IMMUNOLOGICAL RESPONSE OF *LEPTOSPIRA POMONA* BACTERIN IN LABORATORY ANIMALS

by

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INTRODUCTION

For many years the leptospiroses were considered rare diseases and until only a few years ago, they were unrecognized in livestock in the United States. It is now well established that they occur in man and his domestic animals throughout the world and constitute a major problem in certain animals. Leptospiral infections are zoonoses. The infection is transmissible from animal to animal (6) (8) and from animals to humans (46). The past decade in this country has seen an increasing awareness of the prevalence of leptospirosis in man, the domestic animals and the wild life. The public health and livestock sanitary problems posed by these infections and their economic significance to the livestock industry are increasingly appreciated (45). Clinical experience coupled with serological surveys in many parts of the United States indicate that at least 20 to 25 per cent of the swine (129) and 5 to 50 per cent of the cattle (92) have experienced infection with leptospira Pomona. A morbidity rate of 80 per cent is a common finding for an outbreak in cattle or swine (77).

Therefore, tremendous annual economic losses are incurred in dairy and beef cattle as a result of abortion, decreased lactation, stunting of young growing stock and death (65). It has also been recognized that the disease possesses a very serious explosive potential at all times, and it appears that the disease is on the increase and widespread at the present time. United States Department of Agriculture in its review "Losses in Agriculture, 1954" (65) has estimated an annual loss in cattle alone amounting to as much as $112,282,000 to the above causes. The severity of the problem will be more apparent, if similar losses in swine and other domestic animals are estimated, and the potential public health hazards are considered. This

has resulted in an ever-increasing demand for continued inquiry and research. This inquiry into the problems of epidemiology, diagnosis treatment and prophylaxis brought out additional information from time to time, along with many points of disagreement among various workers. In the field of prophylaxis, a number of control programs have been advocated and initiated. Besides, numerous commercial bacterins for the active immunization of cattle and swine to prevent the spread of infection in natural outbreaks of the disease have come into existence. These bacterins are extensively used at the present time in the field with claims and counter-claims on their ability to produce satisfactory immunity in cattle.

Studies of bio-prophylaxis of leptospirosis has always been a controversial subject, ever since such results have been reported by Inado et al. in 1916 (54). However, the commercial bacterins now available for active immunization have all been claimed to produce satisfactory immunity against natural outbreaks in cattle. A critical evaluation of many such reports always disclosed some serious difficulties in proof and stimulated further research. In addition to these doubts, field experiences have failed to convince the research worker. Consequently this study was undertaken to evaluate the immunological response of a widely used commercial leptospira pomona bacterin in laboratory animals.

REVIEW OF LITERATURE

Pathogenic leptospires were isolated first from cases of Weil's disease in 1915 independently by Inado et al. (54) in Japan, Hubner and Reiter (52) in Germany, and Uhlenhuth and Fromme (116) also in Germany. Earlier in 1886, Weil (124) described several cases of "leptospirosis" in humans, although he had no knowledge of the causative agent. Nearly twenty

years later, leptospires were first seen in New Orleans by Stimson (105) in sections of kidney from a patient believed to have died of yellow fever. In 1935, translations appeared concerning Russian findings by Semskow and fellow workers (101) of a spirochete (they) called Leptospira Icterohaemoglobinurea in calves and man. Leptospira Pomona was first recognized in 1937 by Clayton et al. (27) as the cause of illness in a dairy farmer in Pomona, Australia. This was followed by studies in Palestine by Freund, Bernkopf and others (34) (8), concerning a similar organism called Leptospira Bovis that caused disease in cattle and was considered fatal to man.

In the United States, Jungherr (60) from Connecticut in 1937 reported his finding leptospires in the kidneys of three different cattle at autopsy. Marsh (67) in 1945 and Mathews (69) from Texas have reported similar findings. Shortly thereafter, Baker and Little (4) in 1948 published a complete report of leptospirosis in cattle. These workers isolated the organism from a febrile disease in a dairy cow in New Jersey, which they earlier considered as a filterable virus. They soon realized however, from their continued research, that the original agent they had isolated from the milk of the cow and transferred successfully to other cattle, guinea pigs, mice, hamsters and fertile eggs, was actually the leptospira. Following this initial report, the disease has been found to exist in many other parts of the United States. The organism however, could not be classified immediately until extensive comparative serological tests necessary could be developed. Bernkopf and Little (7) in 1948 proved by serologic tests that the American strain of leptospira was different from the more virulent one found in Palestine. Finally Gochenour et al. (44) in 1950 found antigenic similarity between the bovine leptospira in the United States and the one in Australia that had been called Leptospira Pomona.

Thus, leptospirosis is now recognized as an apparently widespread infectious disease of cattle, swine, sheep and horses usually caused by Leptospira Pomona and of dogs caused by Leptospira Canicola (119). Occasionally leptospira Canicola has been reported from bovine cases. The disease is characterized by fever, marked prostration, icterus, hemoglobinuria, bloody milk in the severe form, depression, inappetence and a drop in the milk production in the chronic form (71).

