fig 14)--A plot of the median temperature for dogs injected at 8AM showed that each group had a fairly stable baseline temperature which varied between highs at the AM readings and lows at the PM readings of about 0.55 C (1 F). Following injection, temperatures were within baseline limits at 12 hours but were markedly elevated at 24 hours. The pyrexia persisted for 96 hours in the needle and syringe injected group and for 108 hours in the jet injected group, at which time the temperature returned to baseline values where it remained for the remainder of the experiment. Clinical signs of illness paralleled increased temperature.

PCV (Fig 15)--Median PCV's were initially high with a progressive decline occurring during the initial baseline period through day 4 following injection. The values then became stable during days 5 through 7 and 14. The introduction of virus did not appear to alter the decline in PCV, at least during the initial period following injection. The median values compare closely regardless of mode of injection.

Platelets (Fig 16)--There was a significant variance between jet injected and needle and syringe injected animals regarding their platelet counts. The jet injected group showed a gradual decline in platelet

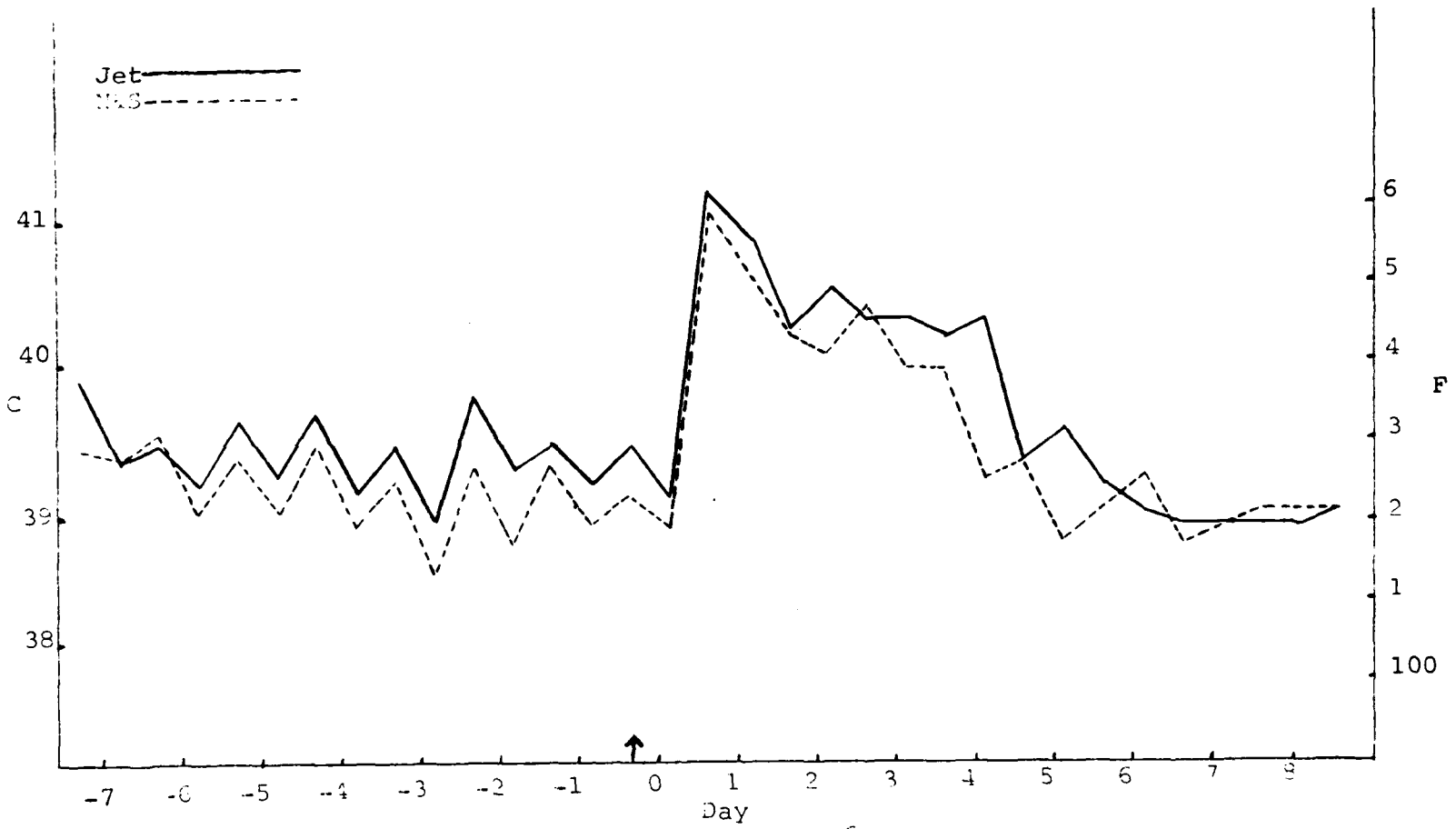


Fig 14--Median Temperatures of Dogs Injected with 10^6 $CCID_{50}$ Virulent VEE Virus--Diurnal Group.

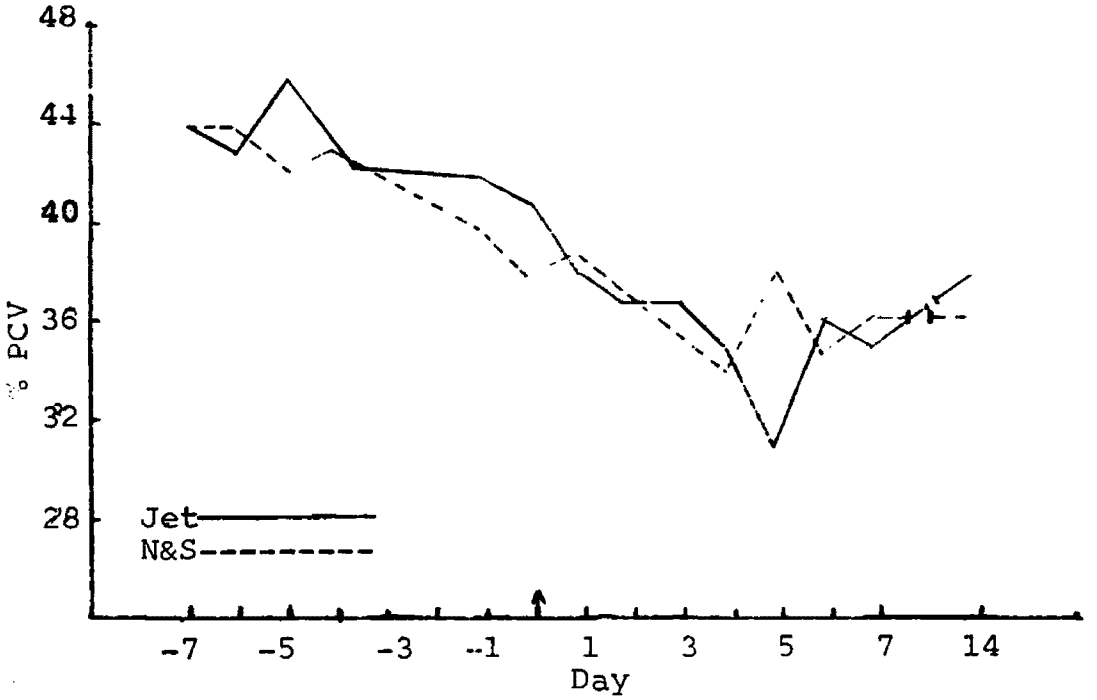


Fig 15--Median packed cell volume of dogs inoculated with 10^6 $CCID_{50}$ VEE virus--diurnal group.

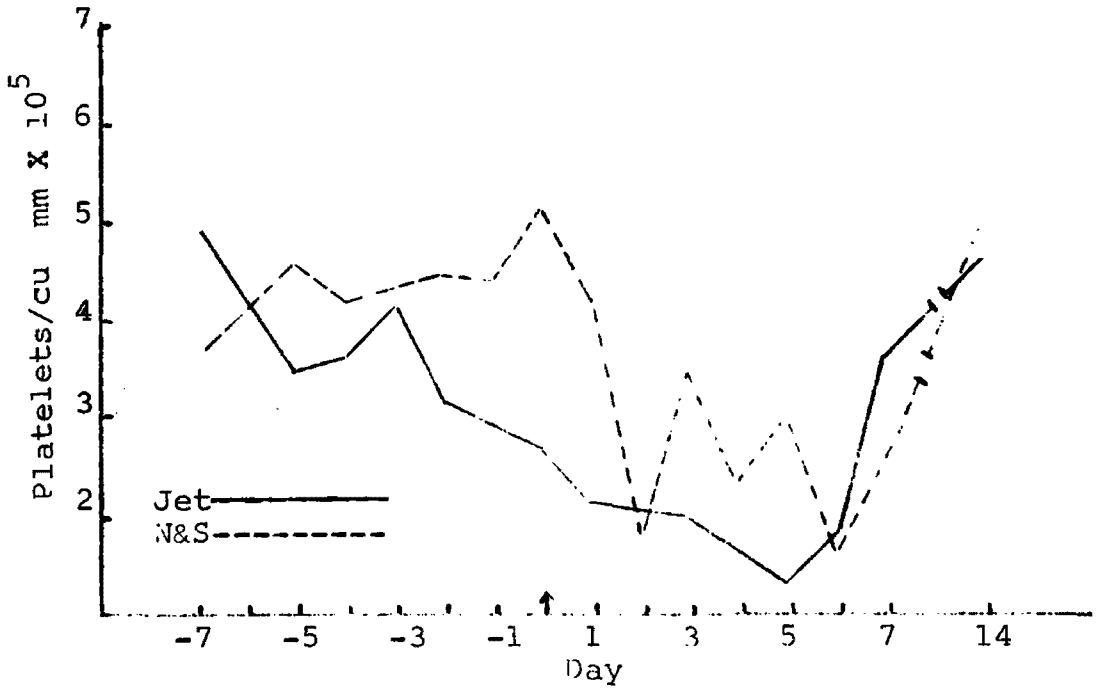


Fig 16--Median platelet counts of dogs inoculated with 10^6 $CCID_{50}$ VEE virus--diurnal group.

values during the baseline period which continued for 5 days post injection (p.i.). The platelet count returned to baseline levels by day 7 and was within those limits at day 14. The needle and syringe injected group maintained a fairly constant baseline count during the baseline period and for 24 hours after virus challenge. On day 2 a low reading comparable to the jet injected group was seen. Days 2 through 7 showed erratic low readings and on day 14 the count was comparable to baseline values.

WBC, lymphocytes, and neutrophiles (Table 8)-- Generally total WBC's remained above 10,000 during the baseline period for both groups. A dramatic drop was seen in the jet injected group at 48 hours p.i., whereas, the needle and syringe injected group did not reach abnormally low counts until 72 hours p.i. Counts remained low in the jet injected group for 24 hours before a rise was noted. Baseline levels were reached by day 6 in the needle and syringe injected group but counts remained low in the jet injected group until day 7. Lymphocyte counts for both groups were comparable throughout, showing a drop at 24 hours p.i. The counts remained depressed through day 5 and on days 6 and 7 returned to baseline levels. Neutrophile counts were very erratic during the baseline period but depression of counts following virus injection was seen in both groups regardless of method of virus injection. Note that in Table 8 and those following

TABLE 8--Median WBC, Lymphocyte, and Neutrophile Counts in Dogs Inoculated with 10^6 $CCID_{50}$ VEE Virus--Diurnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	10.7*	5.6	5.1	10.5	3.6	7.0
-6	12.4	2.9	7.8	12.5	3.6	6.8
-5	14.7	4.3	9.7	11.9	4.2	6.9
-4	17.4	3.2	9.2	13.4	4.1	7.2
-3	22.0	6.2	13.4	11.2	4.2	4.5
-2	15.6	4.5	9.0	17.1	5.0	9.2
-1	12.0	2.9	7.4	14.6	5.1	7.4
0	14.0	6.0	6.7	14.6	4.8	6.8
1	12.3	0.5	10.8	14.0	0.7	11.3
2	5.9	0.5	4.3	13.0	0.5	11.3
3	4.5	1.1	3.1	5.5	0.5	4.3
4	7.4	0.8	5.3	7.5	1.4	4.5
5	6.3	0.9	5.2	8.0	1.3	5.1
6	8.9	2.7	4.9	11.5	3.7	7.0
7	10.9	5.4	6.4	12.3	5.8	5.3
14	14.3	4.7	7.9	13.9	5.2	8.8

*Expressed as counts per cubic mm X 10^3

L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

there are instances in which the sum of the lymphocytes and neutrophiles exceeds the total WBC count due to the use of median values.

Monocytes, eosinophiles, and basophiles--These cell types were very low in number or completely absent in many samples and no trends with respect to virus injection could be established. This was seen in all animal species tested and at all injection times and with all doses of virus tested.

BUN--Blood urea nitrogen was below maximum normal limits (dog <20 mg%, calves <27 mg%, pigs <24 mg%, sheep <20 mg%, goat <28 mg%) in all animal species tested except nocturnal sheep.

Viremia (Table 9)--Harris[b] previously reported viremia titers in dogs. When viremia titers are compared with respect to mode of injection, all 3 jet injected dogs showed viremia on days 1 and 2 p.i., 2 showed viremia on day 3 and 1 on day 4. The virus titer dropped markedly after 48 hours and was negative on day 5. The highest titers were observed on days 1 and 2 following virus injection, with titers exceeding 10^3 median suckling mouse intracerebral doses ($SMICLD_{50}$). Needle and syringe injected dogs had a much lower level of virus titer throughout, with only 1 dog showing greater than 10^3 $SMICLD_{50}$ on day 1 only. Virus titers also disappeared about 24 hours earlier in the needle and syringe injected group compared to the jet injected

TABLE 9--Serum Virus Titers* in Dogs Inoculated with 10^6 $CCID_{50}$** VEE Virus--Diurnal Group.+

Dog #	Injection Method	Day Post-Inoculation 1	2	3	4	5
BA	Jet	$10^{4.2}$	$10^{4.5}$	$10^{2.4}$	0	0
BN	Jet	$10^{3.5}$	$10^{3.5}$	$<10^{0.5}$	$<10^{0.5}$	0
GK	Jet	$10^{3.4}$	$10^{3.3}$	0	0	0
BP	N&S	$10^{2.4}$	$10^{2.2}$	0	0	0
IE	N&S	$10^{0.5}$	$10^{0.5}$	$<10^{0.5}$	0	0
GM	N&S	$10^{3.3}$	$10^{2.5}$	0	0	0

*Median Suckling Mouse Intracerebral Lethal Dose ($SMICLD_{50}$) per 0.025 ml serum.

**Calculated dose. Actual challenge dose as determined by backtitration of inoculum was $10^{6.37}$ $CCID_{50}$ per ml.

+Previously reported by Harris.

N&S - Needle and syringe

group.

Antibody response (Table 10)--The data presented in this table illustrate the magnitude of antibody response and the earliest appearance of antibody as previously reported by Harris.[b] Peak HI antibody titers were seen on day 14 and ranged from 1:2560 to 1:10,240 in jet injected dogs, and from 1:320 to 1:10,240 in needle and syringe injected dogs. Day 21 HI titers were generally lower by twofold or more except in one instance where no decline occurred.

Necropsy--Gross necropsy lesions were absent in all dogs regardless of the day on which they were killed or the method by which they received virus injections.

Dogs: Nocturnal group. Dogs were clinically normal during the baseline period. Following virus injection clinical signs of illness were very similar to those described for the diurnal group of dogs. As Harris[b] previously reported, signs included anorexia, depression, huddling, reluctance to move and gingival vesicle formation. Neither anorexia nor depression were seen after day 6 p.i. Also by day 6 p.i., gingival ulcers were healed in all except 1 dog which had unhealed ulcers until day 11 p.i.

Temperature (Figs 17 and 18)--Temperature response in dogs injected with VEE at 8PM was similar regardless of the method of injection. Generally, there was a rise at

TABLE 10*-Reciprocal of Hemagglutination-Inhibition Titers in Serums of Dogs Inoculated with 10^6 $CCID_{50}$ VEE Virus--Diurnal Group.

Dog #	Injection Method	Day Post-Injection 4	5	6	7	14	21
BA	Jet	N	N	320	2560	10240	1280
BN	Jet	N	20	160	320	2560	---**
GK	Jet	N	10	40	80	2560	320
BP	N&S	N	N	40	80	10240	---**
IE	N&S	N	20	40	80	10240	80
GM	N&S	N	N	40	20	320	320

*Previously reported by Harris.[b]

**Killed on day 14.

N - Negative

N&S - Needle and syringe

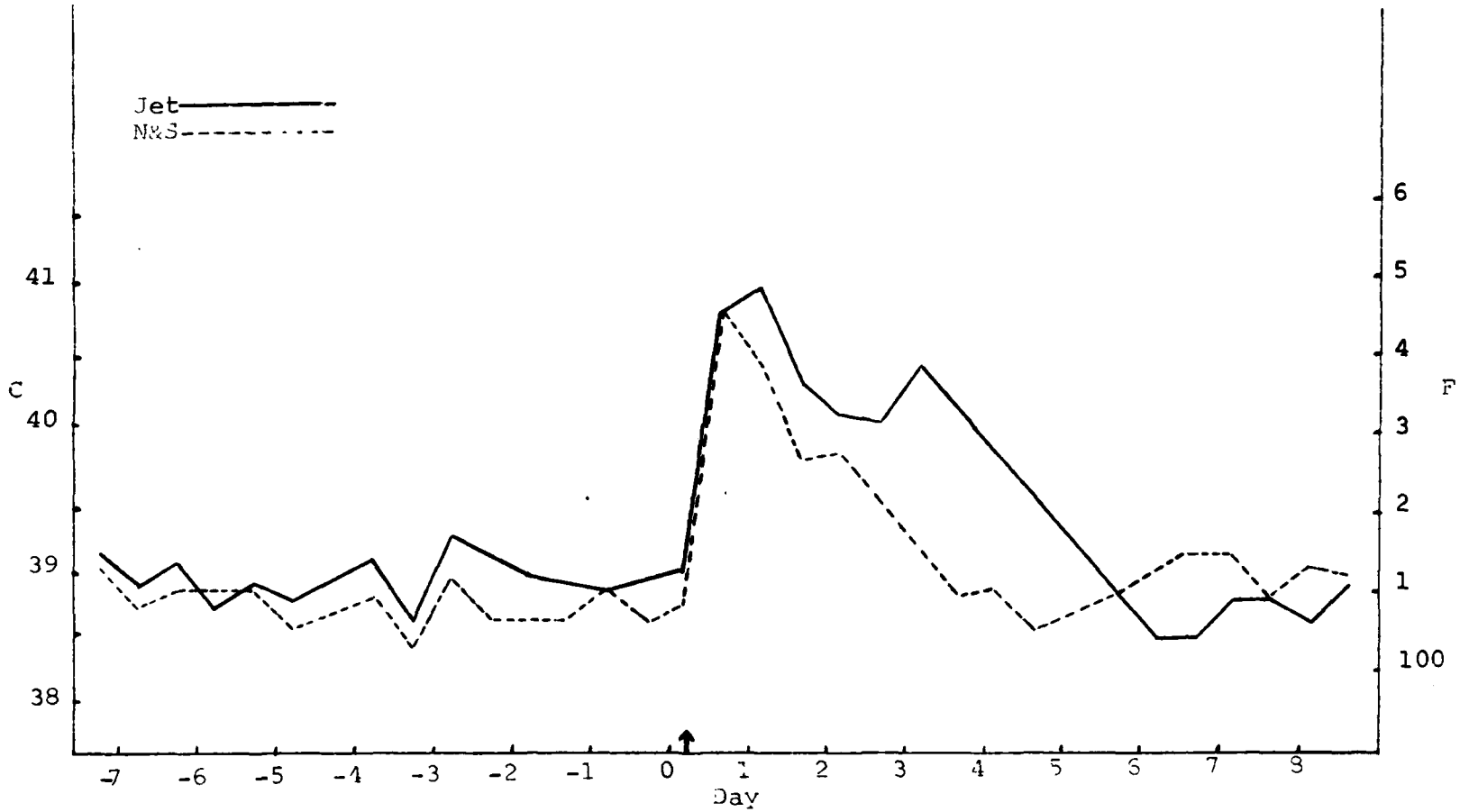


Fig 17--Median Temperature of Dogs Injected with 10^6 $CCID_{50}$ Virulent VEE Virus--Nocturnal Group.

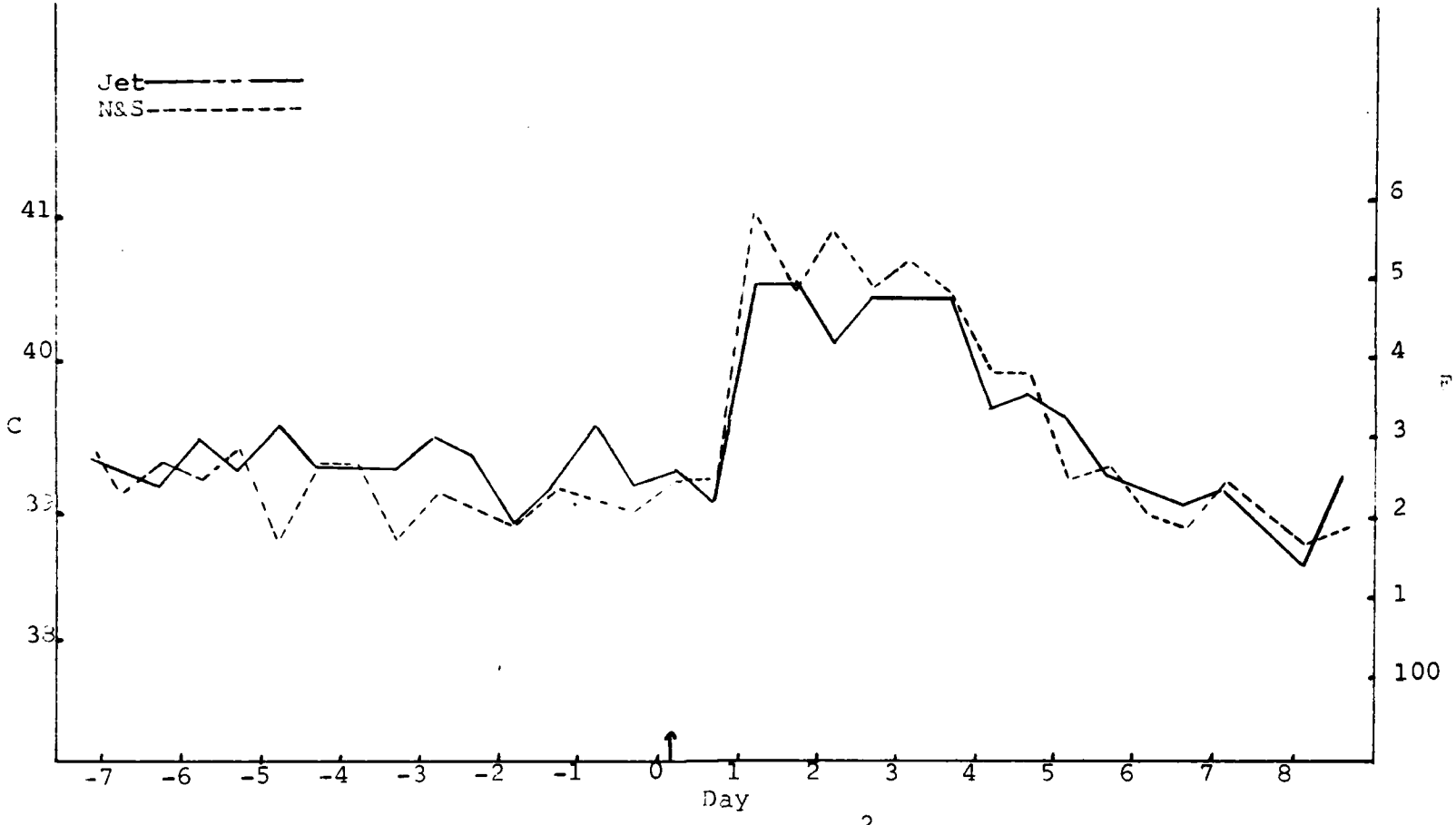


Fig 18--Temperature Response of Dogs Injected with 10^2 $CCID_{50}$ Virulent VEE Virus--Nocturnal Group.

12 hours p.i. followed by a decline which returned to normal baseline levels. Dogs injected with 10^6 median cell culture infectious doses ($CCID_{50}$) via jet injector did sustain a higher temperature for about 48 hours longer than did the needle and syringe injected group. Those dogs receiving 10^2 $CCID_{50}$ VEE virus showed similar fever responses with the needle and syringe injected dog showing a higher maximum temperature, but both returned to baseline values within 12 hours of each other.

PCV (Fig 19 and 20)--Dogs injected with 10^6 $CCID_{50}$ VEE virus by jet injector showed a slight decrease in PCV following injection and a declining pattern during the entire bleeding period. The group receiving 10^6 $CCID_{50}$ VEE virus by needle and syringe remained fairly stable until day 5 p.i. where a decline was seen. Conversely, the dog receiving 10^2 $CCID_{50}$ VEE virus via jet injector had PCV values which remained higher whereas the needle and syringe injected animal showed a gradual decline in PCV values with a somewhat lower reading than the jet injected dog.

Platelets (Fig 21 and 22)--Platelet counts were variable but a general decline was seen at day 1 p.i. in all dogs and the decline persisted throughout the bleeding period. Below baseline counts were still

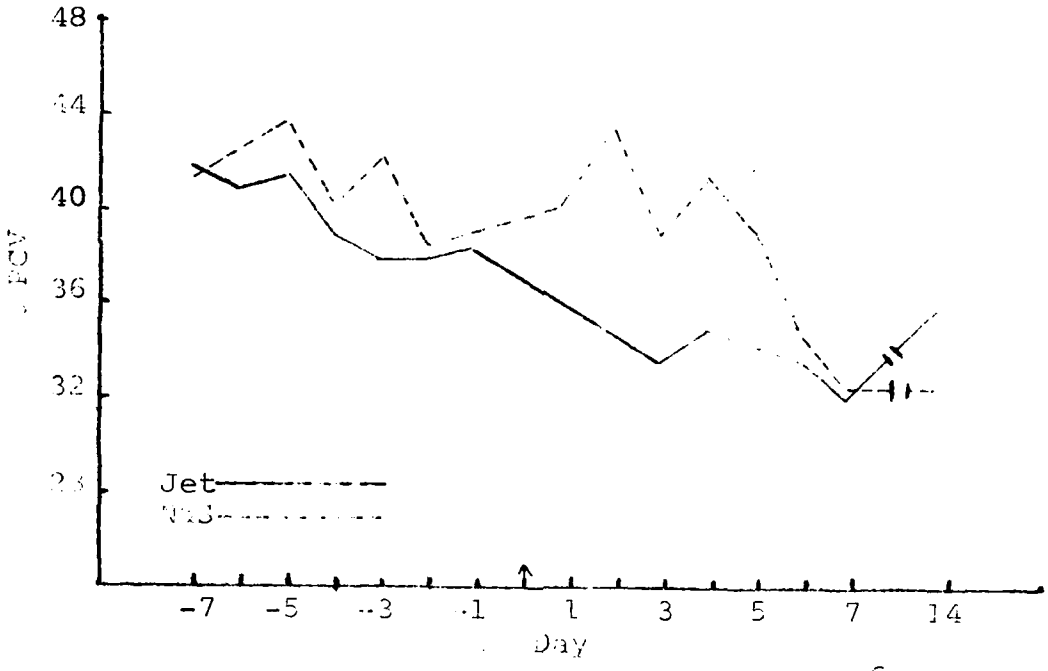


Fig 19--Median PCV values in dogs receiving 10^6 $CCID_{50}$ VEE virus--nocturnal group.

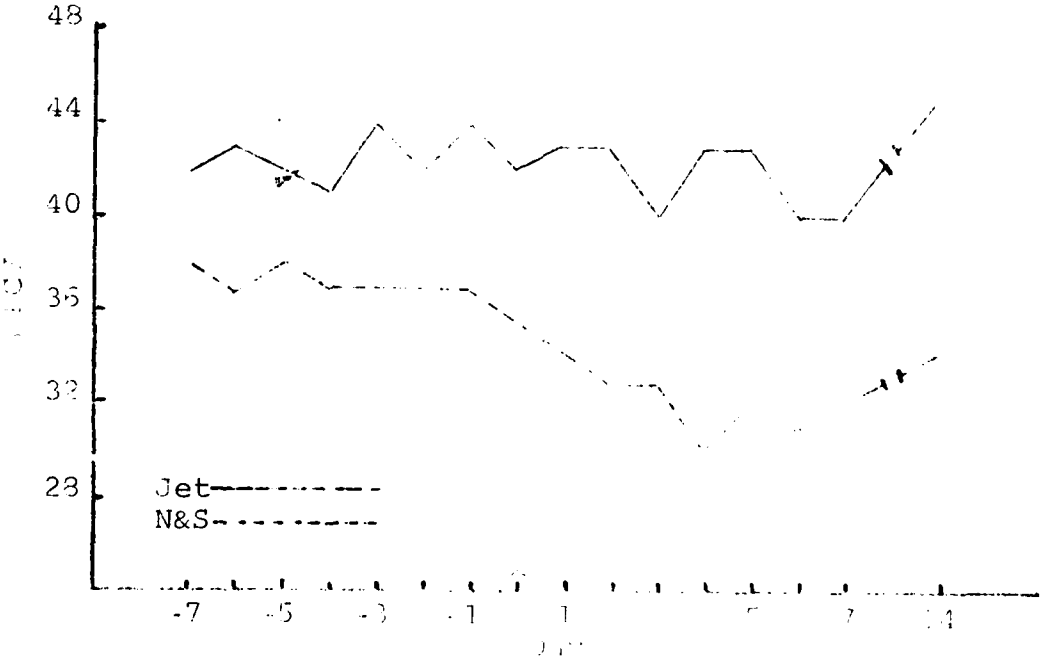


Fig 20--PCV values in dogs [illegible] vi[illegible] nocturnal group.

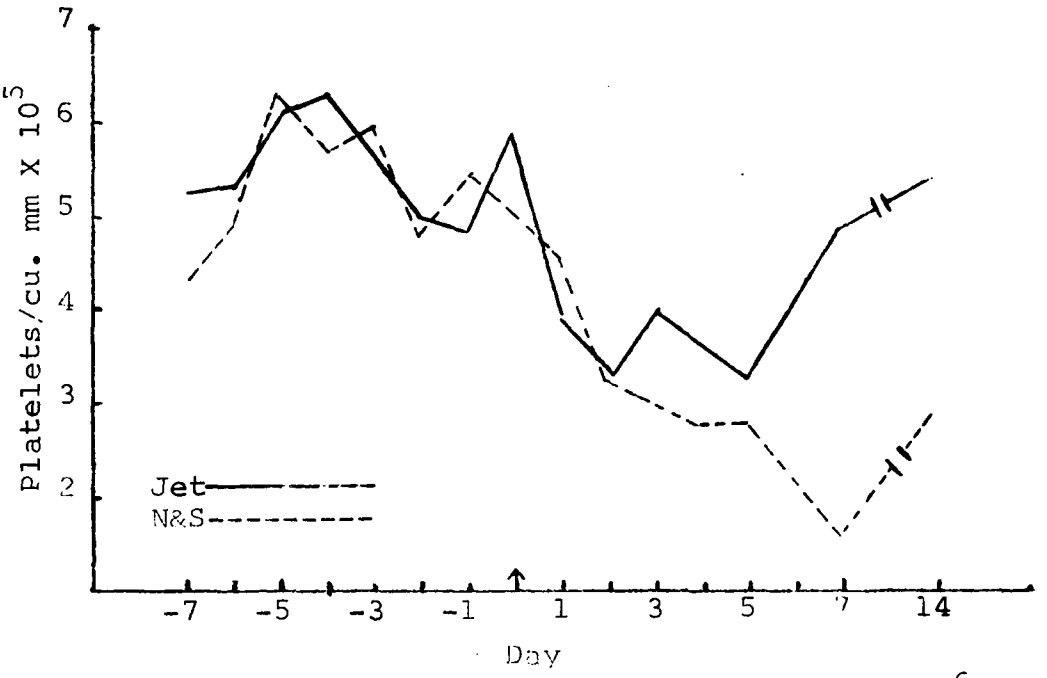


Fig 21--Median platelet counts in dogs receiving 10^6 $CCID_{50}$ VEE virus--nocturnal group.

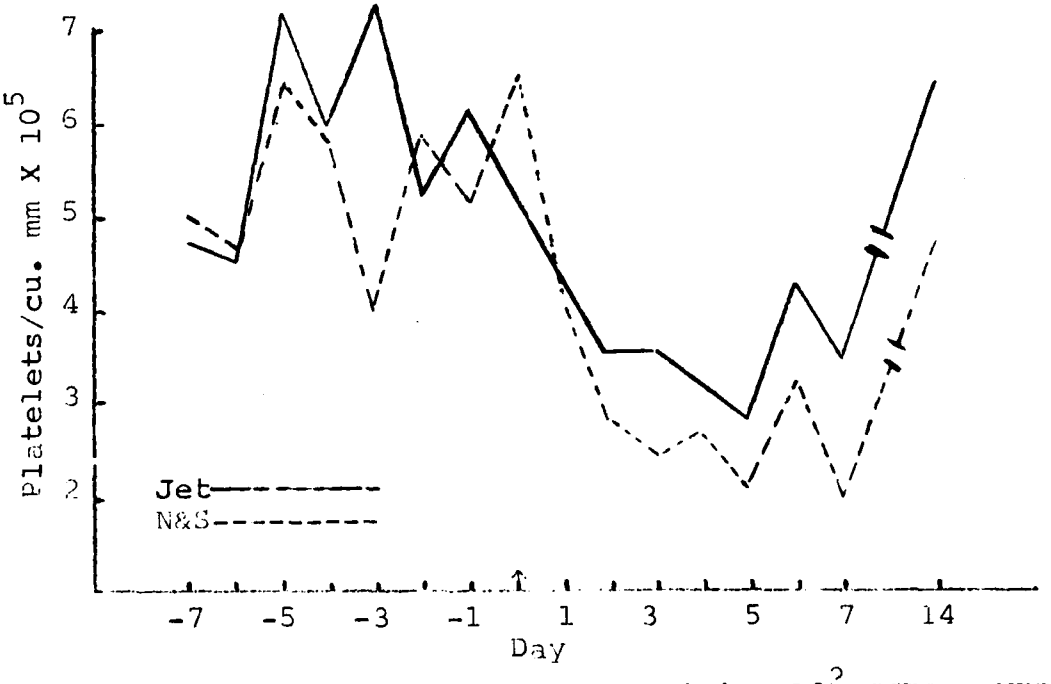


Fig 22--Platelet counts in dogs receiving 10^2 $CCID_{50}$ VEE virus--nocturnal group.

observed at the day 14 bleeding in the needle and syringe injected group receiving 10^6 $CCID_{50}$ VEE virus.

WBC, lymphocytes, and neutrophiles (Tables 11 and 12)- Total WBC counts remained within baseline limits for the first 24 hours following virus injection. Dogs receiving 10^6 $CCID_{50}$ VEE by either jet injector or needle and syringe showed lower than baseline WBC counts on days 2 through 7 p.i. The needle and syringe injected group had returned to baseline on day 14 but the jet injected group was below baseline values. Dogs receiving 10^2 $CCID_{50}$ VEE virus showed a less dramatic drop in WBC counts that the higher dose group, but the drop persisted from days 2 through 6 and 7 for the needle and syringe and jet injected groups, respectively. By day 14, the WBC count was still below baseline values for the jet injected dog and low normal for the needle and syringe injected dog. All dogs regardless of dose or method of injection showed a lymphopenia from day 1 through day 6 p.i. On day 7 and day 14 lymphocyte numbers were within baseline limits. Neutrophile counts were extremely variable, however, there was an initial neutrophilia in all groups during the first 24 hours p.i. In the high dose groups a neutropenia was seen on days 2 through 7 p.i. and was still low on day 14 in the jet injected group. This pattern was also seen in the dog receiving

TABLE 11 --Median WBC, Lymphocyte, and Neutrophile Counts in Dogs Inoculated with 10^6 $CCID_{50}$ VEE Virus--Nocturnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	19.7*	6.5	10.7	14.2	3.2	9.7
-6	23.2	6.8	13.3	16.1	6.8	7.3
-5	24.6	7.1	14.8	18.0	4.6	11.9
-4	19.3	5.4	10.8	12.9	4.1	7.7
-3	23.0	6.5	12.2	14.1	3.4	9.7
-2	25.3	6.0	16.5	15.0	4.0	9.6
-1	21.4	6.8	11.8	12.6	3.1	8.4
0	20.2	5.4	11.0	12.6	3.1	8.7
1	19.2	0.8	16.9	17.4	2.3	14.3
2	11.0	0.1	10.2	9.4	0.4	8.5
3	6.2	0.6	5.1	6.4	0.8	5.1
4	7.5	1.1	5.5	6.3	0.3	5.6
5	11.3	1.4	8.3	7.8	1.0	5.4
6	11.7	3.7	5.6	5.7	1.1	3.3
7	15.9	7.3	6.5	11.8	3.7	6.5
14	13.4	5.1	7.3	20.5	4.8	14.1

*Expressed as counts per cubic mm $\times 10^3$

L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

TABLE 12 --Median WBC, Lymphocyte, and Neutrophile Counts in Dogs Inoculated with 10^2 $CCID_{50}$ VEE Virus--Nocturnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	14.6*	5.1	7.7	16.2	4.8	8.7
-6	20.1	6.2	10.9	20.0	6.0	12.6
-5	21.3	5.5	12.4	22.7	4.8	14.5
-4	14.4	5.6	7.5	19.9	3.8	13.5
-3	22.5	4.7	15.3	17.7	3.7	11.7
-2	15.6	4.2	9.0	13.5	5.9	7.3
-1	22.7	6.1	12.5	11.7	4.7	5.9
0	15.5	2.8	11.0	17.2	3.8	11.5
1	18.2	0.5	14.7	17.7	0.5	16.1
2	9.0	0.6	7.8	13.7	0.0	13.0
3	11.0	0.2	10.6	8.1	0.2	7.7
4	11.0	1.4	8.9	7.9	0.1	6.7
5	14.3	1.4	10.3	7.9	1.9	4.9
6	12.8	2.6	8.1	8.0	2.6	4.1
7	8.8	3.3	3.8	14.3	3.9	8.1
14	11.3	3.7	5.8	11.4	3.9	5.6

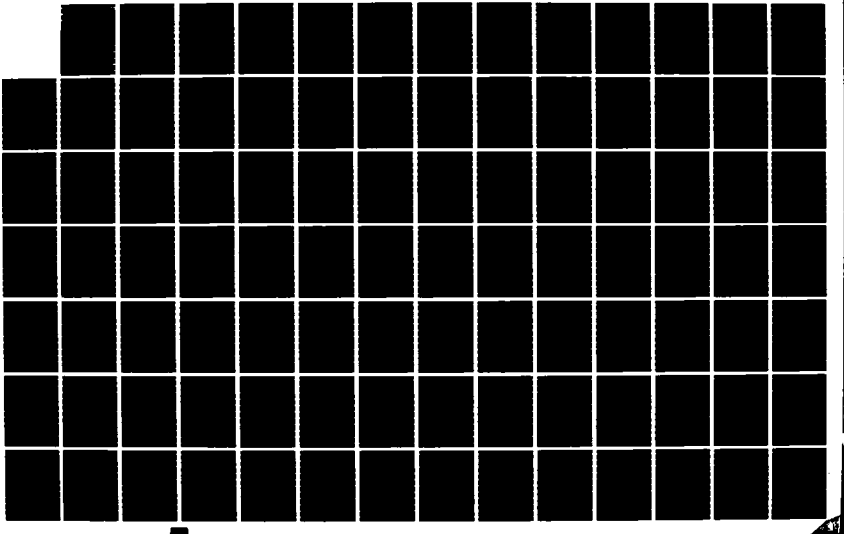
*Expressed as counts per cubic mm X 10^3

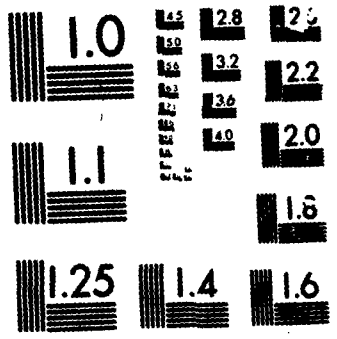
L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

AD-A169 791 THE EVALUATION OF JET INJECTION FOR USE IN VETERINARY MEDICINE(U) TEXAS A AND M UNIV COLLEGE STATION H W WHITFORD MAY 86 DADA17-71-C-1087 2/3

UNCLASSIFIED F/G 6/5 NL




1.0
1.1
1.25
1.4
1.6
2.8
3.2
3.6
4.0
2.2
2.0
1.8

10^2 $CCID_{50}$ VEE virus by needle and syringe but was less evident in the dog receiving the same dose by jet injection.

Viremia (Table 13)--Jet injected dogs showed detectable viremia on days 1 and 2 p.i. The viremia titers ranged from a low of $10^{1.5}$ $SMICLD_{50}$ (dog QB day 1) to a high of $10^{4.5}$ $SMICLD_{50}$ (dog BE day 1). The dog receiving 10^2 $CCID_{50}$ VEE virus had a viremia that was neither higher nor lower than the dogs receiving the higher dose of virus. The needle and syringe injected dogs showed generally higher titers of longer duration than did the jet injected dogs. The virus titers ranged from a high of $10^{5.1}$ (dog IO day 2) to a low of less than $10^{0.5}$ (dog BF day 3). Dog IO had a detectable titer for 5 days p.i. and dog BF had a detectable viremia for 3 days p.i. As in the jet injected group, the dog receiving the low dose of virus (dog BF) showed an intermediate viremia titer compared to the 2 dogs receiving the higher dose of virus.

Antibody response (Table 14)--The antibody response was characterized by a gradual rise in HI titer for all dogs. Five of the 6 dogs had peak HI titers on day 14 with 1 reaching highest titer on day 21. The highest titer was seen in a jet injected dog (dog BE day 14). Dogs receiving 10^2 $CCID_{50}$ VEE virus (dogs IA and BF)

TABLE 13*--Serum Virus Titers** in Dogs Inoculated with 10^2 and 10^6 $CCID_{50}$ VEE Virus--Nocturnal Group.

Dog #	Injection Method	Dosage+	Day Post-Injection 1	2	3	4	5
IA	Jet	10^2	$10^{2.6}$	$10^{2.5}$	0	0	0
IB	Jet	10^6	$10^{1.5}$	$10^{1.7}$	0	0	0
BE	Jet	10^6	$10^{4.5}$	$10^{4.0}$	0	0	0
BF	N&S	10^2	$10^{4.4}$	$10^{3.6}$	$<10^{0.5}$	0	0
IO	N&S	10^6	$10^{4.7}$	$10^{5.1}$	$10^{2.2}$	$10^{0.5}$	$<10^{0.5}$
KK	N&S	10^6	$10^{2.2}$	$10^{1.8}$	0	0	0

*Previously reported by Harris.[b]

**Median Suckling Mouse Intracerebral Lethal Dose per 0.025 ml serum.

+Calculated Challenge dose. Actual challenge dose as determined by back-titration of inoculum was $10^{1.82}$ and $10^{5.16}$ $CCID_{50}$ per ml.

N&S - Needle and syringe

TABLE 14*--Reciprocal of Hemagglutination-Inhibition Antibody Titers in Serums of Dogs Inoculated with 10^2 and 10^6 $CCID_{50}$ VEE Virus--Nocturnal Group.

Dog #	Injection Method	Dosage	Day Post-Injection 4	5	6	7	14	21
IA	Jet	10^2	10	20	160	320	1280	320
QB	Jet	10^6	N	40	160	320	320	---**
BE	Jet	10^6	10	80	320	5120	10240	2560
BF	N&S	10^2	N	10	40	160	640	1280
IO	N&S	10^6	N	40	320	1280	2560	2560
KX	N&S	10^6	N	10	20	40	80	---**

*Previously reported by Harris.[b]

**Killed on day 14.

N - Negative

N&S - Needle and syringe

showed comparable HI antibody titers to those dogs receiving 10^6 $CCID_{50}$ VEE virus.

Necropsy--No gross lesions were seen in any of the dogs regardless of time the animals were killed, dosage of virus, or method of injection.

Calves: Diurnal group. Harris[b] previously compared this group of animals with the group of calves which were injected with virulent virus in the evening. He did not observe any overt signs of clinical illness.

Temperature (Fig 23)--A rise in temperature of almost 1.1 C (2 F) above the baseline was observed at 12 hours p.i. in both jet injected and needle and syringe injected calves. The jet injected group showed a typical diphasic temperature response with a decline in temperature for 36 hours then another rise at 60 hours p.i. The remainder of the temperature readings in the jet injected calves were less than 0.55 C (1 F) above baseline readings. Needle and syringe injected calves showed a steady rise in temperature during the first 24 hours after virus injection. During the next 24 hour period the temperature of the needle and syringe injected group returned to baseline or less than 0.55 C (1 F) above baseline values for the remainder of the experiment.

PCV (Fig 24)--PCV values in all animals generally showed a decline during the baseline period. The decline

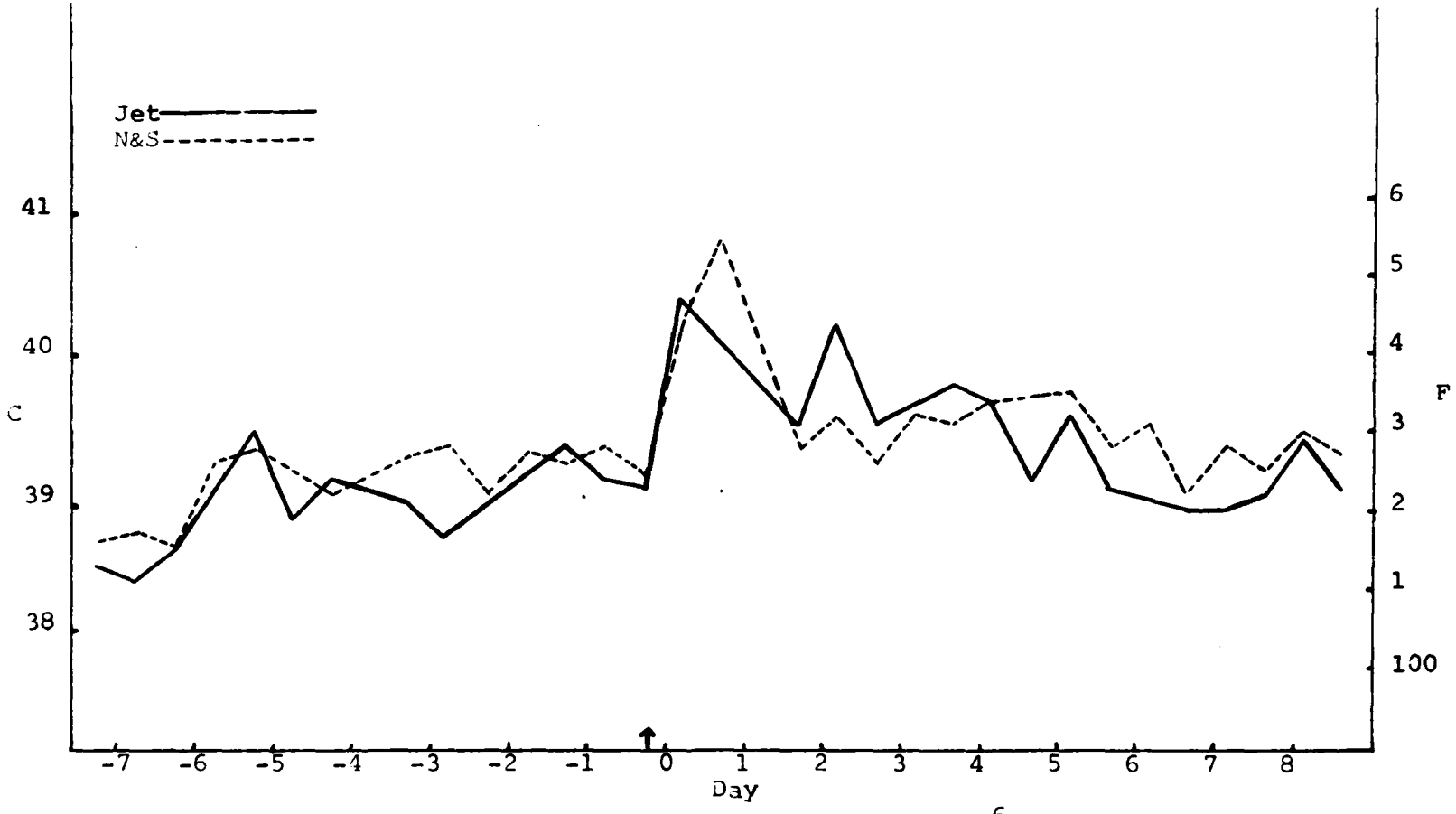


Fig 23--Median Temperature Response of Calves Injected with 10^6 $CCID_{50}$ Virulent VEE Virus--Diurnal Group.

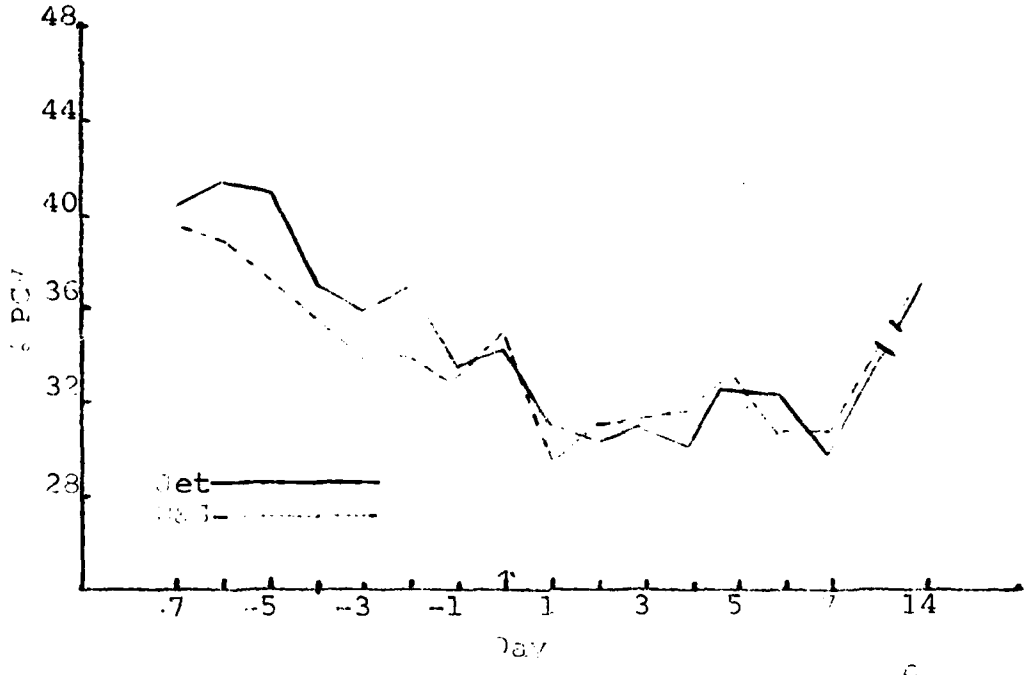


Fig 24--Median PCV values in calves receiving 10^6 $CCID_{50}$ VEE virus--diuenal group.

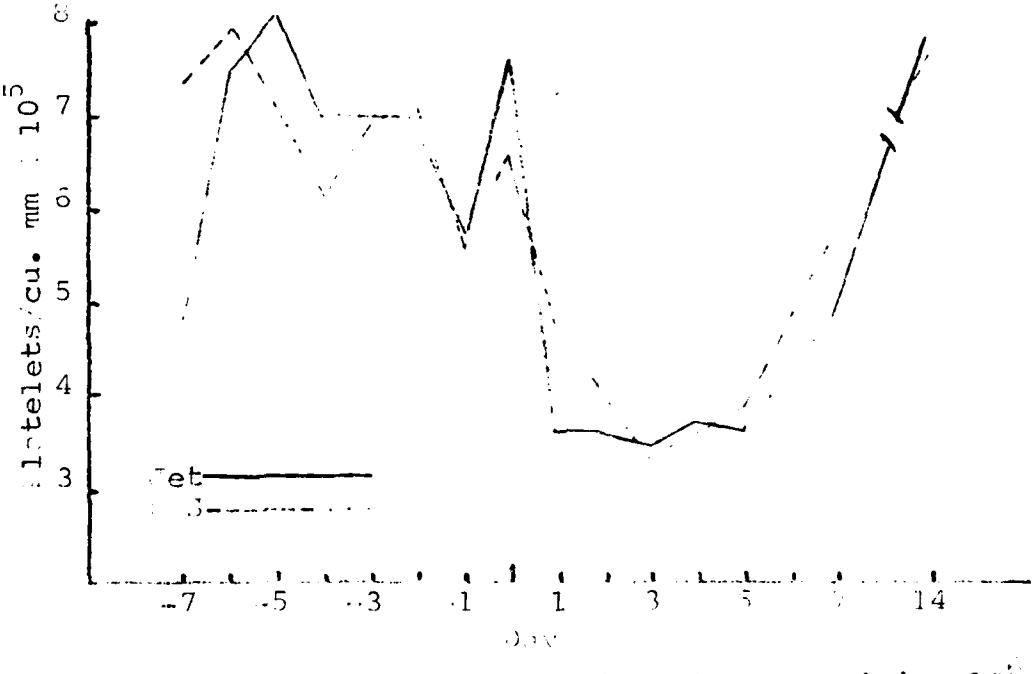


Fig 25--Median platelet counts in calves receiving 10^6 $CCID_{50}$ VEE virus--diuenal group.

continued for 24 hours following virus exposure and tended to reach its lowest point and increase slightly at 24 to 48 hours p.i. A rise in PCV was seen on the day 14 bleeding in both groups.

Platelets (Fig 25)--All animals showed a drop in platelet counts following virus exposure. The needle and syringe injected group's platelets were slower to drop than the jet injected group, but the magnitude of the drop was equivalent to the decrease in the latter group.

WBC, lymphocytes, and neutrophiles (Table 15)--All calves showed a drop in WBC counts at 24 hours p.i. The jet injected group showed a rise in counts over the next 6 days. Baseline levels were recorded on the day 14 bleeding. The needle and syringe injected group also showed rising WBC counts after the initial decrease which reached baseline values by day 6. All calves showed a drop in lymphocytes at 24 hours p.i., followed by a gradual return to baseline values by day 5 in the jet injected group and day 6 in the needle and syringe group. A drop in neutrophiles was seen in both jet injected and needle and syringe injected groups following virus exposure. The jet injected group showed the lowest count at 24 hours p.i. after which time counts rose until day 4. On day 5, 6 and 7 another less

TABLE 15--Median WBC, Lymphocyte, and Neutrophile Counts in Calves Inoculated with 10^6 $CCID_{50}$ VEE Virus--Diurnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	5.6*	4.2	1.0	11.6	9.4	1.2
-6	6.1	3.9	1.9	10.3	8.6	1.2
-5	11.0	3.0	7.8	10.2	7.5	2.4
-4	7.1	4.5	2.0	11.3	8.3	2.6
-3	8.8	6.7	1.8	9.5	6.7	2.1
-2	7.8	5.0	2.5	7.6	5.8	1.4
-1	6.5	3.7	2.4	8.7	6.7	1.5
0	6.3	4.3	1.6	8.2	6.0	1.6
1	2.0	1.6	0.3	3.5	2.1	1.3
2	3.4	2.3	1.0	4.8	4.1	0.2
3	5.3	3.3	1.5	7.0	5.5	1.2
4	5.3	3.3	1.5	5.5	4.2	1.0
5	6.1	4.4	1.4	6.5	4.5	1.6
6	5.0	3.7	1.1	9.3	6.9	1.7
7	5.4	4.1	1.2	9.1	6.5	2.1
14	8.0	5.2	2.3	9.3	6.3	2.7

*Expressed as counts per cubic mm X 10^3

L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

noticeable decline in neutrophiles was seen. The needle and syringe injected group showed a drop at 48 hours p.i. Counts then began to increase and baseline levels were seen by day 5 and afterward.

Viremia (Table 16)--All of the calves responded with detectable levels of circulating virus. Duration of viremia was shorter in the needle and syringe injected group but virus titers were higher or equal to titers in the jet injected calves. Jet injected calves had detectable virus for 3 days following virus injection, whereas needle and syringe injected calves showed only 1 or 2 days of viremia.

Antibody response (Table 17)--On day 6 and 7 HI antibody response was negative in all calves except one needle and syringe injected calf. By day 14 all calves showed positive HI titers of 1:20 or greater. Hemagglutination-inhibition antibody titers in the needle and syringe injected calves on day 14 and 21 were either equal to or higher than those of the jet injected calves.

Necropsy--No gross lesions were seen in any of the calves.

Calves: Nocturnal group. During the experiment all calves were observed to be clinically normal as previously reported by Harris.[b]

TABLE 16*--Serum Virus Titers** in Calves Inoculated with 10^6 $CCID_{50}$+ VEE Virus--Diurnal Group.

Calf #	Injection Method	Day Post-Inoculation 1	2	3	4
123	Jet	$10^{1.5}$	$10^{0.6}$	$10^{0.5}$	0
124	Jet	$10^{2.1}$	$10^{1.8}$	$10^{0.5}$	0
119	N&S	$10^{1.5}$	0	0	0
424	N&S	$10^{3.2}$	$10^{1.7}$	0	0

*Previously reported by Harris.[b]

**Expressed as median suckling mouse intracerebral lethal dose per 0.025 ml.

+Calculated dose. Actual dose as determined by backtitration was $10^{6.83}$ $CCID_{50}$ per ml.

N&S - Needle and syringe

TABLE 17*--Reciprocal of Hemagglutination-Inhibition Titers in Serums of Calves Inoculated with 10^6 $CCID_{50}$ VEE Virus--Diurnal Group.

Calf #	Injection Method	Day Post-Inoculation 6	7	14	21
123	Jet	N	N	20	--**
124	Jet	N	N	20	20
119	N&S	40	160	1280	--**
424	N&S	N	10	20	40

*Previously reported by Harris.[b]

**Killed on day 14.

N - Negative

N&S - Needle and syringe

Temperature (Fig 26)--A rise in temperature was noted in all calves at 12 hours following virus injection. The jet injected group maintained this rise for an additional 12 hours followed by temperatures within baseline limits. Reading above baseline values were noted on day 4 at 8PM and again on day 8. Needle and syringe injected calves showed a rising temperature on day 1 p.i. at the 12 and 24 hour recordings. On days 2 through 6 temperature readings remained higher than baseline values with considerable circadian variation, i.e. low AM readings and high PM readings. On days 7 p.i. and following, the temperature remained within baseline readings but was in the high normal range.

PCV (Fig 27)--All calves showed variable PCV readings during the experiment. The only consistency was that both groups of calves showed a drop in PCV values at 24 hours p.i. when compared to the PCV values at the time of virus challenge (day 0). This was followed by a rise in PCV in both groups so it was not clear as to whether the aforementioned decrease in PCV was due to virus activity.

Platelets (Fig 28)--Platelet counts were extremely variable. No changes in counts could be attributed to mode of injection or virus infection.

WBC, lymphocytes and neutrophiles (Table 18)--WBC counts were depressed at 24 hours p.i. The jet injected

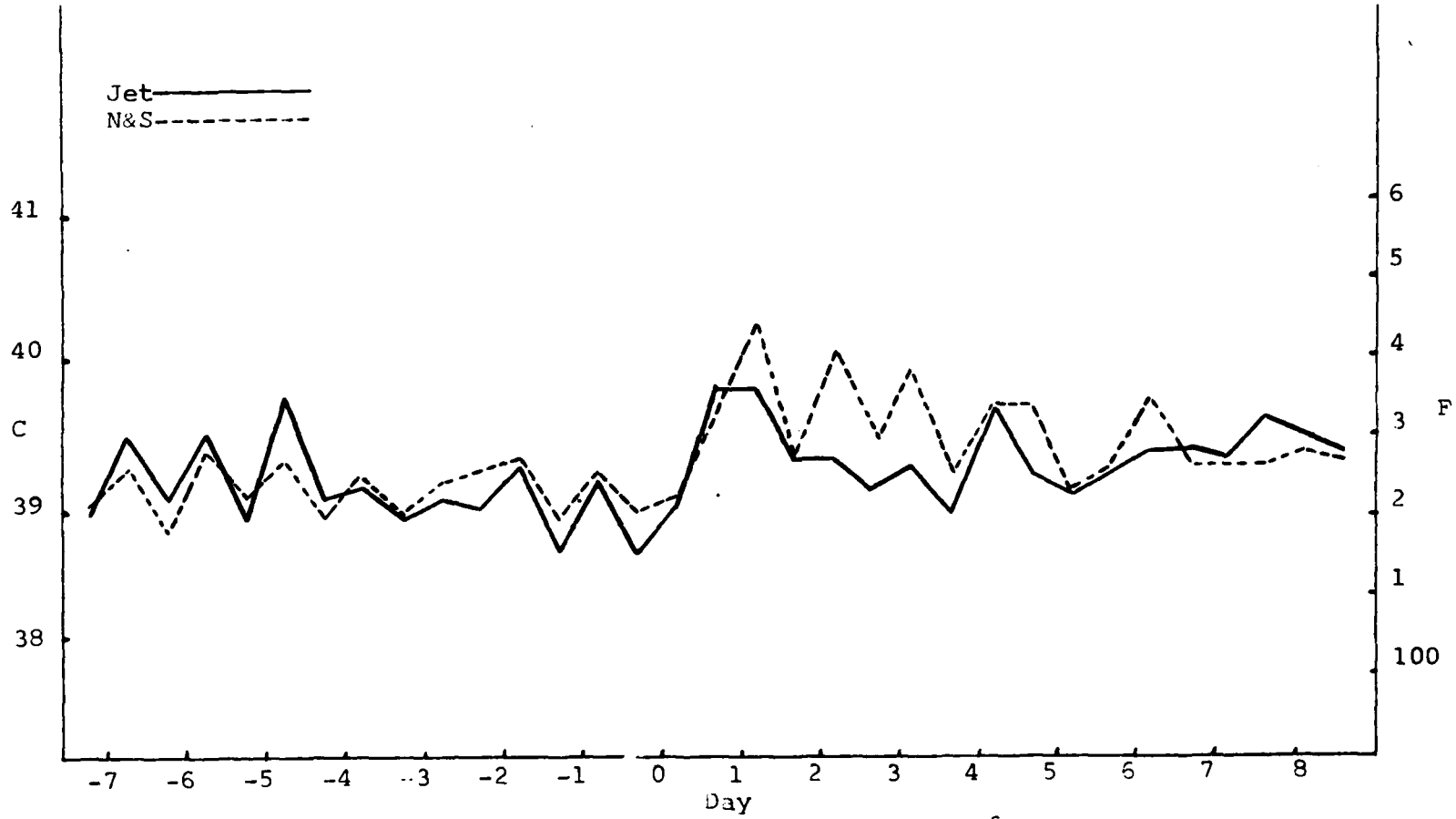


Fig 26--Median Temperature Response of Calves Injected with 10^6 $CCID_{50}$ Virulent VEE Virus--Nocturanl Group.

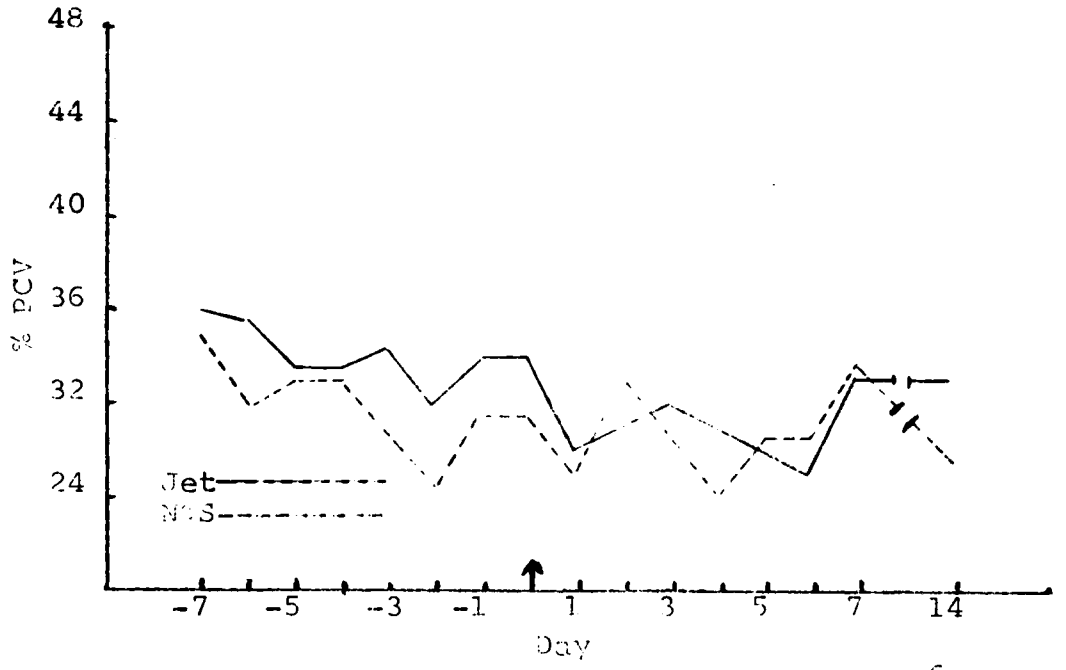


Fig 27--Median PCV values in calves receiving 10^6 $CCID_{50}$ VEE virus--nocturnal group.

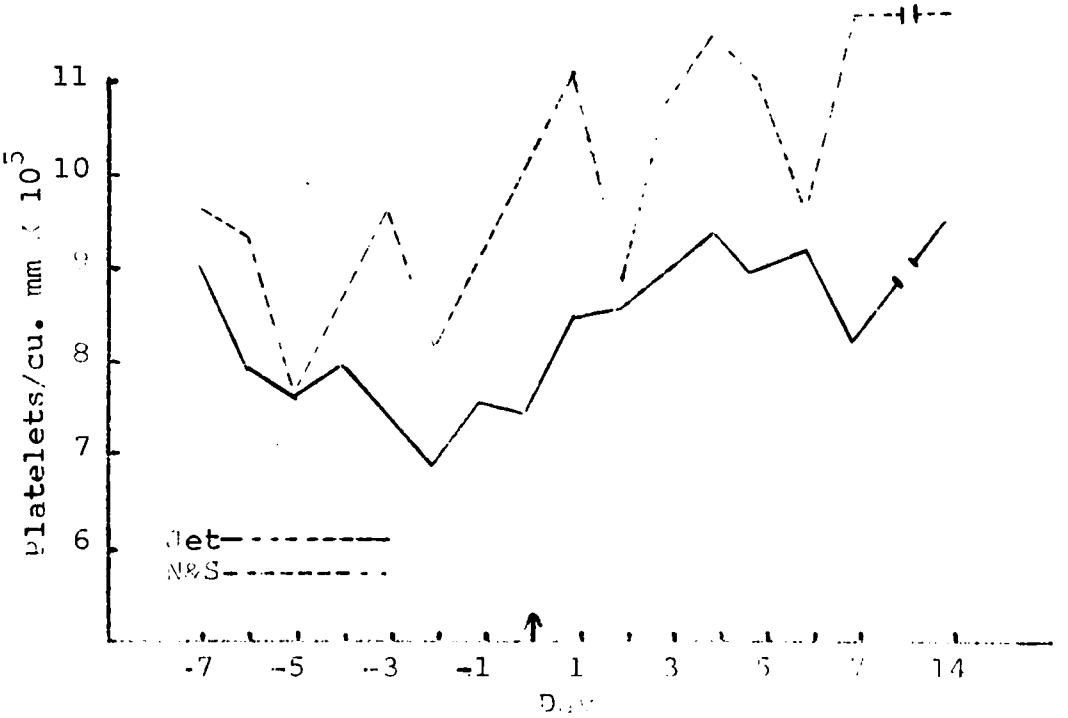


Fig 28--Median platelet counts in calves receiving 10^6 $CCID_{50}$ VEE virus--nocturnal group.

TABLE 18--Median WBC, Lymphocyte, and Neutrophile Counts in Calves Inoculated with 10^6 $CCID_{50}$ VEE Virus--Nocturnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	8.9*	7.4	2.4	15.7	10.9	4.4
-6	7.9	5.1	2.2	12.6	7.6	4.4
-5	8.7	6.0	2.6	11.9	8.7	2.9
-4	9.6	7.0	1.9	14.6	10.7	2.8
-3	9.7	6.9	2.2	14.5	9.1	4.5
-2	9.1	6.5	2.2	14.5	9.7	3.9
-1	12.5	6.5	5.2	14.0	8.4	4.8
0	8.6	6.8	1.5	11.1	7.5	3.0
1	7.7	5.7	1.8	9.0	6.6	1.8
2	9.5	7.8	1.5	8.3	8.7	3.1
3	10.5	7.3	3.0	9.8	6.2	2.3
4	10.0	7.4	2.1	11.2	8.1	2.5
5	8.6	6.8	1.4	10.7	8.3	2.3
6	8.9	6.6	2.1	9.0	6.2	2.4
7	9.7	7.4	2.1	9.5	6.5	2.1
14	11.8	8.1	3.2	14.4	9.7	3.1

*Expressed as counts per cubic mm X 10^3

L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

group showed only a very transient drop at 24 hours p.i. followed by a return to baseline levels at 48 hours p.i. The needle and syringe injected group showed a more dramatic depression of WBC's which remained low for 48 hours before increased counts were seen. A second depression was seen on days 5 through 7 p.i. with baseline ranges seen on the day 14 bleeding. Jet injected calves showed very erratic lymphocyte counts. Although a drop was seen at 24 hours p.i., compared to the count recorded at the time of virus exposure the 24 hour reading was within baseline values. A slight lymphocytosis was seen on days 2 through 4 and on days 7 and 14 p.i. in the jet injected group. Needle and syringe injected calves showed a primary drop at 24 hours p.i., a rise at 48 hours p.i. and a second decrease at 72 hours p.i. A decrease in lymphocytes below baseline was seen on days 6 and 7 also. Jet injected calves showed extremely variable neutrophile counts but there appeared to be a slight neutropenia for 48 hours p.i. Needle and syringe injected calves showed a drop in neutrophiles on day 1 and generally lower than baseline counts on days 2 through 7 p.i.

Viremia (Table 19)--Both jet injected calves showed a low but detectable viremia on day 1 p.i. At 48 hours 1 calf still showed a low serum virus titer. Only one

TABLE 19*--Serum Virus Titers** in Calves Inoculated with 10^6 $CCID_{50}$+ VEE Virus--Nocturnal Group.

Calf #	Injection Method	Day Post-Inoculation 1	2	3	4
2	Jet	$10^{0.5}$	0	0	0
3	Jet	$10^{0.5}$	$10^{0.4}$	0	0
1	N&S	0	0	0	0
4	N&S	$10^{1.6}$	$10^{0.5}$	0	0

*Previously reported by Harris.[b]

**Expressed as median suckling mouse intracerebral lethal dose per 0.025 ml serum.

+Calculated dose. Actual dose as determined by backtitration of challenge inoculum was $10^{6.16}$ $CCID_{50}$ per ml.

N&S - Needle and syringe

needle and syringe injected calf showed a detectable viremia on days 1 and 2 p.i., but the virus titer was higher than either of the jet injected calves.

Antibody response (Table 20)--Hemagglutination-inhibition antibody was not detected until day 14 p.i. All 4 calves showed comparable HI antibody titers on day 14 and 21.

Necropsy--No gross lesions were detected in any of the calves at necropsy.

Pigs: Diurnal group. Following virus injection, all pigs showed inappetance which started at 12 hours p.i. and lasted until day 3 p.i. Blood flecked feces were noted in pig #11 on days 3 and 5, and in pig #10 on day 5. Loose stools were noted in pig #9 on days 3, 5 and 6.

Temperature (Fig 29)--During the 7 day baseline period and after day 6 p.i., it was noted that the median temperatures showed a very definite circadian rhythm which was high in the AM and low in the PM. Both groups of pigs showed the same general temperature curve; a rise at 12 or 24 hours post virus injection, a drop at 36 to 48 hours p.i. and a secondary rise on days 3 and 4 p.i., then a return to baseline readings.

PCV (Fig 30)--PCV's followed the same general trend in both jet injected and needle and syringe injected pigs. There was a drop in values during the baseline period but

TABLE 20*--Reciprocal of Hemagglutination-Inhibition Titers in Serums of Calves Inoculated with 10^6 $CCID_{50}$ VEE Virus--Nocturnal Group.

Calf #	Injection Method	Day Post-Inoculation 6	7	14	21
2	Jet	N	N	80	--**
3	Jet	N	N	10	10
1	N&S	N	N	20	20
4	N&S	N	N	160	--**

*Previously reported by Harris.[b]

**Killed on day 14.

N - Negative

N&S - Needle and syringe

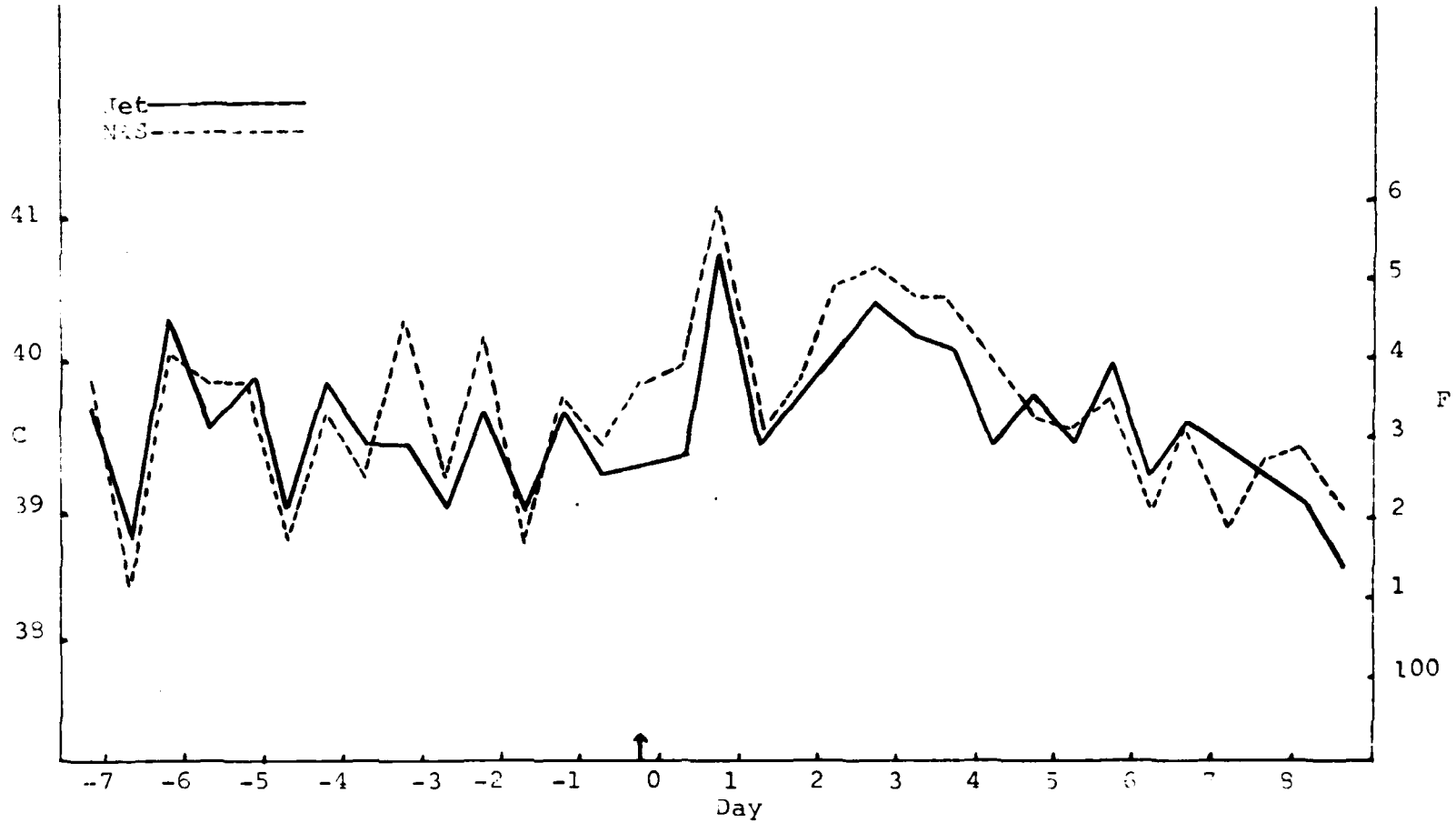


Fig 29--Median Temperature Response of Pigs Injected with 10^6 $CCID_{50}$ Virulent VEE Virus--Diurnal Group.

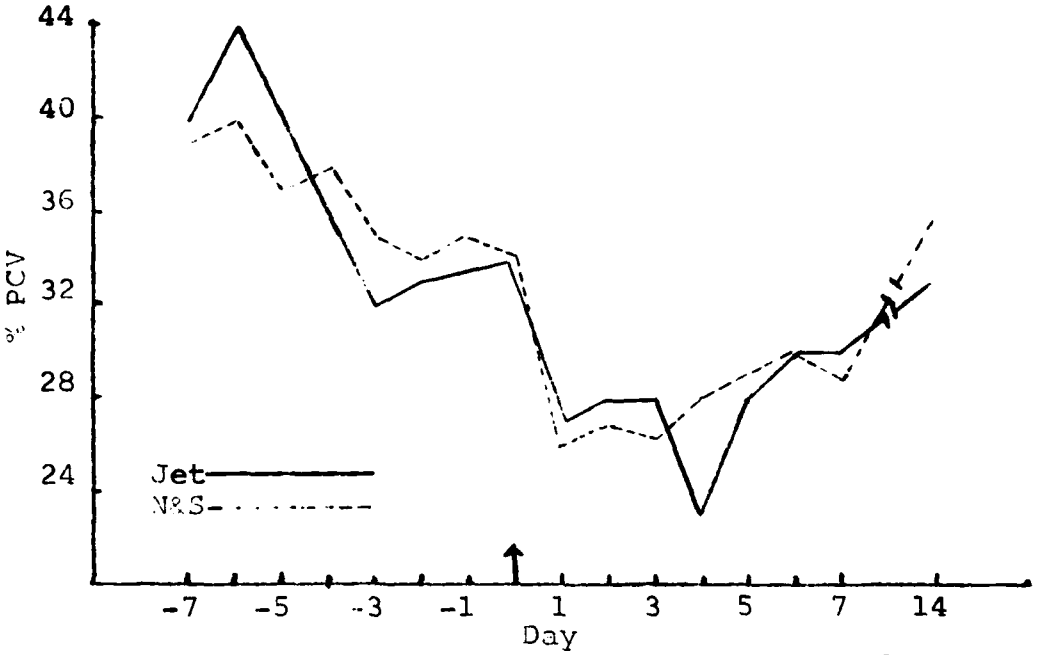


Fig 30--Median PCV values in pigs receiving 10^6 $CCID_{50}$ VEE virus--diurnal group.

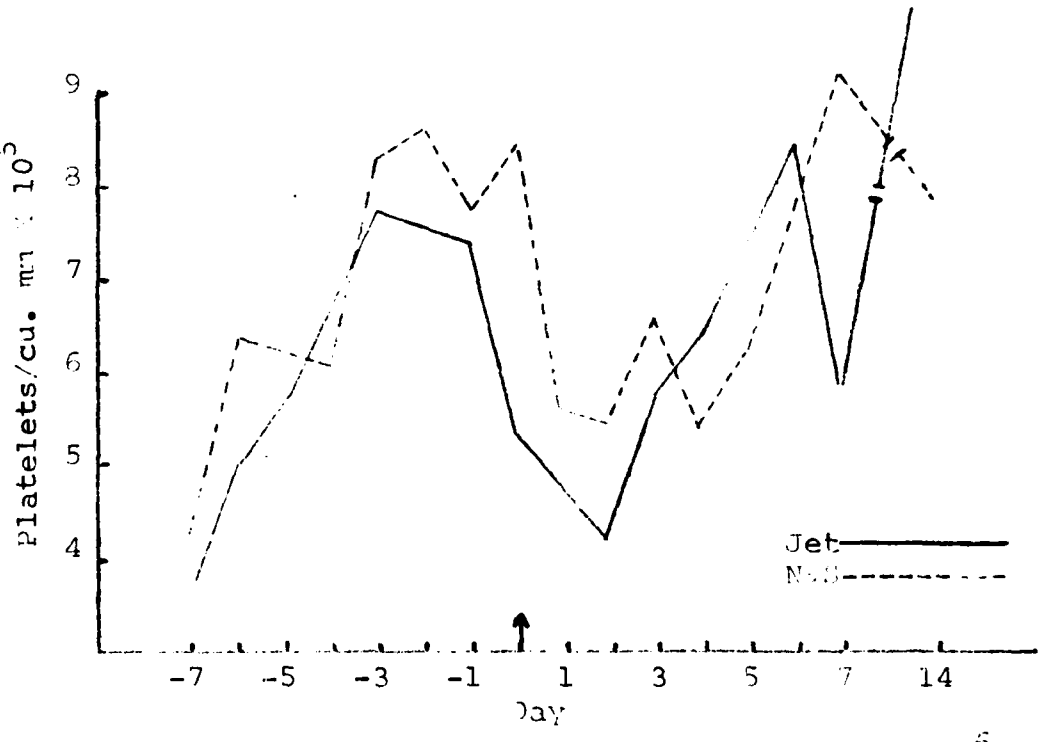


Fig 31--Median platelet counts in pigs receiving 10^6 $CCID_{50}$ VEE virus--diurnal group.

following virus challenge a marked decrease in PCV's was noted at 24 hours. The low readings tended to stabilize during days 1 through 4 followed by a general rising trend on days 5 through 7 and day 14.

Platelets (Fig 31)--Platelet counts followed a similar pattern in both groups. During the baseline period there was a rise in platelet values. After virus challenge, counts dropped on days 1 and 2, but not below initial baseline levels. After day 2, counts tended to rise.

WBC, lymphocytes, and neutrophiles (Table 21)--A depression in WBC's relative to counts obtained on days -6 through 0 was noted on days 1 through 3 p.i. in jet injected pigs and on days 1 and 4 p.i. in needle and syringe injected pigs. A rise in WBC counts was noted on days 5 through 7 and on day 14 in the needle and syringe injected group. Both jet injected and needle and syringe injected groups showed similar lymphocyte counts. A marked drop was noted on day 1 with a rise to above baseline levels on day 5 in jet injected pigs and day 7 in needle and syringe injected pigs. Due to extreme variability in the neutrophile counts, correlation of this cell type in relation to virus challenge was not clear, but there appeared to be an initial neutrophilia followed by a slight neutropenia.

TABLE 21--Median WBC, Lymphocyte, and Neutrophile Counts in Pigs Inoculated with 10^6 $CCID_{50}$ VEE Virus--Diurnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	7.1*	5.0	1.8	8.5	6.0	3.1
-6	17.9	4.7	11.6	15.6	7.6	4.2
-5	20.3	9.7	9.3	15.4	7.5	6.6
-4	20.2	11.3	8.1	17.8	10.5	5.4
-3	14.5	8.7	6.7	19.5	7.8	9.3
-2	16.1	9.0	5.8	18.9	9.4	8.3
-1	26.5	8.4	17.0	18.9	8.1	9.5
0	19.8	9.6	8.0	19.3	10.2	8.3
1	13.4	1.0	12.6	14.6	1.9	12.8
2	13.9	4.2	9.5	15.7	4.4	6.3
3	11.6	4.4	5.8	16.2	5.8	8.9
4	14.6	5.4	8.3	12.5	7.4	5.4
5	17.6	12.1	5.4	21.4	6.1	12.8
6	18.8	9.2	8.3	21.0	7.3	14.3
7	17.9	8.6	8.1	36.5	13.5	21.5
14	26.9	10.8	13.1	32.0	11.0	20.3

*Expressed as counts per cubic mm X 10^3

L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

Viremia (Table 22)--Two of the 3 jet injected pigs showed serum virus on days 1 and 2 p.i. The highest virus titer was $10^{5.3}$ $SMICLD_{50}$ per 0.025 ml (pig 9 day 2) and the lowest detectable titer was $10^{1.5}$ $SMICLD_{50}$ per 0.025 ml (pig 7 day 2). Pig 8 had no detectable viremia. All 3 needle and syringe injected pigs showed viremia on days 1 and 2 p.i. ranging from a high of $10^{4.5}$ $SMICLD_{50}$ per 0.025 ml (pig 10 day 1) to a low of $10^{0.4}$ $SMICLD_{50}$ per 0.025 ml of serum (pig 12 day 2).

Antibody response (Table 23)--Two of 3 jet injected pigs showed HI antibody titers on day 7 p.i., and all 3 demonstrated titers on day 14 which were above day 7 levels. Day 21 HI antibody titers were below day 14 levels. HI titers in jet injected pigs reached a maximum of 1:160 (pigs 7 and 9, day 14). All needle and syringe injected pigs showed titers at day 7 ranging from 1:20 (pig 11), to 1:160 (pig 10). Increased titers were seen on day 14 ranging from 1:80 to 1:320. On day 21, HI antibody titers in needle and syringe injected pigs dropped to 1:20 or 1:40. It was interesting to note that pig #8 showed no detectable viremia, but HI antibody was demonstrated on days 7 and 14; conversely pig #7 showed viremia on days 1 and 2 but no HI antibody until the day 14 bleeding.

Necropsy--The only pig which showed gross lesions at

TABLE 22--Serum Virus Titers* in Pigs Inoculated with 10^6 $CCID_{50}$** VEE Virus--Diurnal Group.

Pig #	Injection Method	Day Post-Inoculation 1	2	3	4
7	Jet	$10^{4.4}$	$10^{1.5}$	0	0
8	Jet	0	0	0	0
9	Jet	$10^{3.5}$	$10^{5.3}$	0	0
10	N&S	$10^{4.5}$	$10^{3.6}$	0	0
11	N&S	10^{2}	10^{1}	0	0
12	N&S	$10^{3.5}$	$10^{0.4}$	0	0

*Expressed as median suckling mouse lethal dose per 0.025 ml serum.

**Calculated dose. Actual dose as determined by backtitration of challenge inoculum was $10^{5.6}$ $CCID_{50}$ per ml.

N&S - Needle and syringe

TABLE 23--Reciprocal of Hemagglutination--Inhibition Titers in Serums of Pigs Inoculated with 10^6 $CCID_{50}$ VEE Virus--Diurnal Group.

Pig #	Injection Method	Day Post-Inoculation 0	7	14	21
7	Jet	N	N	160	--*
8	Jet	N	10	80	40
9	Jet	N	80	160	80
10	N&S	N	160	320	--*
11	N&S	N	20	80	20
12	N&S	N	40	80	40

*Killed on day 14.

N - Negative

N&S - Needle and syringe

necropsy was jet injected pig #8. A peritoneal abscess was noted from which Corynebacterium pseudotuberculosis and Staphylococcus aureus were isolated. No detectable viremia was seen in this pig.

Pigs: Nocturnal group. Clinical observations were complicated by difficulty in handling these animals due to their large size. The snout snare was used which resulted in struggling and excitement when the animals were bled and temperatured. Trauma was noted on the snouts and oral cavity as was some broken canine teeth. No data were obtained on days -6 and -5 of the baseline period. On day 1 p.i., the only clinical sign noted was a depressed appetite in all pigs. On day 2 p.i., the 3 jet injected pigs were still anorectic but 2 of the 3 needle and syringe injected pigs were eating. By day 4 p.i., all were feeding normally. Lameness was noted in pig #1 on day 14. Pig #4 showed an array of clinical sign during the experiment which included inappetance on day 10 p.i., cervical stiffness and hypermetria on day 11, lameness on days 12 through 14. On day 15 paralysis, paddling, convulsions, vomiting, nystagmus and trembling were noted which persisted on day 16, at which time the animal was killed.

Temperature (Fig 32)--At 12 hours p.i., all pigs showed a rise in temperature of greater than 1.1 C (2 F)

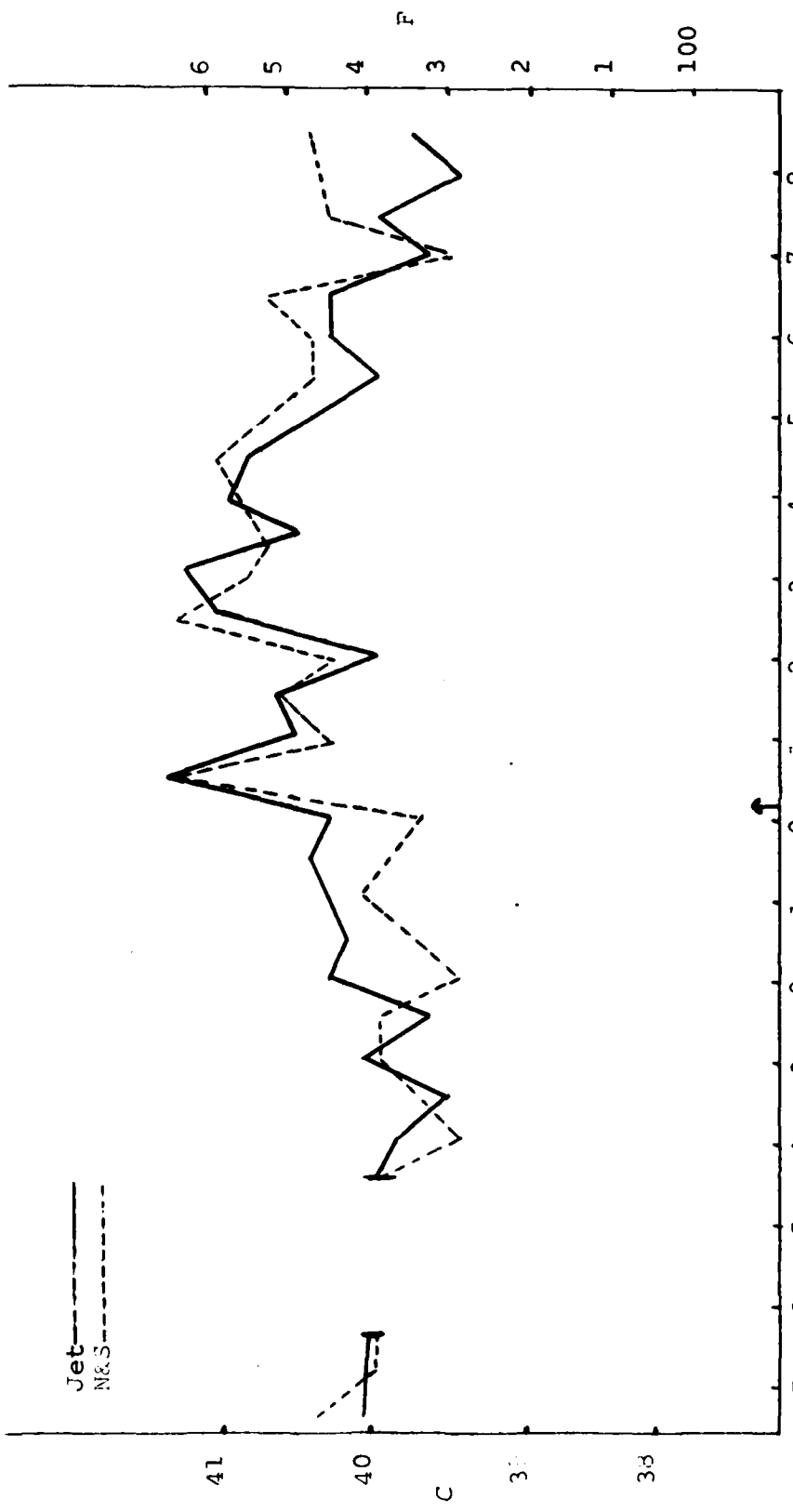


Fig 32--Median Temperature Response of Pigs Injected with 10^6 $CCID_{50}$ Virulent VEE Virus--Nocturnal Group.

above baseline. A drop in temperature was noted at 24 through 48 hours but a second rise was seen on day 3 through 5. Temperatures recorded up to day 5 p.i. had a very similar pattern in both jet injected and needle and syringe injected pigs. On day 6 p.i., the jet injected pigs dropped back to baseline values and remained there. The needle and syringe injected pigs generally continued to show increased temperatures past day 8 p.i.

PCV (Fig 33)--All pigs showed decreasing PCV values until day -1 where they tended to stabilize. Needle and syringe injected pigs showed a decrease in PCV's on days 2 through 7 p.i. and back to baseline values on day 14. Jet injected pigs showed no changes in PCV's until days 3 through 5 p.i. when a rise was noted followed by a drop on days 6 and 7 p.i. with a return to baseline values on day 14 p.i.

Platelets (Fig 34)--Due to the extreme variability of the platelet counts, correlation to virus activity or mode of injection was inconclusive.

WBC, lymphocytes, and neutrophiles (Table 24)--Total WBC's showed a marked depression in both groups at 24 hours p.i. By day 2, WBC's in the jet injected group had returned to baseline values and generally continued to increase to above baseline values on days 6 and 14.

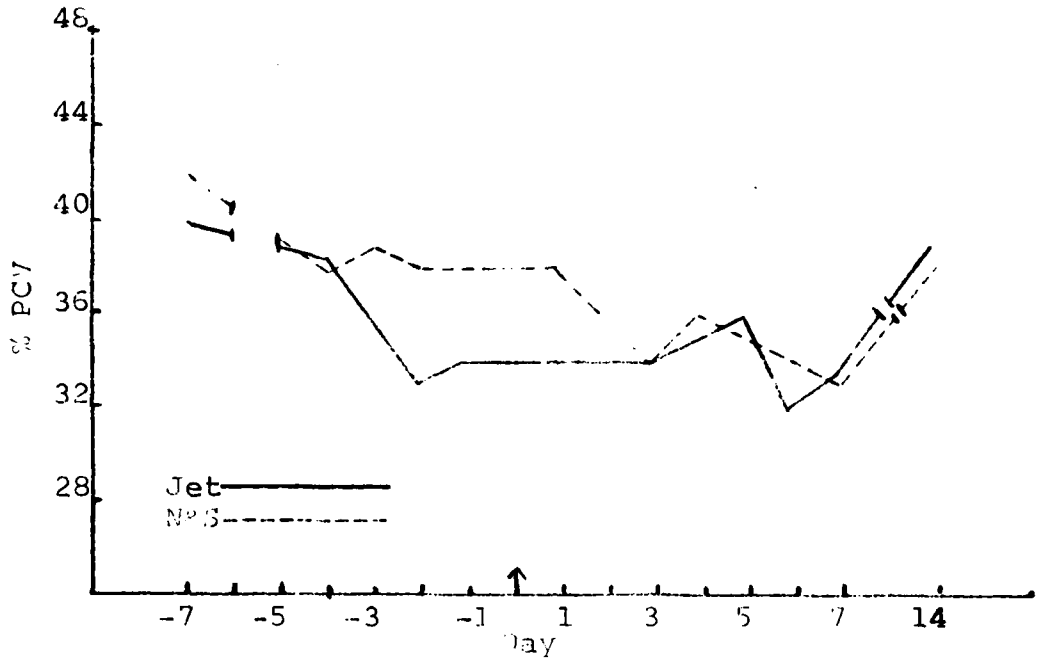


Fig 33--Median PCV values in pigs receiving 10^6 $CCID_{50}$ [illegible] virus--nocturnal group.

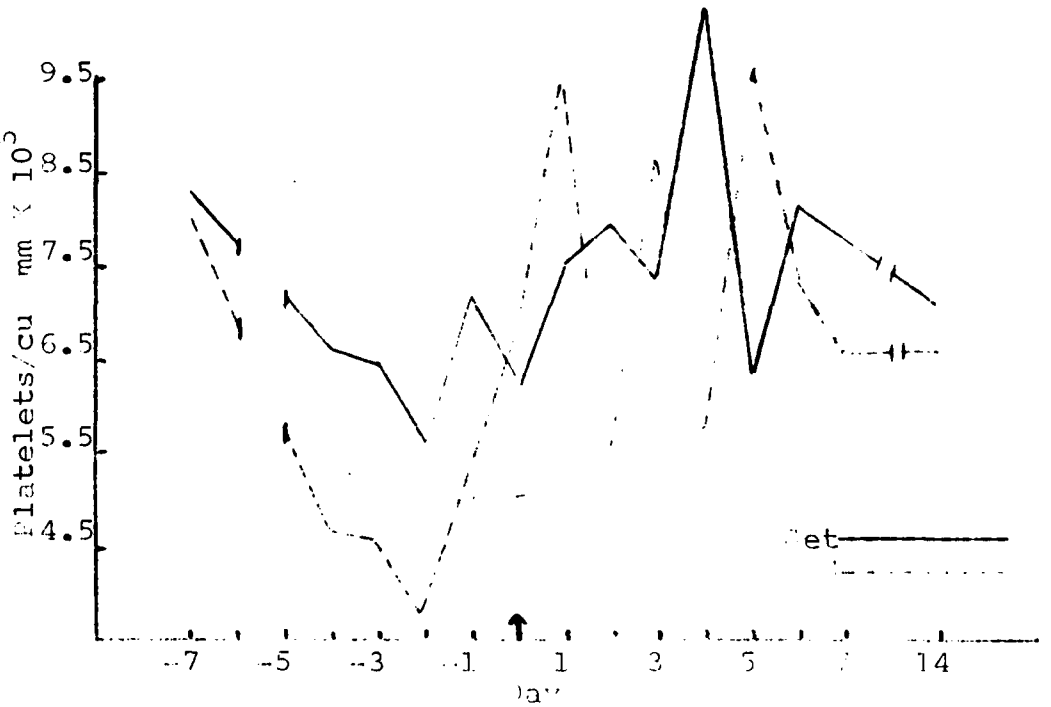


Fig 34--Median platelet counts in pigs receiving 10^6 $CCID_{50}$ [illegible] virus--nocturnal group.

TABLE 24--Median WBC, Lymphocyte, and Neutrophile Counts in Pigs Inoculated with 10^6 $CCID_{50}$ VEE Virus--Nocturnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	27.1*	10.7	8.6	20.4	14.1	8.2
-6	----**	----**	----**	----**	----**	----**
-5	----**	----**	----**	----**	----**	----**
-4	18.2	9.7	6.0	19.9	10.9	7.5
-3	16.8	8.7	8.6	16.6	7.6	7.4
-2	15.5	8.5	6.1	20.1	11.6	8.0
-1	23.1	9.9	9.8	17.9	10.1	6.1
0	18.3	9.7	6.6	18.2	12.5	4.8
1	12.6	4.8	8.8	8.9	5.3	3.6
2	16.7	5.4	11.9	18.0	7.0	10.1
3	16.5	6.7	9.5	14.8	7.6	5.6
4	19.1	6.4	13.0	17.9	10.2	7.3
5	18.7	10.3	13.5	23.7	11.9	11.1
6	28.0	7.2	20.2	21.7	11.3	7.3
7	22.9	7.1	14.2	20.4	8.3	10.0
14	31.6	8.8	22.4	22.2	8.3	11.8

*Expressed as counts per cubic mm X 10^3

**No sample taken.

L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

Needle and syringe injected pigs showed a slightly depressed WBC count on day 3 p.i. and generally higher than baseline values on days 5 through 7 and 14 p.i. Lymphopenia was marked at 24 hours in all pigs. On day 2 p.i. and following, rising lymphocyte counts were noted with return to baseline values on day 4 in the needle and syringe injected pigs and on day 5 in the jet injected pigs. The jet injected pigs showed another drop in lymphocytes on days 6 and 7. The jet injected group showed a neutrophilia on days 2, 4 through 7 and 14 p.i. The needle and syringe injected pigs showed a transient neutropenia on day 1 p.i. and a neutrophilia on days 2, 5, 7, and 14 p.i.

Viremia (Table 25)--All jet injected pigs showed a viremia on days 1 and 2 p.i. Virus titers ranged from a high of $10^{1.86}$ $SMICLD_{50}$ per 0.025 ml serum (pig #3 day 1) to a low of $10^{0.41}$ $SMICLD_{50}$ per 0.025 ml serum (pig #2 day 1). Two of the needle and syringe injected pigs showed a viremia on day 1 and all 3 were viremic on day 2 p.i. Titers in the serum ranged from a high of $10^{2.5}$ $SMICLD_{50}$ per 0.025 ml (pig #4 days 1 and 2) to a low of $10^{0.4}$ $SMICLD_{50}$ per 0.025 ml (pig #6 day 2).

Antibody response (Table [illegible])--All pigs showed HI antibody titers on day 7 ranging from 1:20 (pig #5) to 1:80 (pig #3). All titers were equal or increased on day

TABLE 25--Serum Virus Titers* in Pigs Inoculated with 10^6 $CCID_{50}$** VEE Virus--Nocturnal Group.

Pig #	Injection Method	Day Post-Inoculation 1	2	3	4
1	Jet	$10^{1.2}$	$10^{1.5}$	0	0
2	Jet	$10^{0.4}$	$10^{0.5}$	0	0
3	Jet	$10^{1.9}$	$10^{1.5}$	0	0
4	N&S	$10^{2.5}$	$10^{2.5}$	0	0
5	N&S	0	$10^{0.5}$	0	0
6	N&S	$10^{0.5}$	$10^{0.4}$	0	0

*Expressed as median suckling mouse intracerebral lethal dose per 0.025 ml serum.

**Calculated dose. Actual dose as determined by backtitration of challenge inoculum was $10^{6.37}$ $CCID_{50}$ per ml.

N&S - Needle and syringe

TABLE 26--Reciprocal of Hemagglutination-Inhibition Titers in Serums of Pigs Inoculated with 10^6 $CCID_{50}$ VEE Virus--Nocturnal Group.

Pig #	Injection Method	Day Post-Inoculation 0	7	14	21
1	Jet	N	40	80	--*
2	Jet	N	40	40	40
3	Jet	N	80	160	160
4	N&S	N	40	320	--**
5	N&S	N	40	640	--*
6	N&S	N	40	320	160

*Killed on day 14.

**Killed on day 16.

N - Negative

N&S - Needle and syringe

14 and titers remained the same or were decreased in pigs remaining on day 21.

Necropsy--Pig #1 killed on day 14 showed no gross lesions. Pig #5 also killed on day 14 showed pus in the carpal and hock joints. Pig #4 was killed on day 16 and showed lung congestion with foreign body pneumonia from inhalation of vomitus. On histopathologic examination, a brain abscess and meningitis were seen. Pig #3, killed on day 21, showed adhesions of the spleen and intestines and an abscess in the abdominal cavity. Pig #'s 2 and 6, also killed on day 21, showed no gross lesions.

Sheep: Diurnal group. Clinically, all sheep remained apparently healthy throughout the experiment.

Temperature (Fig 35)--An elevated temperature response was seen in the needle and syringe injected sheep on day 2 p.i. (48 hour reading), which returned to baseline levels 12 hours later. The jet injected group showed two minor temperature responses. One at 12 hours p.i. and the other 18 to 30 hours later. However, these temperature changes did not appear to be significantly elevated.

PCV (Fig 36)--PCV's showed a decline during the baseline period and remained essentially stable following virus injection.

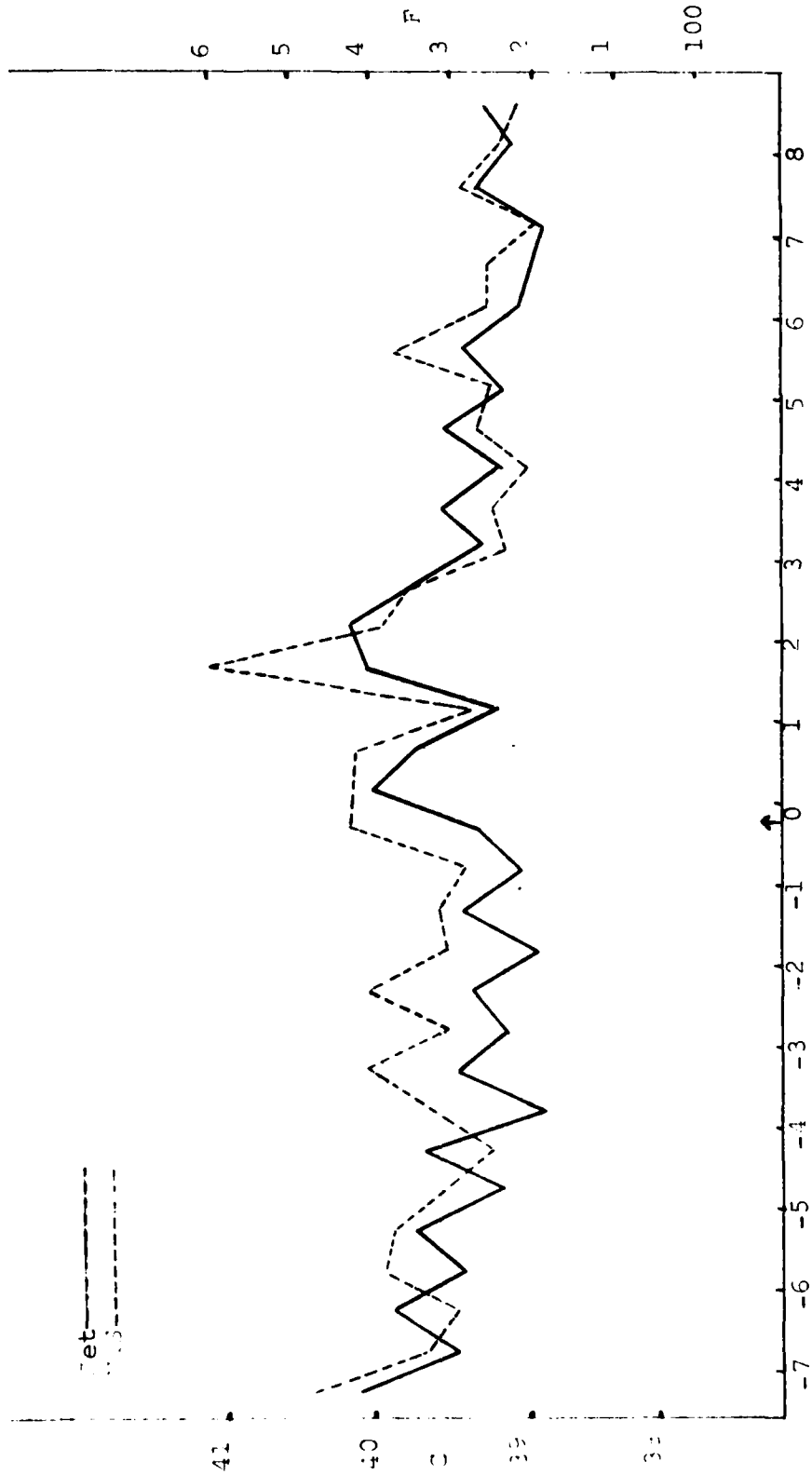


Fig 35--Median Temperature Response of Sheep Injected with 10^6 $CCID_{50}$ Virulent VEE Virus--Diurnal Group.

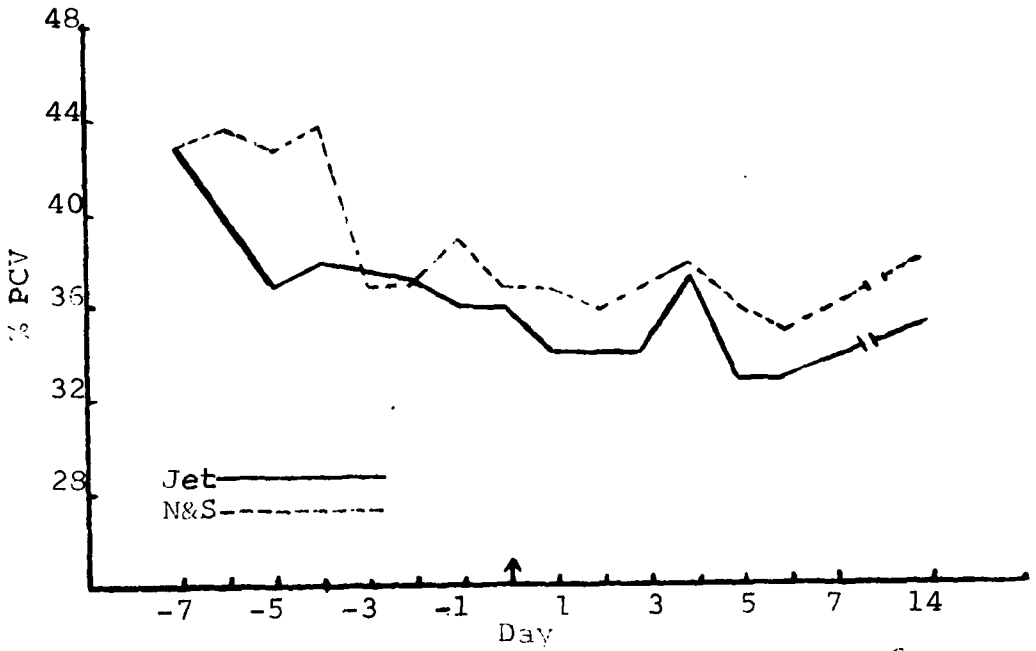


Fig 36--Median PCV values in sheep receiving 10^6 $CCID_{50}$ VEE virus--diurnal group.

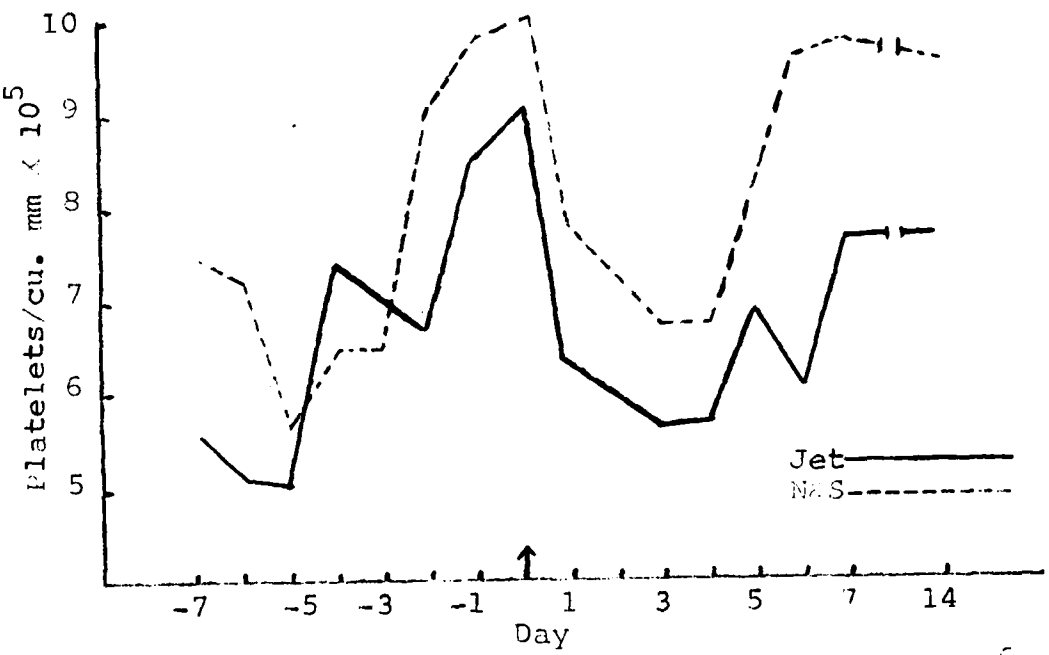


Fig 37--Median platelet counts in sheep receiving 10^6 $CCID_{50}$ VEE virus--diurnal group

Platelets (Fig 37)--Platelet counts showed a rather noticeable rise during the latter part of the baseline period and a drop was noted in both groups on days 1 through 4 p.i., followed by a rise in counts comparable to previously recorded high levels. The lowest counts recorded after virus injection still exceeded initial counts recorded for the baseline values.

WBC, lymphocytes and neutrophiles (Table 27)--Fluctuations in WBC counts made interpretation difficult. There appeared to be a transient drop on day 1 p.i. in the jet injected group and on day 2 in the needle and syringe injected group. Lymphopenia was observed on the same days as the leukopenia occurred. Decreased neutrophile counts were seen on day 4 p.i. in both groups. The jet injected sheep showed slight neutropenia on days 3, 5, and 6 p.i., also a rising trend was noted thereafter.

Viremia (Table 28)--The sheep were not very responsive to VEE virus challenge. Only 2 of the 4 showed moderate levels of viremia; one jet injected sheep and 1 needle and syringe injected sheep. Circulating virus was found only on days 1 and 2 p.i. Of the remaining 2 sheep, the needle and syringe injected sheep had a barely detectable viremia on day 1 p.i. of less than 1 $SMICLD_{50}$ per 0.025 ml serum. The other jet

TABLE 27--Median WBC, Lymphocyte, and Neutrophile Counts in Sheep Inoculated with 10^6 $CCID_{50}$ VEE Virus--Diurnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	7.8*	5.5	2.1	8.6	4.4	3.6
-6	8.0	7.3	2.5	6.4	4.0	2.2
-5	8.6	6.2	2.0	8.3	5.2	2.5
-4	11.2	8.1	2.9	8.9	6.0	2.1
-3	9.5	7.0	2.2	7.8	5.6	2.0
-2	11.4	6.9	3.9	9.5	6.4	2.8
-1	9.0	6.1	2.5	11.5	7.0	3.7
0	8.9	6.4	2.4	10.1	5.4	4.3
1	7.4	4.7	2.4	8.8	5.5	3.1
2	8.4	5.4	2.8	6.8	4.1	2.4
3	9.5	7.9	1.6	9.0	6.5	2.1
4	8.1	7.3	0.8	8.9	7.4	1.1
5	8.2	6.8	1.3	8.7	4.7	2.9
6	7.1	5.0	1.7	7.5	4.6	2.2
7	8.5	6.2	2.0	8.6	6.0	1.9
14	9.8	6.3	2.8	9.7	7.1	2.2

*Expressed as counts per cubic mm X 10^3

L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

TABLE 28--Serum Virus Titers* in Sheep Inoculated with 10^6 $CCID_{50}$** VEE Virus--Diurnal Group.

Sheep #	Injection Method	Day Post-Inoculation 1	2	3	4
212	Jet	0	0	0	0
215	Jet	$10^{2.8}$	$10^{2.7}$	0	0
219	N&S	$<10^0$	0	0	0
182	N&S	$10^{2.5}$	$10^{1.8}$	0	0

*Expressed as median suckling mouse intracerebral lethal dose per 0.025 ml serum.

**Calculated dose. Actual dose as determined by backtitration of challenge inoculum was $10^{5.5}$ $CCID_{50}$ per ml.

N&S - Needle and syringe.

injected sheep showed no detectable viremia.

Antibody response (Table 29)--Hemagglutination-inhibition antibody titers were seen in all sheep on day 7 p.i. All except needle and syringe injected sheep #219 showed increased antibody titers on day 14. Day 21 HI titers were less than those recorded on day 14 on the 2 animals available for testing. Titers ranged from 1:20 to 1:40 on day 7 for both groups; 1:40 to 1:60 on day 14 in jet injected sheep; and 1:10 to 1:80 in needle and syringe injected sheep on day 14. On day 21, the jet injected sheep had a titer of 1:10 and the needle and syringe injected sheep had a titer of 1:20.

Necropsy--On necropsy, nasal bots were noted in sheep #219. Sheep #215 had a pale area on the liver surface which did not extend into the liver parenchyma.

Sheep: Nocturnal group. On days 1 and 2 p.i., all sheep appeared slightly depressed and reluctant to move. At 8AM on day 3 p.i. and following the sheep appeared normal.

Temperature (Fig 38)--Both groups of sheep showed similar temperature patterns although the needle and syringe injected group showed generally higher median values. Twelve hours p.i. a rise in temperature of almost 1.1 C (2 F) was noted in needle and syringe injected sheep and more than a 1.7 C (3 F) rise was seen in the jet

TABLE 29--Reciprocal of Hemagglutination-Inhibition Titers in Serums of Sheep Inoculated with 10^6 $CCID_{50}$ VEE Virus--Diurnal Group.

Sheep #	Injection Method	Day Post-Inoculation 0	7	14	21
212	Jet	N	20	40	10
215	Jet	N	40	160	--*
219	N&S	N	20	10	--*
182	N&S	N	20	80	20

*Killed on day 14.

N - Negative

N&S - Needle and syringe

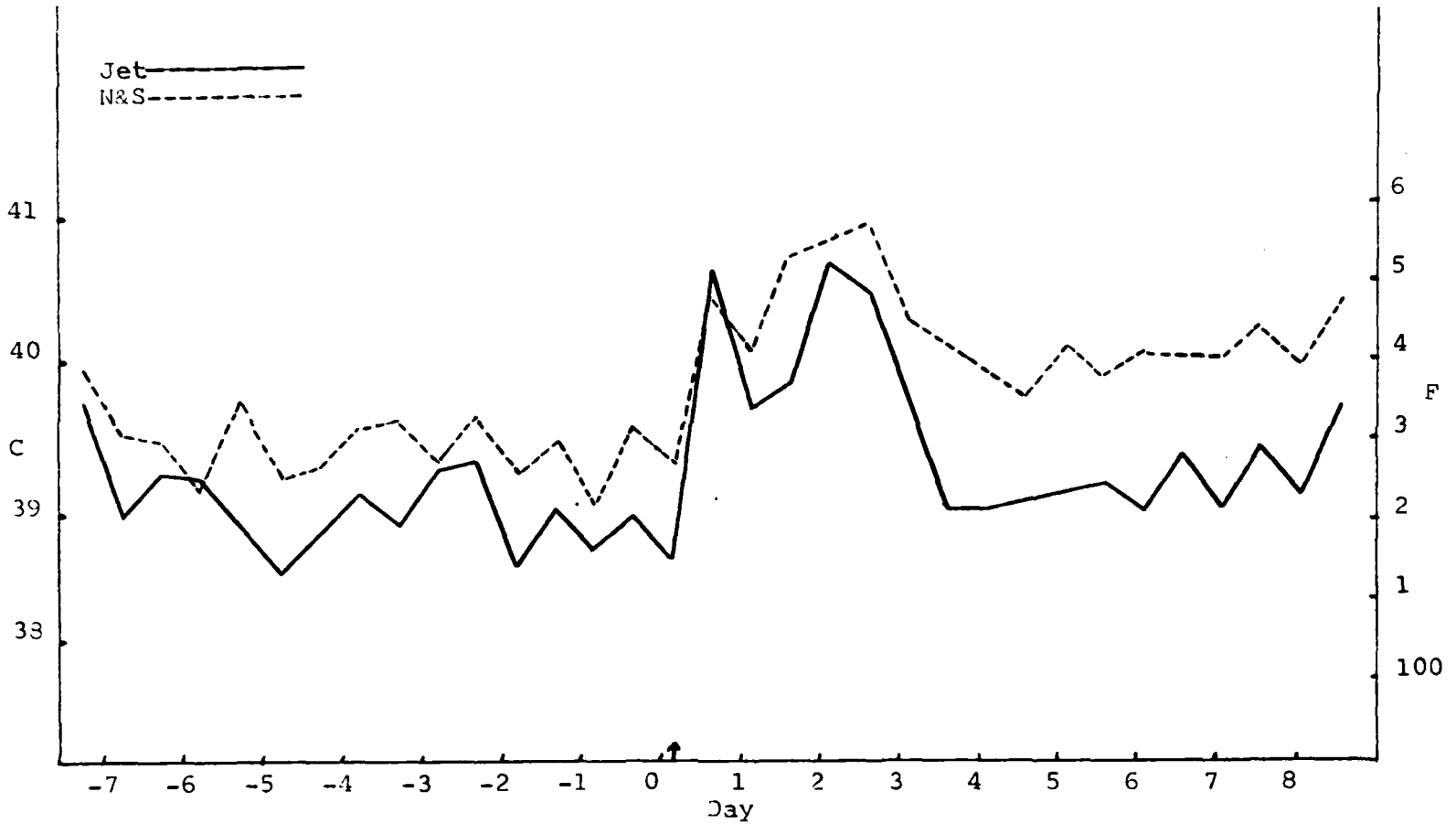


Fig 38--Median Temperature Response of Sheep Injected with 10^6 $CCID_{50}$ Virulent VEE Virus--Nocturnal Group.

injected group. A slight drop in temperature was noted at the 24 hour p.i. reading, followed by a second rise at 36 hours p.i. in the needle and syringe injected group and 48 hours p.i. in the jet injected group. Temperatures were still elevated on day 3 but declined on day 4. Temperatures after day 4 p.i. were slightly above baseline values in both groups.

PCV (Fig 39)--PCV's were similar in both groups and showed a general decrease throughout the experiment that did not correlate with time of virus exposure. PCV values were also low at the day 14 bleeding.

Platelets (Fig 40)--Platelet counts showed a rising trend in both groups during the baseline period. After virus injection counts were within baseline values except for day 14 counts which were higher. Generally, the rise in platelets correlated with a decline in PCV.

WBC, lymphocytes and neutrophiles (Table 30)--WBC counts were somewhat variable during the baseline period especially in the jet injected group. A definite drop in WBC's was seen in the needle and syringe injected group on days 1 and 2 p.i. A slight drop was seen in the jet injected sheep on day 1 p.i. with a return to baseline values on day 2 p.i. The same general pattern that was seen in the WBC counts was observed in the lymphocyte counts i.e. a drop on days 1 and 2 p.i. in both groups;

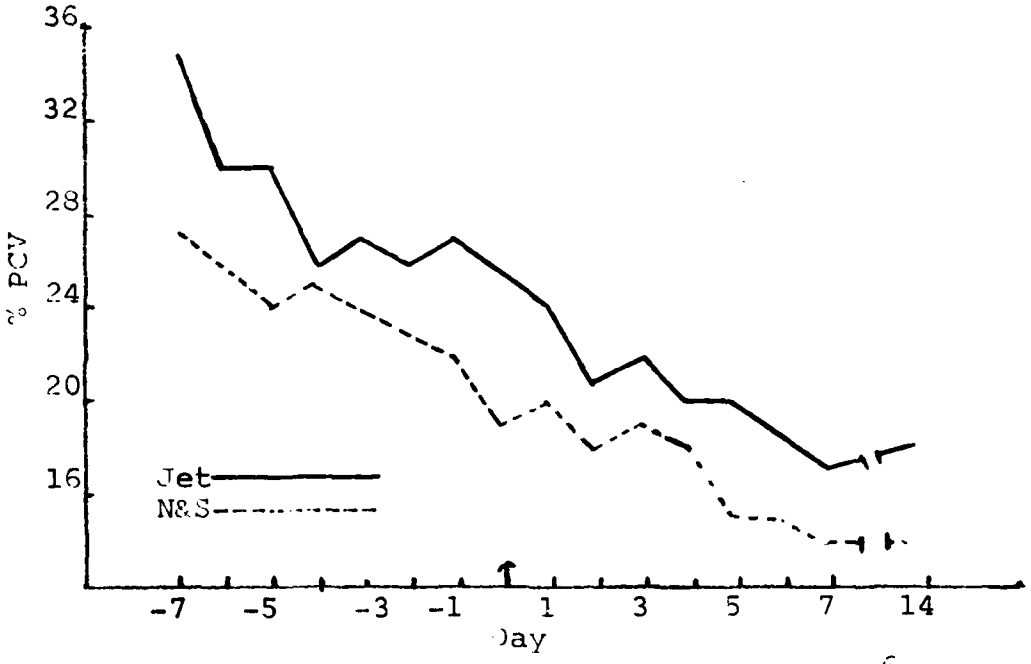


Fig 39--Median PCV values in sheep receiving 10^6 $CCID_{50}$ VEE virus--nocturnal group.

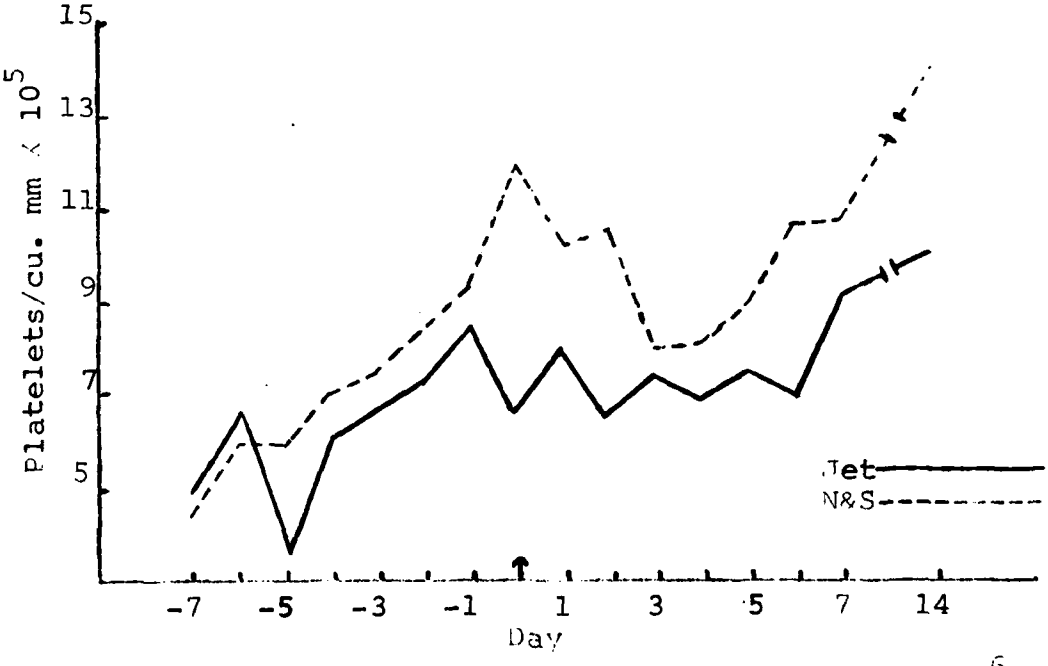


Fig 40--Median platelet counts in sheep receiving 10^6 $CCID_{50}$ VEE virus--nocturnal group.

TABLE 30--Median WBC, Lymphocyte, and Neutrophile Counts in Sheep Inoculated with 10^6 $CCID_{50}$ VEE Virus--Nocturnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	7.6*	3.9	2.9	8.2	7.5	0.2
-6	8.2	4.8	2.7	8.5	3.4	4.5
-5	6.1	3.1	2.3	6.5	2.8	3.1
-4	6.9	4.4	2.1	5.3	2.8	2.0
-3	6.5	3.2	2.8	6.5	3.8	2.8
-2	7.1	4.3	2.1	5.8	3.3	2.5
-1	8.7	4.8	2.8	6.1	2.7	3.1
0	11.2	8.4	1.9	5.5	4.0	1.3
1	5.4	2.6	2.5	3.7	2.1	1.3
2	6.4	4.1	2.2	3.8	2.3	1.4
3	8.6	5.8	2.7	6.0	5.3	0.6
4	7.0	5.5	1.3	6.2	4.5	1.4
5	5.9	2.9	2.6	5.5	2.8	2.4
6	6.8	4.9	1.5	7.1	3.7	3.2
7	5.7	4.0	1.5	7.6	2.9	4.4
14	7.1	4.4	2.6	7.5	3.7	3.6

*Expressed as counts per cubic mm. X 10^3

L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

lymphopenia in the jet injected animals was also noted on day 5 p.i. The needle and syringe injected group of sheep showed a decreasing neutrophile count on days 1 through 3 p.i. whereas the jet injected group had low neutrophile counts on days 4, 6, and 7 p.i.

Viremia (Table 31)--Measurable levels of circulating virus were found in all sheep on days 1 and 2 p.i. regardless of mode of injection. Minimal viremia titers were detected on day 3 in 2 sheep and moderate levels in a third sheep. On each of the 3 days of viremia, the virus titer in the needle and syringe injected group was slightly higher than the titer in the jet injected group.

Antibody response (Table 32)--Only one animal in each group developed a detectable HI antibody response. Sheep #175 in the needle and syringe injected group had a 1:20 HI titer on days 7 and 14, and a 1:160 titer on day 21. Sheep #198, injected by the jet injector, did not have an HI titer on day 7 but had a 1:20 on day 14 and a 1:40 titer on day 21.

BUN--Of all animals tested only this group of sheep had BUN values which exceeded published norms of 20 mg%.[41] Two sheep (#175 and #213) had BUN values of 22 to 25 mg% recorded during the baseline period whereas the other 2 (#198 and #286) had no elevated BUN values. All 4 sheep had BUN values ranging from 30 to 37 mg% at

TABLE 31--Serum Virus Titers* in Sheep Inoculated with 10^6 $CCID_{50}$** VEE Virus--Nocturnal Group.

Sheep #	Injection Method	Days Post-Inoculation 1	2	3	4
198	Jet	$10^{1.4}$	$10^{1.2}$	$< 10^0$	0
213	Jet	$10^{2.4}$	$10^{2.5}$	0	0
175	N&S	$10^{3.2}$	$10^{3.2}$	10^2	0
286	N&S	$10^{2.5}$	$10^{3.1}$	$< 10^0$	0

*Expressed as median suckling mouse intracerebral lethal dose per 0.025 ml serum.

**Calculated dose. Actual dose as determined by backtitration of challenge inoculum was $10^{5.5}$ $CCID_{50}$ per ml.

N&S - Needle and syringe

TABLE 32--Reciprocal of Hemagglutination-Inhibition Titers in Serums of Sheep Inoculated with 10^6 $CCID_{50}$ VEE Virus--Nocturnal Group.

Sheep #	Injection Method	Day Post-Inoculation 0	7	14	21
175	N&S	N	20	20	160
286	N&S	N	N	N	--*
198	Jet	N	N	20	40
213	Jet	N	N	N	--*

*Killed on day 14.

N - Negative

N&S - Needle and syringe

various times after virus injection.

Necropsy--Gross lesions were absent on all 4 sheep.

Goats: Diurnal group. The only clinical sign observed in the goats after virus challenge was inappetance on day 1 p.i. and at the morning observation on day 2 p.i.

Temperature (Fig 41 and 42)--Rectal temperature response corresponded to challenge dose of virus. Those goats receiving 2 logs of VEE challenge virus showed no temperature elevation, whereas those receiving 6 logs of virus showed elevated temperatures at 12 hours p.i. The needle and syringe injected goats had an almost 1.7 C (3 F) rise while the jet injected group had an almost 1.1 C (2 F) rise. Temperatures for the remainder of the experiment were not significantly above baseline readings.

PCV (Fig 43 and 44)--PCV's in the needle and syringe injected goat which received 2 logs of virus did not vary from baseline values. The jet injected goat which received the 2 logs of virus showed a drop in PCV at day 3 p.i. In goats receiving 10^6 $CCID_{50}$ VEE virus, the PCV of the jet injected group did not vary from baseline. The needle and syringe injected goats which received the high dose of virus showed a drop in PCV values on days 2 and 3 p.i., and a higher than baseline value on day 5 p.i.

Platelets (Fig 45 and 46)--There were no significant

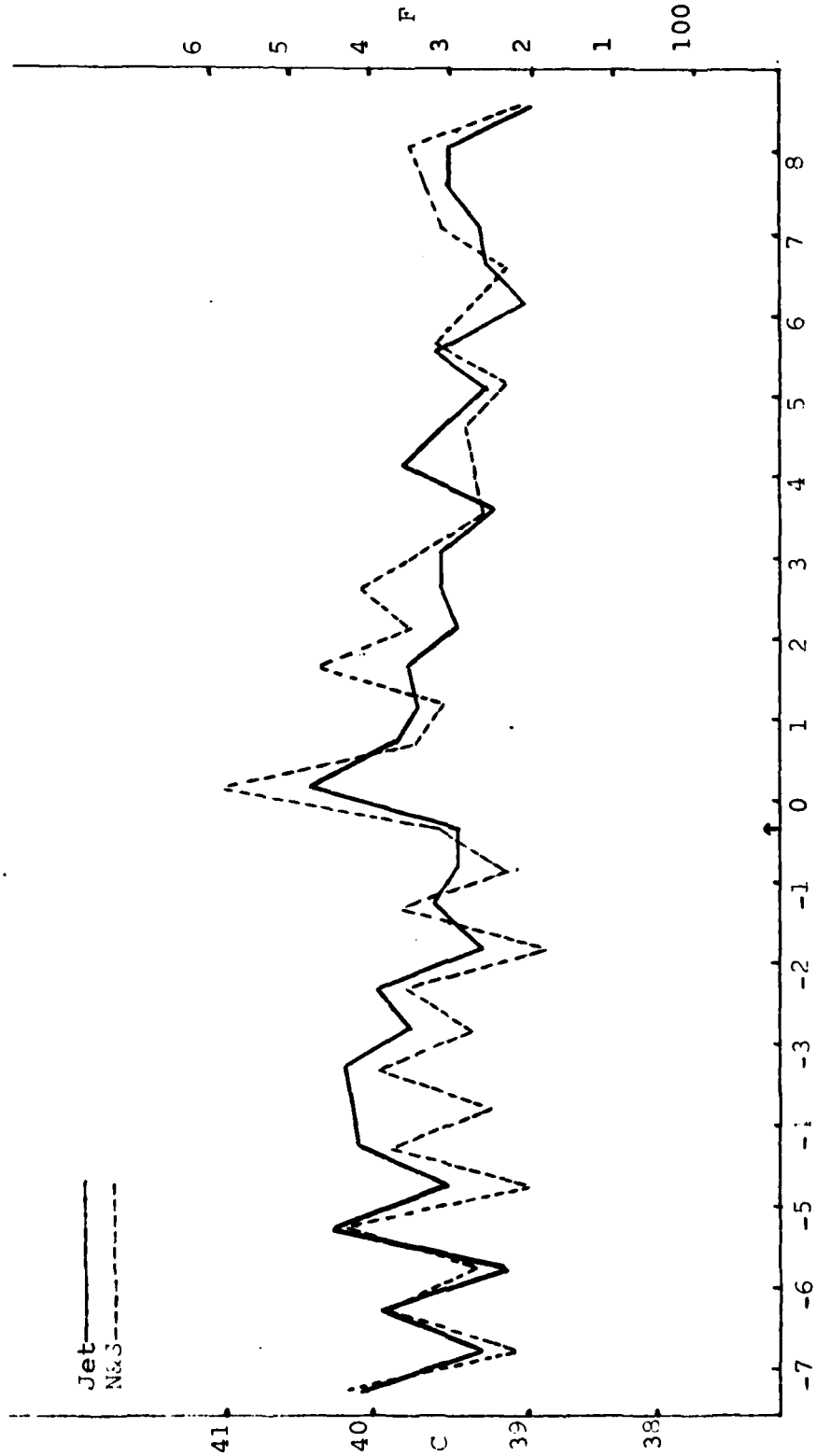


Fig 41--Median Temperature Response of Goats Injected with 10^6 $CCID_{50}$ Virulent VEE Virus--Diurnal Group.

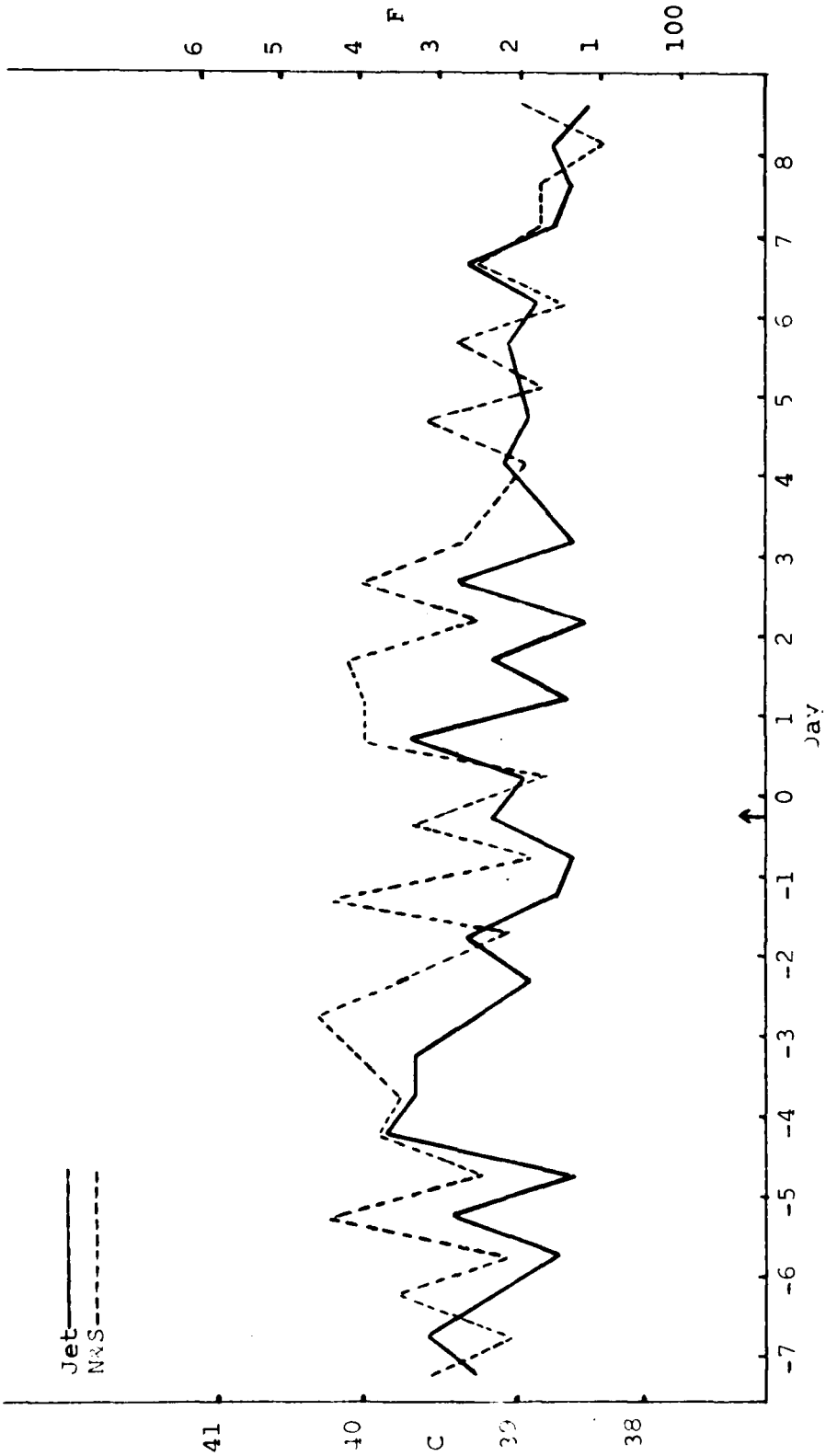


Fig 42--Temperature Response of Goats Injected with 10^2 $CCID_{50}$ Virulent VEE Virus--Diurnal Group.

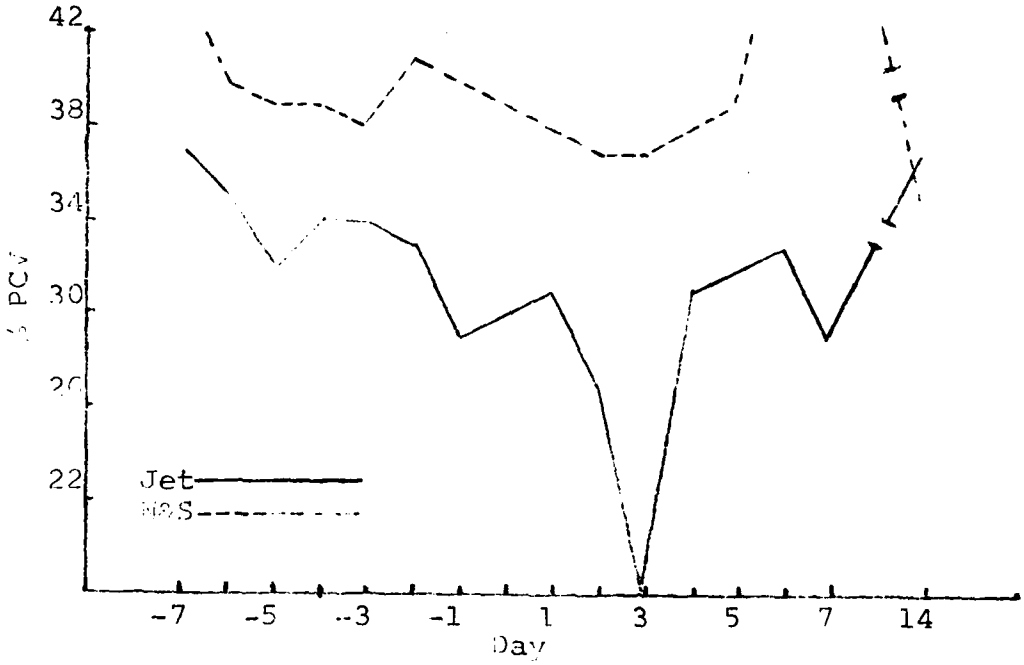


Fig 43--PCV values in goats inoculated with 10^2 $CCID_{50}$ VEE virus--diurnal group.

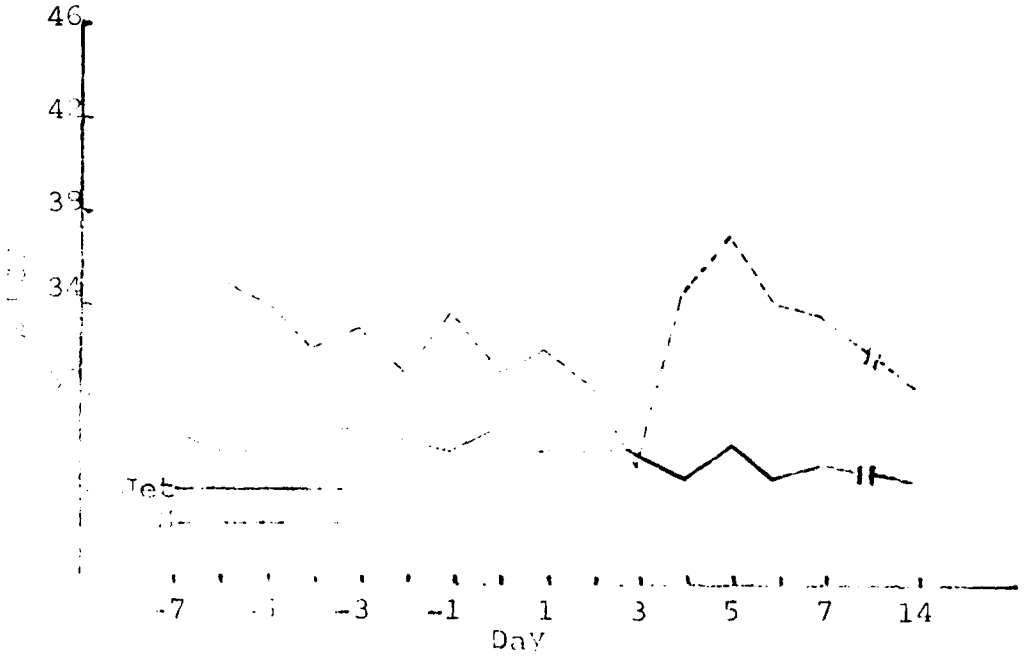


Fig 44--Median PCV values in goats inoculated with 10^5 $CCID_{50}$ VEE virus--diurnal group.

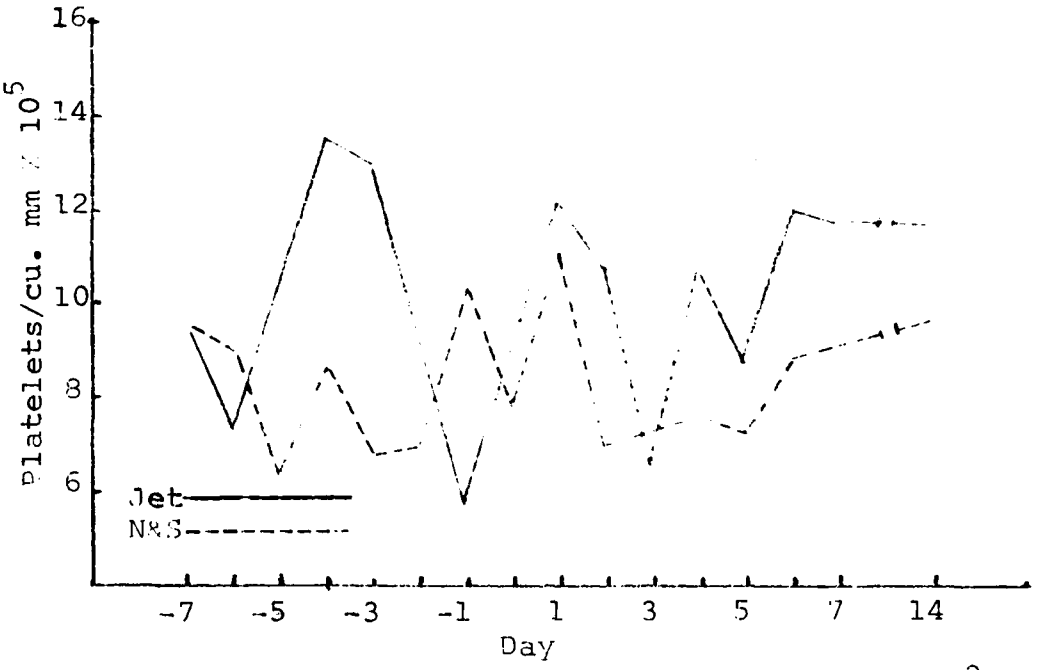


Fig 45--Platelet counts in goats inoculated with 10^2 $CCID_{50}$ VEE virus--diurnal group.

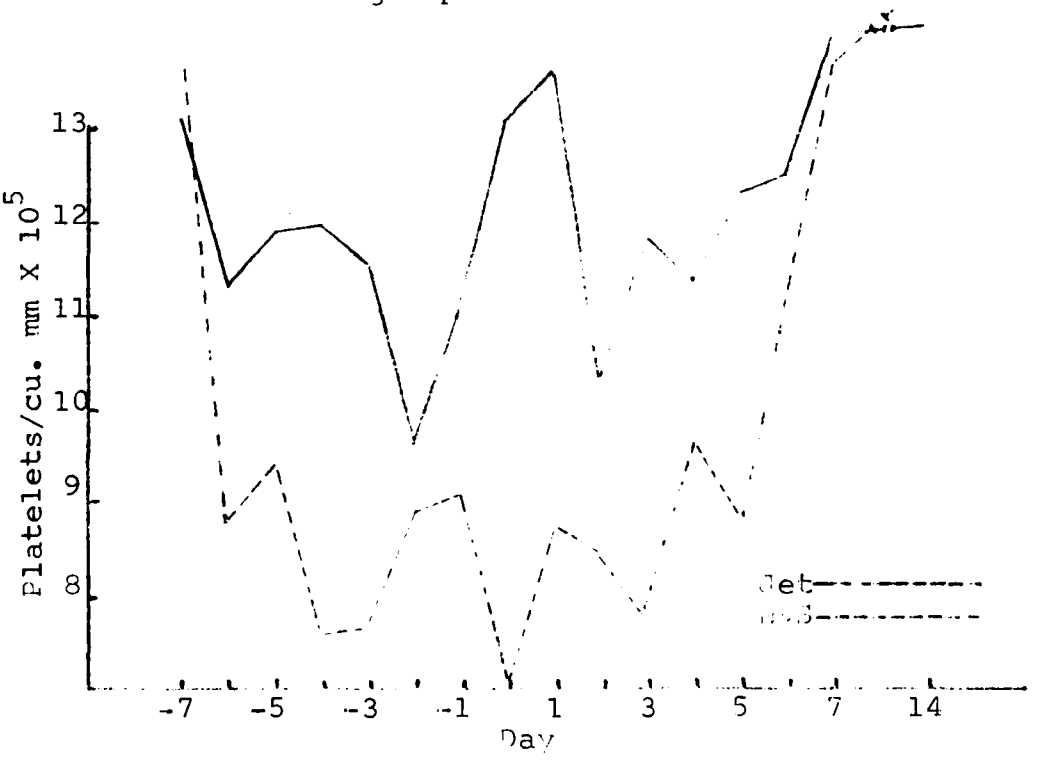


Fig 46--Median platelet counts in goats inoculated with 10^6 $CCID_{50}$ VEE virus--diurnal group.

variations from baseline platelet counts in either group of goats at either dose of virus.

WBC, lymphocytes and neutrophiles (Table 33 and 34)- Goats receiving 10^2 $CCID_{50}$ VEE virus showed extremely variable baseline WBC values and except for one low reading in the jet injected goat on day 5 p.i., there were no significant deviations from baseline values. Both needle and syringe injected and jet injected groups of goats that received 10^6 $CCID_{50}$ VEE virus showed a significant drop in WBC's. The jet injected group had low counts on days 1 through 3 p.i., and again on day 14, whereas the needle and syringe injected goats had low counts on days 1 through 6 p.i. Lymphocyte counts in goats which received 2 logs of virus showed no changes from the baseline counts except for one low reading in the jet injected goat on day 5 p.i. Goats receiving 6 logs of virus had somewhat variable lymphocyte counts. The needle and syringe injected group had lymphocyte counts within pre-injection levels while the jet injected group had a lymphopenia on day 3 p.i. and a lymphocytosis on day 6 p.i. The jet injected goat that received 10^2 $CCID_{50}$ VEE virus had a neutropenia on days 3 through 5 p.i., whereas the needle and syringe injected goat had only a slight neutropenia on day 3. Both groups of goats which received the high dose of

TABLE 33 - WBC, Lymphocyte, and Neutrophile Counts in Goats Inoculated with 10^2 $CCID_{50}$ VEE Virus--Diurnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	20.2*	13.5	5.9	10.8	5.9	4.9
-6	19.1	7.8	10.3	12.2	4.5	5.5
-5	17.6	10.2	7.0	11.6	5.2	5.8
-4	17.2	6.7	10.3	21.4	5.6	15.4
-3	23.8	19.0	4.8	11.1	6.8	4.3
-2	13.6	10.1	3.5	11.7	5.4	6.1
-1	9.9	6.7	3.2	15.9	6.0	9.2
0	11.8	8.1	3.4	11.8	5.4	6.1
1	11.7	6.4	5.1	13.6	7.9	6.7
2	12.0	8.2	3.6	13.7	7.7	6.0
3	10.1	9.3	0.8	10.1	6.0	3.9
4	12.2	9.3	2.9	13.0	5.6	6.5
5	7.1	3.8	3.1	13.2	8.4	4.2
6	12.4	8.8	3.5	15.0	5.7	9.0
7	11.6	5.9	5.3	16.1	7.7	8.4
14	22.8	9.6	11.9	9.8	5.0	4.8

*Expressed as counts per cubic mm X 10^3

L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

TABLE 34--Median WBC, Lymphocyte, and Neutrophile Counts in Goats Inoculated with 10^6 $CCID_{50}$ VEE Virus--Diurnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	12.7*	6.1	5.7	14.6	6.3	7.4
-6	14.2	6.6	7.3	12.8	5.9	6.2
-5	15.2	5.9	9.0	13.1	6.3	6.3
-4	17.4	6.8	10.1	14.4	5.6	8.5
-3	23.0	7.8	15.2	14.2	6.6	7.2
-2	11.9	5.2	6.6	13.4	5.4	7.5
-1	12.3	6.3	5.7	12.4	4.9	6.9
0	12.7	8.3	4.0	14.0	3.9	9.7
1	8.4	5.0	3.0	9.7	5.7	3.6
2	8.6	5.5	2.7	8.2	4.3	3.5
3	7.9	3.9	3.9	7.5	4.1	3.1
4	12.1	6.8	5.0	8.4	4.8	3.1
5	13.8	5.1	8.7	8.2	5.5	2.7
6	16.0	10.6	5.4	9.9	5.6	4.3
7	16.5	5.4	10.9	13.2	4.9	7.8
14	9.9	5.4	4.3	12.2	4.9	7.3

*Expressed as counts per cubic mm X 10^3

L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

challenge virus had a neutropenia. The jet injected group had lower than baseline neutrophile counts on days 1 through 3 p.i. and the needle and syringe injected group had low neutrophile counts on days 1 through 6 p.i.

Viremia (Table 35)--Both goats (#179 and #182) which received 10^2 $CCID_{50}$ VEE virus showed only a trace of serum virus (less than 1 $SMICLD_{50}$ in 0.025 ml serum) on day 1 p.i. only. Goats receiving 10^6 $CCID_{50}$ VEE virus by needle and syringe had a low level of serum virus (less than 1 $SMICLD_{50}$ in 0.025 ml of serum) on days 1 and 2 p.i. Only 1 of the 2 jet injected goats (#181) showed a demonstrable serum virus titer on days 1 p.i. ($10^{1.4}$ $SMICLD_{50}$ per 0.025 ml serum) and day 2 p.i. ($10^{1.1}$ $SMICLD_{50}$ per 0.025 ml serum).

Antibody response (Table 36)--Goats receiving 2 logs of virus did not show HI titers until day 14. The jet injected goat (#179) had a 1:160 titer and the needle and syringe injected goat (#182) had a 1:20 titer. All goats receiving 6 logs of VEE virus showed HI titers by day 7. Jet injected goats had titers of 1:80 and needle and syringe injected goats had HI titers ranging from 1:40 to 1:80. HI titers were increased on day 14, ranging from 1:160 to equal to or greater than 1:640 in jet injected goats and from 1:320 to equal to or greater than 1:640 in needle and syringe injected goats. Day 21

TABLE 35--Serum Virus Titers in Goats Inoculated with 10^2 and 10^6 $CCID_{50}$ VEE Virus--Diurnal Group.

Goat #	Injection Method	Dosage	Day Post-Inoculation 1	2	3
179	Jet	10^2	$<10^0$	0	0
180	Jet	10^6	0	0	0
181	Jet	10^6	$10^{1.4}$	$10^{1.1}$	0
182	N&S	10^2	$<10^0$	0	0
283	N&S	10^6	$<10^0$	$<10^0$	0
295	N&S	10^6	$<10^0$	$<10^0$	0

*Expressed as median suckling mouse intracerebral dose per 0.025 ml serum.

**Calculated dose. Actual dose as determined by backtitration of challenge inoculum was $10^{2.3}$ and $10^{5.8}$ $CCID_{50}$ per ml.

N&S - Needle and syringe

TABLE 36--Reciprocal of Hemagglutination-Inhibition Titers in Serums of Goats Inoculated with 10^2 and 10^6 $CCID_{50}$ VEE Virus--Diurnal Group.

Goat #	Injection Method	Dosage	Day Post-Inoculation 7	14	21
179	Jet	10^2	N	160	40
130	Jet	10^6	80	160	160
181	Jet	10^6	80	>640	--*
182	N&S	10^2	N	20	10
283	N&S	10^6	40	320	160
295	N&S	10^6	80	>640	--*

*Killed on day 14.

N - Negative

N&S - Needle and syringe

HI titers were decreased or the same as titers on day 14. Unfortunately, the highest titered goats were killed on day 14 and not available for testing on day 21.

Necropsy--The only gross lesions noted at necropsy were an abscessed prescapular lymph node in goat #295 and visceral adhesions in the abdominal cavity of goat #179.

Goats: Nocturnal group. No clinical signs of illness were noted in any goat following virus exposure.

Temperature (Fig 47 and 48)--In goats receiving 2 logs of VEE virus, the jet injected goat did not show a temperature rise until 72 hours p.i. The rise was about 1.1 C (2 F) above the previous reading on day 0, and about 0.55 C (1 F) above the highest pre-exposure temperature. The temperature remained slightly elevated at 84 hours p.i. and then dropped to within pre-exposure levels. The needle and syringe injected goat showed a temperature rise at 12 hours p.i. or more than 1.1 C (2 F) above the previous reading 12 hours earlier. At 24 hours after virus exposure, the temperature was back to within baseline values. In goats which received 6 logs of VEE virus, the jet injected goats showed a slight rise at 12 hours p.i. of 0.55 C (1 F) above the day 0 temperature but only 0.11 C (0.2 F) above the highest baseline temperature. The temperature continued to rise slightly over the next 24 hours to peak out at about

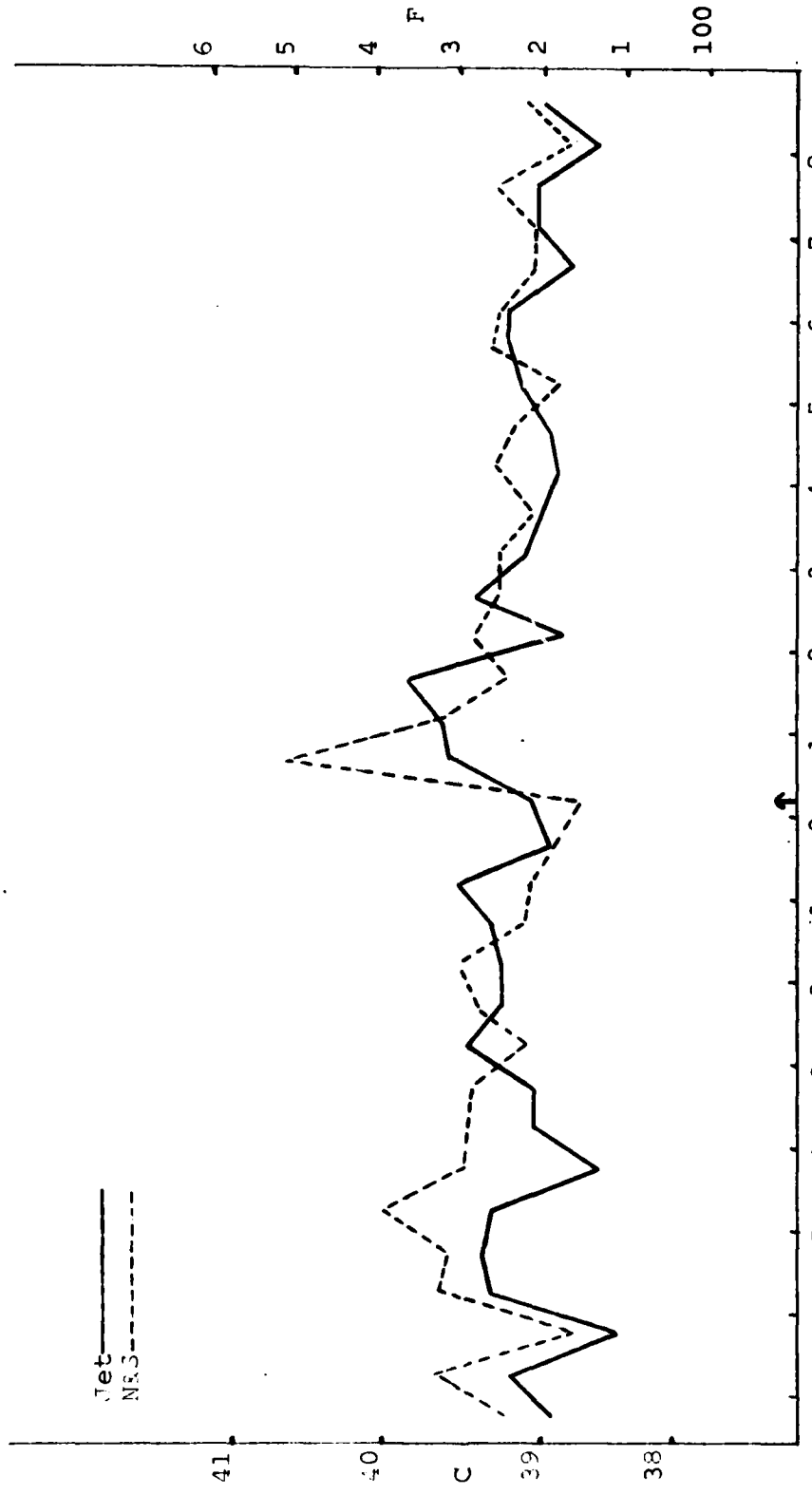


Fig 47--Median Temperature Response of Goats Injected with 10^6 $CCID_{50}$ Virulent VEE Virus--Nocturnal Group.

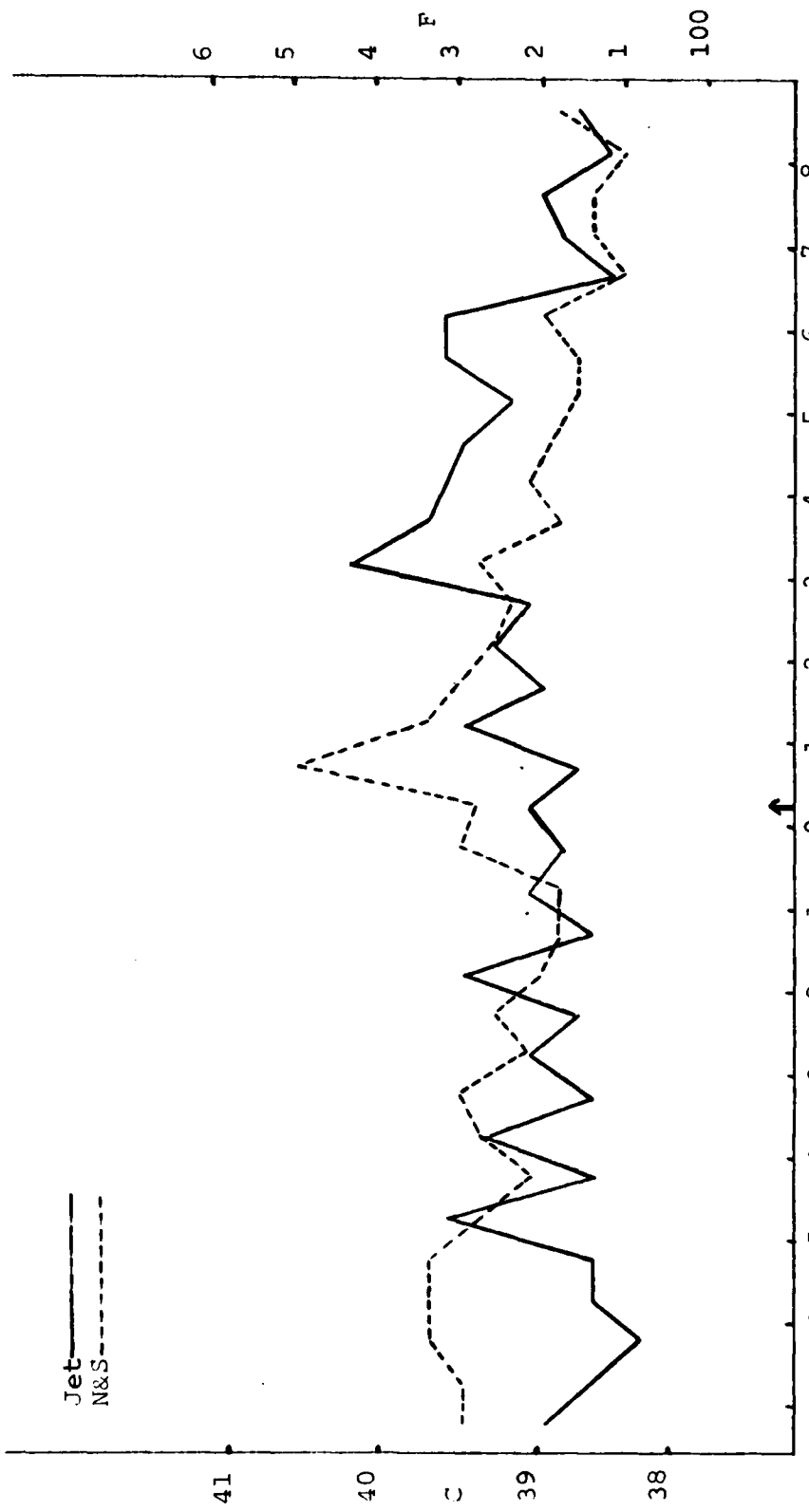


Fig 48--Temperature Response of Goats Injected with 10^2 $CCID_{50}$ Virulent VEE Virus--Nocturnal Group.

0.33 C (0.6 F) above the highest pre-exposure temperature. By 48 hours p.i., the temperature had returned to within baseline limits. The needle and syringe injected goats showed a significant temperature spike at 12 hours p.i. of more than 1.1 C (2 F) above the highest baseline reading. At 24 hours p.i., the temperature had dropped to slightly above baseline and was within baseline readings on day 2 p.i. and following.

PCV (Fig 49 and 50)--PCV's of the goats receiving 2 logs of VEE virus showed no pattern attributable to virus injection. The goats which received 10^6 $CCID_{50}$ VEE virus by needle and syringe showed a drop at 24 hours p.i. Decreased PCV values in the needle and syringe injected goats was evident both before and after virus exposure. The jet injected group showed a slight rise in PCV's on days 2 and 3 p.i., but a general downward trend was noted immediately before and after injection of virus.

Platelets (Fig 51 and 52)--Goats which received the low virus dose showed extremely variable baseline counts and the platelet counts did not vary from baseline limits except for a drop on day 5 p.i. in the needle and syringe injected goat. Goats receiving the 6 logs of virus did not show a difference from baseline values except for a higher count in the needle and syringe injected group on

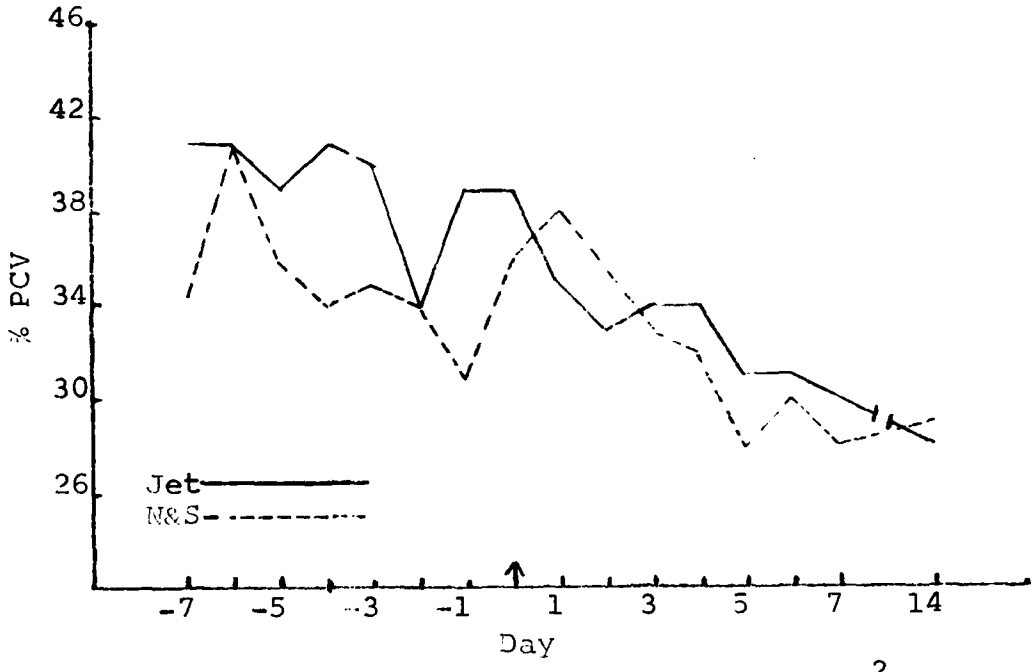


Fig 49--PCV values in goats inoculated with 10^2 $CCID_{50}$ VEE virus--nocturnal group.

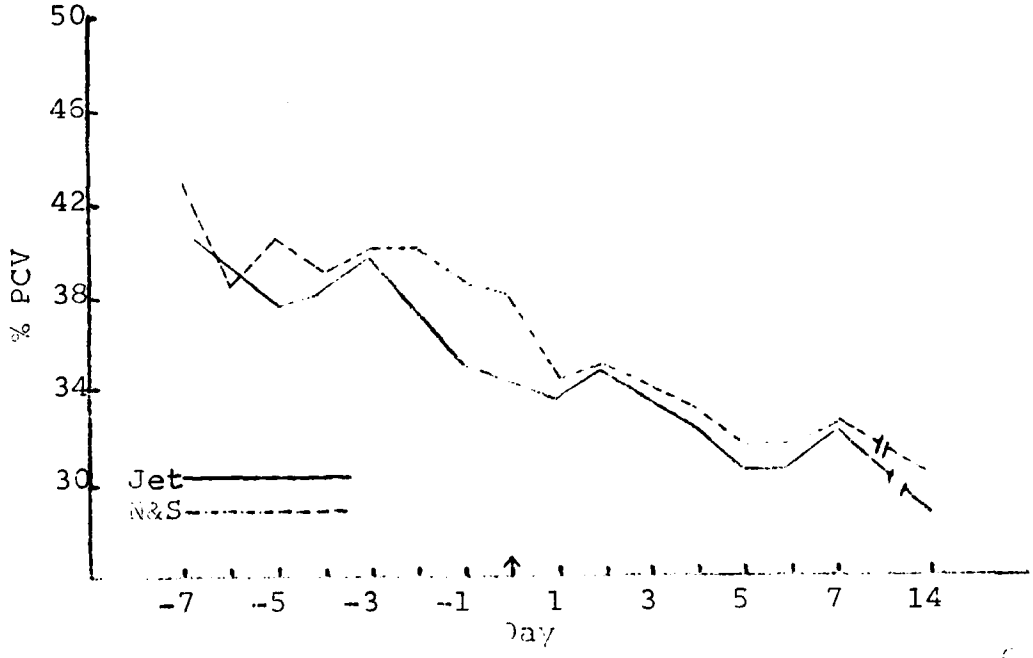


Fig 50--Median PCV values in goats inoculated with 10^{6} $CCID_{50}$ VEE virus--nocturnal group.

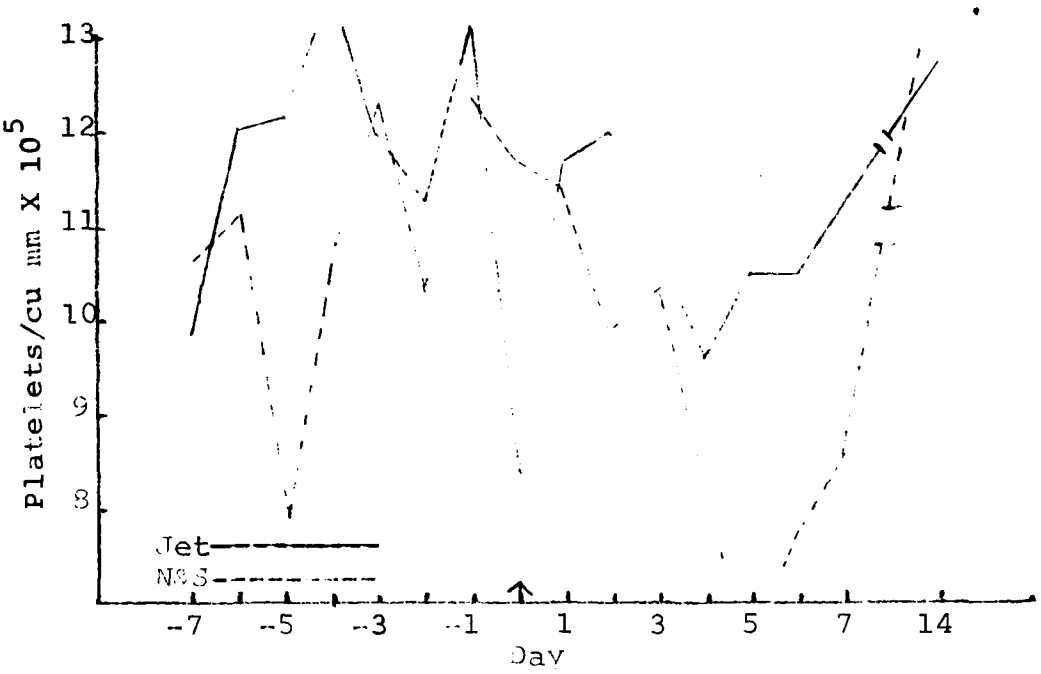


Fig 51--Platelet counts in goats inoculated with 10^2 $CCID_{50}$ VEE virus--nocturnal group.

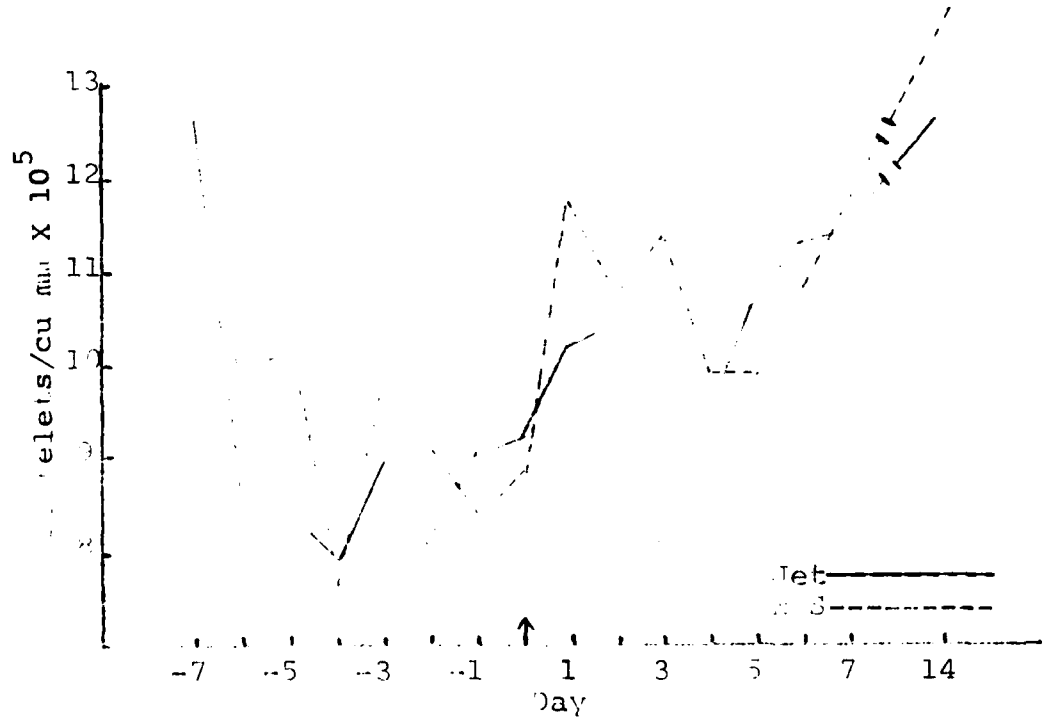


Fig 52--Median platelet counts in goats inoculated with 10^3 $CCID_{50}$ VEE virus--nocturnal group.

day 14 p.i.

WBC, lymphocytes and neutrophiles (Tables 37 and 38)-- The goat which received the lower dose of VEE virus by jet injector (Table 37) did not show a significant drop in WBC's, but did show a rise above baseline on day 4 p.i. The needle and syringe injected goat receiving the lower dose showed a drop in WBC's on day 2 p.i. which persisted through day 7, and was still low on day 14. Goats which received the higher dose of virus (Table 38) showed a drop in WBC's after virus injection. The jet injected group showed a significant drop on day 1, a rise to just below baseline values on days 2 through 5 p.i., following by counts within baseline limits. The needle and syringe injected goats showed a slight transient drop in WBC's on day 1 only. The goat receiving the low dose of virus by jet injector (Table 37) had lymphocyte counts essentially within baseline limits. The needle and syringe goat showed a perceptible drop on day 5 which persisted to just below baseline values through day 7. In goats receiving higher dose of virus (Table 38), a decrease in lymphocytes was seen at different times. Jet injected goats showed a significant drop on day 1 p.i. which returned to baseline counts on day 2, followed by a less perceptible drop on days 3 through 5. The needle and syringe injected goats showed only a slight drop in

TABLE 37 --WBC, Lymphocyte, and Neutrophile Counts in Goats Inoculated with 10^2 $CCID_{50}$ VEE Virus--Nocturnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	15.2*	9.9	5.2	15.9	8.7	6.7
-6	18.4	9.9	7.7	33.7	7.1	26.6
-5	23.9	7.6	15.8	19.2	9.8	9.4
-4	23.8	10.5	11.9	21.7	13.5	7.8
-3	19.4	14.7	4.3	15.2	9.1	5.2
-2	23.3	7.9	13.3	12.3	7.0	4.8
-1	29.3	15.2	13.2	20.5	12.3	7.0
0	20.1	10.5	9.2	20.3	8.3	11.0
1	22.7	15.4	6.6	15.7	9.7	5.3
2	18.8	10.0	8.6	10.6	6.9	3.5
3	21.0	7.6	12.8	12.6	6.9	5.3
4	33.7	12.1	20.2	11.3	7.2	3.8
5	22.1	9.3	12.6	10.0	5.3	4.5
6	20.7	9.1	10.8	12.0	5.6	6.4
7	19.3	12.7	6.3	12.5	6.8	5.6
14	17.7	11.3	4.8	14.7	7.4	6.0

*Expressed as counts per cubic mm X 10^3

L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

TABLE 38 --Median WBC, Lymphocyte, and Neutrophile Counts in Goats Inoculated with 10^6 $CCID_{50}$ VEE Virus--Nocturnal Group.

	Jet Injected			N&S Injected		
Day	WBC	L	N	WBC	L	N
-7	13.4*	8.9	4.0	10.7	6.5	4.0
-6	10.9	6.6	4.0	11.6	6.9	4.4
-5	14.0	7.1	6.6	22.0	7.2	14.2
-4	11.9	5.9	5.9	15.8	5.3	10.1
-3	12.4	6.7	5.1	15.7	6.1	9.2
-2	12.9	8.1	4.3	14.6	5.9	8.1
-1	12.4	8.9	3.2	12.0	4.6	6.5
0	11.3	5.9	5.0	11.0	6.2	4.3
1	7.1	3.2	3.6	10.2	4.9	4.8
2	10.7	6.2	4.3	11.0	4.6	6.2
3	9.9	5.4	4.0	10.6	4.9	5.4
4	9.6	5.3	4.0	13.4	6.6	6.3
5	10.3	5.5	4.4	11.0	4.1	6.7
6	11.9	6.4	5.1	14.2	4.7	9.4
7	19.5	7.1	11.5	17.5	4.7	12.6
14	17.2	13.2	3.5	13.4	7.3	5.8

*Expressed as counts per cubic mm X 10^3

L - Lymphocytes, N - Neutrophiles

N&S - Needle and syringe

lymphocytes on day 5 p.i. The goat receiving 10^2 $CCID_{50}$ VEE virus by jet injection (Table 37 p. 143) had marked fluctuations in pre-exposure baseline neutrophile counts, and except for one high count on day 4 p.i., neutrophiles remained within baseline limits. The low virus dose needle and syringe injected goat showed a neutropenia on days 2, 4, and 5 p.i. Goats receiving the higher dose of VEE virus (Table 38 p. 143) showed essentially no variation from baseline neutrophile counts except on day 7 p.i. the jet injected group showed a neutrophilia.

Viremia (Table 39)--Neither of the goats which received 10^2 $CCID_{50}$ VEE virus had a serum virus titer following virus exposure. One of the 2 goats (#284) which received 10^6 $CCID_{50}$ VEE virus by jet injector had a serum virus titer of $10^{0.5}$ $SMICLD_{50}$ per 0.025 ml serum on days 1 and 2 p.i. Likewise, only 1 of the 2 goats (#285) which received the same dose of virus by needle and syringe had a detectable viremia ranging from $10^{1.77}$ to $10^{1.38}$ $SMICLD_{50}$ per 0.025 ml serum on days 1 and 2 p.i., respectively.

Antibody response (Table 40)--The goats receiving 2 logs of virus did not show HI antibody titers until day 14. The jet injected goat (#183) showed a 1:80 HI titer which dropped to 1:10 on day 21. The goat (#184) that received the low dose via needle and syringe had a 1:320

TABLE 39--Serum Virus Titers* in Goats Inoculated with 10^2 and 10^6 $CCID_{50}$** VEE Virus--Nocturnal Group.

Goat #	Injection Method	Dosage	Day Post-Inoculation 1	2	3
183	Jet	10^2	0	0	0
185	Jet	10^6	0	0	0
284	Jet	10^6	$10^{0.5}$	$10^{0.5}$	0
134	N&S	10^2	0	0	0
285	N&S	10^6	$10^{1.8}$	$10^{1.4}$	0
292	N&S	10^6	0	0	0

*Expressed as median suckling mouse intracerebral lethal dose per 0.025 ml serum.

**Calculated dose. Actual dose as determined by backtitration of challenge inoculum was $10^{1.5}$ and $10^{5.5}$ $CCID_{50}$ per ml.

N&S - Needle and syringe

TABLE 40--Reciprocal of Hemagglutination-Inhibition Titers in Serums of Goats Inoculated with 10^2 and 10^6 $CCID_{50}$ VEE Virus--Nocturnal Group.

Goat #	Injection Method	Dosage	Day Post-Inoculation 7	14	21
183	Jet	10^2	N	80	10
185	Jet	10^6	10	80	--*
284	Jet	10^6	40	80	40
184	N&S	10^2	N	320	40
285	N&S	10^6	40	320	--*
292	N&S	10^6	40	40	1:20

*Killed on day 14.

N - Negative

N&S - Needle and syringe

HI titer on day 14 and a 1:40 on day 21. The goats which received 6 logs of virus had HI antibody titers on day 7 ranging from 1:10 to 1:40 when jet injected and 1:40 when needle and syringe injected. On day 14, the HI titers had risen to 1:80 in the jet injected goats and on day 21 had dropped to 1:40 in the one remaining goat. The needle and syringe injected goats had a 1:40 to 1:320 range in HI titers on day 14 and a drop to 1:20 on the remaining goat on day 21.

Necropsy--No significant gross lesions were observed in any of the goats.

Field Study Evaluation of the Jet Injector

The efficacy of the jet injector for administration of vaccines to large groups of animals in a short period of time was evaluated in 2 different studies involving dogs and feedlot cattle.

Dogs

VEE vaccine was injected into a group of 131 dogs. The immune response was measured using serological techniques. The antibody response was determined in serums collected on day 0 and day 35 post vaccination by measuring both SN and HI antibody titers (Table 41).

TABLE 41--Serological Response of Dogs to VEE Vaccination

Serological Test	No. of serums positive / No. of serums tested	Seroconversion rate
SN	120/124*	96%
HI	118/131	90.1%

*Seven serums were not tested due to technicial difficulties.

All of the day 0 serums (124) tested for SN antibodies were negative. One hundred-twenty of the 35 day serums were positive for SN antibody for a seroconversion rate of 96%. Of the 131 paired serums tested for HI antibodies, 118 had a twofold or greater increase in titer when compared to day 0 serums, and 13 samples showed no increase in titer. Antibody titers ranged from 1:4 to equal to or greater than 1:1,280. The 7 serums not tested by SN were all HI positive. The seroconversion rate was determined to be 90.1% by HI vs 96% by SN.

Approximately 1 year after the dogs were initially vaccinated, 6 of the 120 SN positive dogs were randomly selected and rebled. Serums collected on day 0, day 35, and 1 year were tested and compared for evidence of SN antibodies and the SN titers calculated by the method of Reed and Muench[46] (Table 42).

TABLE 42--Log Neutralization Indices (LNI) for VEE Vaccinated Dogs.

Dog #	Prebleed Virus Titer*	--LNI	35 day serum Virus Titer	--LNI	1 year serum Virus Titer	--LNI
001	8.83	<0.5	6.0	2.83	5.48	3.35
005	9.5	<0.5	5.5	4.0	5.68	3.82
019	9.5	<0.5	6.78	2.72	7.5	2.0
040	8.17	<0.5	6.63	1.54	5.38	2.79
050	8.68	<0.5	6.17	2.51	5.0	3.68
099	8.5	<0.5	5.5	3.0	5.75	2.75

*Virus titer expressed as log 10.

As can be seen in the table, all pre-vaccination serums were negative for SN antibody. Five of six 35 day post vaccination serums had neutralizing indices (LNI) of 2.0 logs or greater whereas all 6 samples obtained a year later showed LNI's of 2.0 logs or greater.

Cattle

Two hundred head of light feedlot calves were vaccinated with IBR modified live virus vaccine. Fifty of the calves were randomly selected for serological studies, and paired samples were collected from 47 head. Serum was collected immediately prior to and 35 days after vaccination. The serological results (SN) of these calves

are shown in Table 43.

TABLE 43--SN Response of 47 Calves to IBR Vaccination with the Jet Injector.

Day Serum Collected	# Positive* / # Tested	% Positive
0	1/47	2
35	13/47	28

*Determined by the method of Bitsch[10] using 1 part IBR virus (10^1 $CCID_{50}$) to 4 parts serum.

When compared to pre-bleed serums, day 35 serums had a 26% greater incidence of IBR SN antibodies.

DISCUSSION AND CONCLUSIONS

In human medicine, the jet injector has been shown to be a valuable tool for the inoculation of large populations of people. In terms of economy of time, personnel, and money, the technique cannot be equalled by the conventional methods of parenteral drug administration. The purpose of this study was to show that jet injection can be used in veterinary medicine with the same advantages as have been obtained in human medicine.

Dose delivery characteristics were determined, not only to show that replicate injections would be within acceptable limits, but also to establish and standardize the dosage setting on the prototype apparatus for subsequent adjustment when changes in dosage amounts were made. Hingson[32] reported that delivery of the inoculum was within 4 to 5% of the set dose when he tested the Hypospray jet injector. Data collected in this study showed that except for the 0.5 ml dose, the mean dose delivery of 1 ml and 2 ml dose settings was well within a 5% limit. The mean dose delivery of the 0.5 ml setting was, however, within 6% of expected delivery. This study confirmed that the repeatability of the volume delivered by the jet injector was accurate to within 5 to 6% of calculated dose.

Figge and Barnett[20] reported that there were only

two variables affecting the depth of penetration in humans--spring pressure and skin thickness. The animal studies added other variables, in that the skin thickness and density vary between species at accessible injection sites, and external barriers such as hair, wool, bristles, feathers, or scales are encountered. Another difference in domestic animals is that the skin is somewhat loosely attached to the underlying subcutaneous tissue. This affects the tautness of the skin and allows the inoculum to diffuse laterally into the subcutaneous space rather than penetrate the denser muscle tissue. For the most part these obstacles were overcome by redesign of the nozzle, increase in spring pressure using an intensifier, and increase in diameter of the orifice. These changes also allowed decreased injection time when the dose was increased from 1 ml to 2 ml by increasing the size of the dosage barrel. Several additional animal vaccines which require a 2 ml dose could thus be delivered by the jet injector.

The changes made were in response to problems recognized during the course of testing the equipment. Repeated injections in the dog showed that the uncrowned nozzle designed for use in humans gave poor, inconsistent penetration. This was probably due to the inability to establish a firm skin to nozzle contact. The crowned

nozzle appeared to alleviate the problem by "bunching" the skin under the nozzle which gave better contact. At the same time, the skin was pulled tighter under the nozzle and pressed more firmly against the underlying musculature resulting in deeper and more consistent penetration. An additional advantage of the crowned nozzle was that less chance of slippage and resultant cutting of the skin was attained due to the gripping action of the crown.

Previous work[20,30] using the jet injector established that orifice diameters of between 75u (0.003 inch) to 125u (0.005 inch) were best suited for injections into humans. In cattle and horses, a significant amount of inoculum was left on the skin surface with nozzles designed for human injections and with #5 and #9 crowned nozzles when used without the intensifier. Less residual inoculum was left on the skin when the intensifier was added, but some inoculum was still present. Three additional sizes of orifice diameters were tested i.e. #10, #11, and #12, and with these the best results were attained with the #10 and #11 orifice diameters. Results with the #12 nozzle was not as good and appeared to have exceeded the upper limit in diameter size which would effect good inoculum delivery. Another advantage gained by the use of larger diameter orifices plus intensifier

was the reduction in the time required to eject 2 ml doses from the dosage barrel.

Radiographic technique to study penetration and dispersion patterns of jet injected materials in humans was first reported by Figge and Barnett[20] in 1947. They also injected radiopaque material with needle and syringe and made comparisons between the two methods of injections. Using the nozzle designed for animal use, the same type of experiment was performed in dogs to compare inoculum dispersion and depth of penetration between jet injection and needle and syringe injection. The major difference between a jet injection and a needle and syringe injection was that with the latter all of the dye was deposited at the same depth depending on the length of the needle used. The deposition by the jet injector was a dispersion pattern which tends to be along fascial planes and other areas of lesser resistance, whereas the needle and syringe injection tends to be deposited in a localized teardrop pattern.[17,20] Another difference was that the jet injector produced a "tailing effect" in that the inoculum extended from the point of contact on the skin to the maximum penetration depth. This allows a certain minute quantity of inoculum to be deposited ID, a larger amount SC, and the bulk of the dose deposited IM.

Studies designed to test the jet injector for uses

other than immunization were for the most part rather disappointing. Ziff[62] showed that cortisone could be injected into arthritic joints of humans. Attempts to force methylene blue dye into the joints and caudal spinal nerve of horses and goats were thwarted not only by the thick unyielding skin found covering these areas, but also by the dense ligaments, bone, fascia, and synovial membrane protecting these structures. It was, therefore, concluded that these procedures were not practical for the jet injector as equipped at the present time. It does not, however, preclude the use of the jet injector for injection of local anesthetics into the skin and SC tissues prior to operations such as biopsy, firing, removal of small skin tumors, or anesthetizing lacerated areas for suturing and debridement.

Skin testing in the human using the jet injector has been reported with tuberculin,[25] coccidioidin,[22] and histoplasmin.[44] Skin testing in the bovine with needle and syringe has been routinely performed in the caudal fold. Attempts to introduce dye intradermally into the caudal fold using the jet injector equipped with intradermal nozzle were not successful. The procedure was not attempted in the skin of the neck which has been reported of be more sensitive to the tuberculin test than the skin

of the caudal fold.[39]

According to a report by Dr. Keith Farrell at the U. S. Animal Health Association meeting in 1974, permanent and unalterable identification of the equine has become increasingly important in light of recurring VEE epizootics and the possibility of erradication of equine infectious anemia through test and slaughter. The use of the jet injector to permanently identify equine by injecting insoluble dyes into the mucosa of the upper lip appeared promising, but the permanence of the markings was not adequately determined due to the untimely death of the test animal.

A published reference to the use of the jet injector for injecting water-in-oil adjuvants was not found in the literature. The tests conducted during this study showed that the technique was successful in that demonstrable precipitin antibody was produced in a sheep against the TMV.

Laboratory studies to compare the similarities and/or differences between jet injection and conventional needle and syringe injection were made using vaccine models in dogs and calves, and a virulent VEE virus model in several species of animals. In dogs receiving VEE vaccine by jet injection and needle and syringe injection, SN serological results were quite comparable. There was a slight

difference in the HI serology which favored jet injection. When calves were given IBR vaccine via jet injection and needle and syringe injection, SN results appeared to favor needle and syringe injection. However, in neither study did the degree of difference overwhelmingly favor one technique over the other.

The hypothesis that the antigenic and structural makeup are altered by the increased pressures and shearing forces encountered in the jet injector was tested. Data on biological, virological, and serological parameters were collected and compared from 5 species of animals that received virulent VEE virus by the conventional needle and syringe method and by jet injector.

Clinical response following virus inoculation showed that dogs had the most pronounced signs associated with virus exposure. Depression, anorexia, and reluctance to move were noted in all dogs. Pigs, sheep and goats showed minimal signs of virus infection consisting of a day or two of inappetance, slight depression, and increased respiration. Calves showed no outward clinical signs of virus infection. There were no observable differences in the clinical signs expressed by jet injected animals when compared to needle and syringe injected animals.

Rectal temperature response was somewhat dependent on the species of animal, the time of injection, and the

titer of the challenge virus. Minor differences were seen between jet injected and needle and syringe injected animals which were probably not significant. A diphasic temperature response occurred with enough regularity throughout the species to consider this as a farily consistent finding. Out of the 26 groups of animals recorded, a diphasic temperature (primary rise during the first 24 hours p.i., followed by a secondary rise at 48 through 60 hours p.i.) was seen in 15 of the groups. Three other groups, all needle and syringe injected, showed a tertiary temperature rise (low dose nocturnal dog, nocturnal calves, and high dose diurnal goats). The exceptions to the diphasic temperature pattern was seen predominantly in the goats, with only 1 of the 8 groups showing a clear-cut diphasic response and another showing a lesser defined diphasic response. The jet injected calves (nocturnal) and needle and syringe injected sheep (diurnal) also did not show the diphasic response. The typical diphasic response usually showed a higher initial temperature rise followed by a lesser secondary rise, except in the sheep which showed a higher secondary rise.

In goats receiving low dosage of VEE virus, only 1 goat (nocturnal needle and syringe injected) showed a typical diphasic temperature response, whereas diurnal groups showed no elevated temperature response. The jet

injected goat (nocturnal) showed a temperature response at day 3 and a secondary rise on day 6 which may reflect a delayed response to a low dose of virus, although no viremia was detected on these days.

The hematological parameters were compared to detect similarities or differences between jet injected and needle and syringe injected animals.

Since the basophile, eosinophile, and monocyte counts, were low to absent, these measurements were not used for comparative purposes in evaluation of jet vs needle and syringe injection. Some of the parameters studied showed extreme fluctuation with erratic values both before and after virus exposure and, as a result were very difficult to interpret in terms of response to virus challenge using different modes of injection. However, in other parameters, rather clear-cut responses were seen to occur following virus challenge. Where possible, attempts were made to establish trends from those parameters that showed limited or inconsistent values.

PCV measurements tended to fit 3 broad categories: 1) generally decreasing throughout the bleeding period; 2) decreasing during baseline which continued after virus injection, followed by a leveling off or rising PCV; and 3) variable PCV values. Four groups fit into category 1, 10 groups into category 2 and 10 into category 3. Two

other groups showed little change throughout the bleeding period. Those groups that showed a continual decline in PCV values throughout the experiment (category 1) may have been due to bleeding diathesis, but high PCV measurements obtained early in the experiment may have been due to excitement to the animal during the bleeding process. According to Schalm et al,[50] the spleen contracts during excitement and may dump a significant amount of RBC's into the circulation causing higher than normal PCV measurements. A rise in PCV values after virus injection as seen in category 2 was difficult to explain. Whether the increase was due to a physiological rebound mechanism or directly due to virus exposure was not determined. In general, interpretation of PCV values in relation to the effect of virus challenge or mode of injection was difficult to determine. The only groups to show a significant drop in PCV at 24 hours p.i. were the diurnal pigs. However, similar decreases were noted in the baseline data and the same phenomenon was not observed in the nocturnal group, so it would appear that this was not a consistent finding in the pig.

Platelet counts were extremely variable and no definite trend could be established in the nocturnal pigs, nocturnal needle and syringe injected sheep and all goats. All dogs, calves (diurnal), pigs (diurnal), sheep (diurnal),

and sheep (nocturnal jet injected) typically showed a depression of thrombocytes following virus challenge with most groups recovering to within baseline values by day 7. A notable exception to this trend was seen in the calves (nocturnal) which showed an increase in platelets following virus exposure. Thrombocytopenia has been reported to be caused by virus infections.[50] Since VEE virus does replicate in endothelial cells, it is conceivable that damage to these cells causes adherence of platelets and a resultant thrombocytopenia. Decreased thrombocyte counts were relative to the counts recorded during the baseline period and not below published normal values.[50] The thrombocytosis seen in the nocturnal group of calves was unique when compared to the diurnal group of calves. Causes of thrombocytosis have been related to splenic removal, acute hemorrhages, trauma, fractures, injection, malignancy and iron deficiency.[50] Since none of these conditions were seen in the calves, cause of the thombocytosis in the nocturnal group of calves was not ascertained. The extreme variability seen in platelet counts in some of the groups made interpretation difficult. These counts may have been the result of technique since platelets tend to stick to surfaces and are rapidly incorporated into a clot if there is any delay in transferring the blood sample to the diluent. Generally

the platelet counts showed a similar pattern in both needle and syringe and jet injected groups of animals were a discernible pattern could be ascertained. Only minor variations were detected and no significant differences could be attributed to mode of injection.

The classic response of the WBC's to a virus infection is a leukopenia due to both a lymphopenia and neutropenia.[50] The effect of VEE virus on the leukocytic cells of dogs and calves has been previously reported by other investigators.[54,57,b] In dogs, there were no detectable differences in the WBC's, lymphocytes, or neutrophiles in jet injected vs needle and syringe injected animals. When median values were tabulated a rather clear cut pattern emerged in all groups of dogs regardless of time of inoculation, method of injection, or dose of virus. Following virus injection there was an initial lymphopenia with a concurrent neutropenia which did not noticeably affect the total WBC count. This was followed by a lymphopenia and neutropenia which caused a marked drop in WBC's. This contrasts Taber et al[54] who reported that there was no neutropenia in dogs given virulent VEE virus.

There were minor differences between jet injected and

needle and syringe injected virus with respect to response of the leukocytic cells in calves. The jet injected groups generally returned to baseline counts approximately 24 hours sooner than the needle and syringe injected calves. The nocturnally injected calves (needle and syringe) also showed a second slight drop on days 5 through 7 p.i. which was not seen in the jet injected group of calves. The general leukocytic response in calves after virus injection was an initial depression of lymphocytes with neutrophile counts also dropping or remaining the same. This was followed by an increase of one or both cell types thus bringing the total WBC back to baseline values. These data show that the lymphocyte is the cell type which is consistently depressed by VEE whereas Walton and Johnson[57] reported that VEE infected calves had a leukopenia due to a neutropenia.

No references could be found on the effect of VEE virus infection on the hemogram of the pig. Published values for the pig show a wide normal range of WBC, lymphocytes and neutrophiles.[50] Normal ranges for pigs are: WBC's, 11,000 to 22,000; lymphocytes, 4,300 to 13,600 and neutrophiles, 3,100 to 9,000 per cu mm. In the experimental pigs, all showed a leukopenia at 24 hours which was due entirely to a depression of lymphocytes.

The neutrophile counts were increased in the diurnal groups but either remained the same or were depressed in the nocturnal groups. The leukopenia-lymphopenia was seen only at 24 hours p.i. By 48 hours p.i. WBC counts had begun to rise to baseline levels. There were no detectable differences in leukocytes attributable to mode of injection.

All of the sheep showed a depressed WBC count following virus exposure, and, except for the diurnal needle and syringe group, depression of WBC counts was seen at 24 hours p.i. The leukopenia was due entirely to a lymphopenia. Although a neutropenia was seen at either day 3 or 4 p.i. it was not reflected in a leukopenia because the depression was relatively minor due to the low normal neutrophile counts characteristic of the sheep. In comparing results of the experimental sheep with those of published normal ranges (WBC's, 4,000 to 12,000; lymphocytes, 2,000 to 9,000; neutrophiles, 700 to 6,000),[50] it was noted that all counts were within these published ranges. Since the changes were rather small and of short duration, it would appear that VEE virus exposure had little effect upon the leukocytic cells in the sheep. Differences between jet injected and needle and syringe injected groups were also insignificant.

Dosage of VEE virus appeared to affect the leukocytic response seen in the goats. Except for sporadic changes in the counts the low dose of virus did not appear to significantly affect the leukocyte counts. In the goats receiving high doses of virus, relative changes were apparent in the form of a leukopenia following virus exposure. This depression of WBC's was most noticeable in the diurnal groups of goats in which the leukopenia was due to a neutropenia which is in contrast to that seen in the dogs, calves, and pigs. This observation was not consistent in the nocturnal groups of goats which showed a transient leukopenia at 24 hours due to a depression of the lymphocytes. Many of the leukocyte and neutrophile counts exceeded published normal values for goats (WBC, 4,000 to 13,000; lymphocytes, 2,000 to 9,000; neutrophiles, 12,200 to 17,200)[50] especially in goats injected with low dose of virus, however Schalm[50] admits that the literature is scanty with respect to goat hematology. As in the sheep, the cell counts did not drop below published norms nor did the mode of injection appear to have much influence on the leukocyte counts.

Except for the nocturnal group of sheep which showed minimal elevations, all BUN values were within normal range and it would appear that VEE virus infection causes little if any functional impairment in kidney. Gross

lesions were not seen in the kidneys of the sheep with elevated BUN's.

All dogs developed a detectable viremia regardless of mode of injection, time of exposure, or dosage of virus. There was not a consistent difference between duration or magnitude of viremia in the dogs which could be attributable to method of injection except for the one needle and syringe dog having a low viremia.

Although the needle and syringe injected calves had higher titers than jet injected calves, one needle and syringe injected calf did not develop a detectable viremia. The jet injected calves had a viremia of longer duration than the needle and syringe injected calves. Viremia titers in calves experimentally infected with subtype I variety E^{16} ranged from $10^{2.5}$ to $10^{5.5}$ $SMICLD_{50}$ per ml serum. Most of the calves in the nocturnal group infected with the Texas isolate had lower viremia titers by about 0.4 logs ($10^{2.1}$ $SMICLD_{50}$ per ml), but the diurnal group had titers comparable to those cattle infected with the I-E strain of VEE virus.

The duration of viremia in pigs was not prolonged past 2 days, but the pigs showed extremely high viremia titers, and 2 animals developed viremia which exceeded 10^6 $SMICLD_{50}$ per ml. Although the jet injected pigs showed higher titers, one jet injected pig failed to

develop a demonstrable viremia. The diurnal group of pigs generally showed higher virus titers than did the nocturnal groups. This may have been due in part to the difference in ages of the pigs since the diurnal groups were younger than the nocturnal groups of pigs. A previous paper reported that pigs inoculated with virulent VEE developed antibody titers ranging from $10^{3.7}$ to $10^{4.8}$ $SMICLD_{50}$ per ml.[16] Our data showed that viremia titers for diurnally inoculated pigs ranged from $10^{2.0}$ to $10^{6.9}$ $SMICLD_{50}$ per ml and nocturnally inoculated pigs ranged from $10^{2.0}$ to $10^{4.1}$ $SMICLD_{50}$ per ml.

In contrast to pigs, the sheep showed a more consistent viremia that was of longer duration in the nocturnally inoculated animals than in the diurnal groups. In comparing jet injected with needle and syringe injected sheep, the viremia response was very similar. One diurnal jet injected sheep failed to develop a demonstrable viremia whereas a needle and syringe injected sheep showed a very low titer. The other 2 sheep (diurnal group) and the nocturnal groups had comparable titers.

Prior to this experiment it was thought that the goat was the most likely reservoir and amplifying host for VEE of the animals tested. This assumption was based on the presence of large goat populations in Latin American countries where the animal is both a companion

and food animal. However, based on the viremia response which was quite minimal or absent in 10 of 12 animals injected with VEE, the hypothesis was not substantiated. The goats showed a more consistent viremia in the diurnal groups with 5 of 6 showing viremia, but titers were very low in all except one jet injected animal and absent in another jet injected goat. In the nocturnal groups only 1 jet injected and 1 needle and syringe injected animal responded with a viremia; the nocturnal needle and syringe injected animal being the only one to develop virus titers comparable to the other animal species tested. Our data does not agree with a report by Erickson et al[19] in which they were able to detect viremia titers up to $10^{4.1}$ $SMICLD_{50}$ per ml and showed viremia in 5 of 6 goats inoculated. The highest titer seen in our study was $10^{3.4}$ $SMICLD_{50}$ per ml of serum, and only 3 of 12 had titers greater than 10^{0} $SMICLD_{50}$ per .025 ml.

It was also interesting to note that the low dose of virus ilicited a barely detectable ($<10^{0}$) viremia in the diurnal group of goats whereas the nocturnal low dose group of goats showed no viremia. However, backtitration of the low dose inoculum for the nocturnal group showed virus titers of $10^{1.5}$ $CCID_{50}$ per ml whereas the diurnal low dose backtitration was $10^{2.3}$ $CCID_{50}$ per ml and this

may have accounted for the lack of viremia in the nocturnal group of goats.

Hemagglutination-inhibition antibody response in dogs was quite uniform regardless of mode of injection.

In calves (diurnal jet injected group and both nocturnal groups) antibody was detected initially on the 14 day bleeding. Earlier antibody response was seen in the diurnal needle and syringe group of calves on days 6 and 7 p.i. Whether this was due to mode of injection or time of exposure is speculative.

In pigs, all except 1 of the 12 exposed to virus showed an HI antibody response at day 7 and all 12 were positive on day 14. All of day 14 titers except 1 were increased from the previous HI titer. On day 21 all were decreased from the day 14 HI titer except one. With the exception of the one jet injected pig which showed no titer at day 7, there was no significant differences between the groups regardless of time of exposure or method of virus injection.

All 4 diurnally inoculated sheep showed HI antibody on day 7 bleeding. One jet injected sheep (nocturnal) had HI antibodies on day 7; the other 3 (1 jet injected, 2 needle and syringe injected) were negative. On day 14 the same jet injected sheep and one needle and syringe injected sheep in the nocturnal group has HI titers.

Two of the nocturnally exposed sheep (1 jet injected and 1 needle and syringe injected) failed to develop a detectable HI antibody titer. From these data, it would appear that a diurnal exposure is most likely to illicit an antibody response. Another observation noted was that antibody titers of the nocturnally exposed sheep were still increasing on day 21 and this may reflect a delay in antibody response associated with a nocturnal exposure rather than lack of response. The differences in HI response with respect to mode of injection were minor consisting of one early response in the jet injected nocturnal group, and slight differences in HI titer.

In all goats HI antibody response was seen on day 14 bleeding, and all of the goats receiving the high dose of VEE virus showed antibody at 7 days. Day 21 HI titers were all decreased except 1 which was the same as the day 14 bleeding. The appearance of HI antibody in those goats which failed to develop a detectable viremia was of interest. Backtitration of the nocturnal low dose was lower than the diurnal low dose ($10^{1.5}$ vs $10^{2.3}$) but there was apparently sufficient virus in the nocturnal low dose inoculum to stimulate an antibody response even though there was no viremia detected. Whether this was due to handling procedures or individual host response was not determined. It was equally possible

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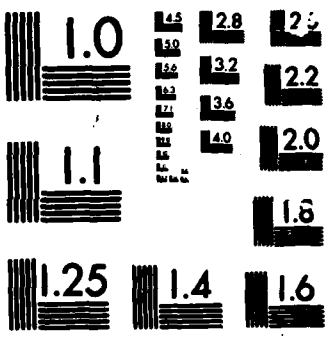
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why none of the 4 high dose nocturnal goats showed detectable viremia, but had at least $10^{5.5}$ $CCID_{50}$ virus exposure (as determined by backtitration) and developed HI antibody titers at 7 days p.i.

Gross necropsy and histopathological findings were attributable to causes other than virus infection. Attempts to isolate VEE virus from tissues collected at 14 and 21 days p.i. were unsuccessful which indicated that the virus was eliminated with appearance of antibody and did not remain as a reservoir of infection, at least in the tissues examined.

In summarizing the laboratory comparison of jet injected vs needle and syringe injected antigens, there was no overall consistent differences between the two methods. Where differences were detected by one method the converse was usually seen in another group of animals or at another exposure time.

The possibility of the 5 species tested with virulent VEE virus being reservoir or silent amplifying hosts for the spread of VEE virus was also studied. Harris[b] previously reported the role of dogs and calves in the perpetuation of VEE in nature. None of the 5 species studied could be considered as long term reservoir hosts for VEE since threshold infection levels

of viremia never existed for more than 3 days following virus exposure. Sudia and Newhouse[53] have reported that potential for vector infection is poor if viremia is less than 4.5 logs; fair, 4.6 to 5.5 logs; good, 5.5 to 6.4 logs; excellent, 6.5 to 8.5 logs. Our data indicate that dogs[b] and young pigs infected diurnally have good to excellent potential for vector infection, whereas older pigs infected nocturnally have poor potential. Sheep and calves[b] have poor to fair potential for vector infection and goats have poor potential. This contrasts Sudia and Newhouse[53] which states that the pig has poor potential for infection.

The limited field studies using jet injected VEE vaccine in a group of 131 dogs was very successful in stimulating an immune response in a population of animals. The high seroconversion rate for both SN and HI antibodies affirmed the capability of the jet injector for mass immunization in the canine. These findings extend those of Bertotti and Randolph[10] reported earlier.

Another interesting aspect that emerged from this study is the apparent long-standing immunity which the modified VEE virus vaccine appears to confer on immunized dogs. The apparent increase in LNI's in 3 of the serums collected at 1 year, compared with 35 day serums, may be inaccurate since the day 35 serums were stored frozen for

a considerable length of time thus reducing the serum neutralizing antibody titer.

In contrast to the high seroconversion rates with the VEE vaccine, seroconversion in cattle jet injected with IBR vaccine was comparatively low. The field trial involving 47 vaccinates resulted in a 26% seroconversion rate, which compared favorably to the more controlled study (Table 7 p. 55) comparing jet injection vs needle and syringe injection. In the latter study the rate of seroconversion was 20 to 50%. The factors that contributed to these low seroconversion rates were not determined. Factors to be considered were a) antigenic quality of the vaccine used, b) sensitivity of the test system for antibody assay, c) technical difficulties in handling the serum during collection, shipment and subsequent storage, d) host response to attenuated IBR virus may be minimal and samples were not collected during peak antibody production, and e) method of vaccine administration was ineffective.

In conclusion, the jet injector system performed equally as well as the conventional needle and syringe method of inoculation. From an economic standpoint in terms of cost and time, the jet injector system would be preferred since it does not require the use of relatively expensive disposable needles and syringe, and more

animals can be injected over a shorter period of time.

SUMMARY

Jet injection, as a technique in human medicine for the parenteral deposition of a number of substances has been well documented. Jet injection has proven to be safer, more economical and faster than the conventional needle and syringe method when large numbers of people were immunized. Jet injection has had only limited use in veterinary medicine. In order to better evaluate the usefulness of jet injection, the technique was tested in several animal models in parallel with the conventional needle and syringe method of inoculation. The effects of the equipment on live and modified live viruses deposited parenterally by this technique were critically evaluated.

Physical characteristics of the jet injector were studied. These consisted of dose delivery characteristics and dye penetration studies. Statistical evaluation of the dose delivery of the jet injector showed that inoculum volumes of 0.5 ml, 1.0 ml and 2.0 ml were within 5 to 6% of expected dose. Dye penetration studies in dogs, cattle, pigs, sheep, goats, chickens and fish were also performed. Optimal combinations of dosage barrel, nozzle design, orifice diameter and intensifier pressure were determined for each species of animal tested. In

domestic mammals, it was found that for a dose of 1 ml or 2 ml, the combination consiting of the intensifier (containing the 600 psi rated spring), crowned nozzle with an orifice diameter of 225 to 275u (0.009 to 0.11 inch) gave the deepest and most consistent penetration of dye. The optimal injection site was either the hip (dogs, goats, and sheep) or the neck (horses and cattle). In chickens only the 1 ml dose was tested. The 225u (0.009 inch) crowned nozzle without the intensifier worked best in chickens and the optimal inoculation site was in the leg. Injections into catfish were difficult and largely unsuccessful due to the slime buildup on the skin.

Adjunct studies consisting of intra-articular injections, nerve block injections, skin testing, permanent identification and adjuvant injections were conducted. Intra-articular and nerve block injection target sites were too deep to effectively penetrate with available equipment. Intradermal injections into the caudal fold of the bovine were not successful. Permanent identification in the equine appeared promising, however, the animal died after a three week observation period. A sheep which received jet injections of a water-in-oil adjuvant mixed with tobacco mosaic virus over an 8 week period resulted in the production of tobacco mosaic

specific antibodies.

Comparison studies to evaluate jet injection vs needle and syringe injections were conducted using radiopaque dye, modified live virus vaccines, and virulent VEE virus as inoculums. Radiographic studies in dogs showed that needle and syringe injected radiopaque dye was deposited in a teardrop pattern whereas jet injected dye tended to follow fascial planes and be deposited in muscle, subcutaneous tissue and skin.

Modified live virus vaccines were given to dogs and calves by jet injector and needle and syringe. VEE vaccine in dogs elicited a high seroconversion rate regardless of mode of injection. On the other hand IBR vaccine was less effective in that it elicited a seroconversion rate of 20 to 40% in the jet injected calves and a 50% rate in the needle and syringe injected calves.

Virulent VEE virus was given to dogs, calves, pigs, sheep and goats to further compare jet injection with needle and syringe injection. Data on clinical response, hematological, serological and virological parameters were collected and compared to show that the virus was not altered by the increased forces generated in the jet injector. The studies showed that there were no appreciable differences in the response of animals due to

mode of injection.

The epidemiology of VEE was investigated in pigs, sheep and goats. Young pigs that were injected diurnally were found to be good to excellent potential sources for vector infection based on height of viremia obtained. Sheep, goats, and older pigs (nocturnally injected) showed poor to fair potential for virus infection. These studies supplement those of Harris[b] who examined the role of dogs and calves in this respect.

Studies to examine the usefulness of jet injection under conditions of everyday use were conducted. VEE vaccine was given to 131 dogs and IBR vaccine was given to 200 calves using only the jet injector. Vaccinated animals were tested for a specific antibody response. Seroconversion rates for dogs given VEE vaccine was 96% and 90.1% respectively for SN and HI antibody response. Additionally it was shown that 6 dogs maintained significant SN antibody titer to VEE a year after vaccination. IBR vaccinated calves (47) had an SN antibody seroconversion rate of 26%.

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