With the result, extensive studies have been carried out by various workers on the epidemiology, pathology, immunology and control of this disease during the past few years.

Epidemiology

Epidemiologically, it has been brought out by several workers (72) (118) (46) (57) that leptospirosis is primarily a disease of rodents and it is upon these hosts that the leptospires depend for their survival, and that man and the domestic animals become infected by coming in contact with excreted leptospires. Experimental work conducted in a search to determine the natural reservoir host for Leptospira Pomona among the domestic animals besides the rodents, indicate swine as the natural reservoir host. It was thought that infection in cattle did not appear to be as widespread as it does in swine. Furthermore Leptospira Pomona transferred readily by contact exposure from pig to pig and from pig to cattle, a finding apparently made possible by the tremendous numbers of organisms eliminated in the urine over long periods in carrier swine. Although rodents and swine are considered to be the primary animal carriers, recent investigations have revealed a variety of hitherto unrecognized wild mammal hosts (1) (119). Present studies seem to indicate additional sylvatic leptospiral carriers

in many parts of the world (3). It has also been confirmed that these organisms display an affinity for the renal cortex and may be found nesting in the lumina of the convoluted tubules of the kidneys of clinically recovered animals and in others with an inapparent infection (3). They may be shed in the urine of these carrier animals for varying periods of time. It is not very clear why certain animals become permanent carriers, while in others the urinary elimination of leptospires is only a transitory event. The present knowledge with regard to the exact epidemiological significance of each species in any outbreak of this disease is also not complete. However the epidemiology of infection seems to be governed to a great extent by the availability of suitable hosts and an external environment favorable to the survival of leptospires.

Diagnosis

Varyinus methods of diagnosis of the disease are available at present, though many are cumbersome and some are of doubtful value. It is essential in establishing a definite diagnosis that the clinical findings be confirmed not only by the serological evidence but should be supported by the isolation and demonstration of the organism, followed by animal inoculation procedures to determine the virulence of the organism (isolated) and its capacity to produce pathological changes in experimental animals. In essence, Koch's postulates should be established, for, many times clinical symptoms are inadequate and the value of serological tests are confusing.

Serologically, the test of classical significance is the agglutination-lysis test of Schuffner and Mochtar (100), one of the earliest leptospiral sero-diagnostic tests devised. It has been, and is today, the most widely used procedure in this respect. This is a microscopic agglutination test

in which living leptospires are employed as antigens. This is also considered a standard procedure against which all other serological methods devised later have generally been evaluated. Recently, complement fixation test and a capillary tube agglutination test have been described. In many such procedures killed organisms as antigens have been used. Randall et al. (87) have employed leptospires subjected to sonic vibration as antigen in the complement fixation test, and claimed positive reactions from serums taken as early as fifth day of illness. York (127) devised a complement fixation test in which formalinized infected chorioallantoic fluid is used as antigen. The agglutination test devised by Stoenner (109) is performed by formalin killed concentrated antigen.

Thus various serodiagnostic technics have been developed and perfected, but at present, the value of any serological test, however, is limited to a considerable extent by the presence of various serotypes of the organism and many nonspecific reactions encountered in these procedures. Clinically it has been observed that leptospirosis is one of the most variable diseases ever studied. Even in a single herd under close observation, all animals do not show exactly the same degree of illness which may vary all the way from inapparent infection to death. Isolation of the organism from blood, urine and other sources, identifying the organism, and demonstration of its pathogenicity in suitable animals therefore is the only way to establish a diagnosis. Isolation of the organism again is by no means easy to accomplish. Leptospires, unlike many other bacteria, are not visible with ordinary staining methods and by the usual microscopic examination. They would not or could not be seen unless they are specifically searched for with suitable equipment from any of the body fluids. They do not grow and multiply in the usual bacteriological media under

ordinary laboratory conditions. Some of these variabilities of leptospirosis as a disease and the peculiarities of leptospira as an organism, makes it all the more difficult to isolate and establish the organism for appropriate tests. However, bacteriological evidence has to be established by animal inoculation and culture methods in support of clinical and serological evidence in view of the present inadequate serological techniques. This is particularly essential if knowledge of the epidemiology is to be obtained and a specific prophylaxis and control program is to be instituted.

Prophylaxis and Management

With mounting evidence as to the economic significance of the disease in domestic animals, it is only natural that various prophylactic programs should have been initiated. Prevention and control of leptospirosis as in the case of any other infectious disease depends to a large extent on the background of information available on the various aspects of the disease accumulated by a number of research workers. Factors such as the ecology of the carrier host, the carrier state in the recovered animals or in the inapparent infections, the mode of transmission of the disease, the number of susceptible animals in an outbreak and the conditions peculiar to each outbreak, should be taken into consideration in planning any control program. It was shown experimentally that infection could occur by contact exposure between susceptible animals. Baker and Little (4) have observed that infection could also occur by infected urine excreted by a standing animal onto the concrete floor of a barn thereby causing a spread of droplets to arise, which in turn are inhaled by nearby healthy animals. Organisms can also gain entrance through conjunctiva in such a case. This mode of infection could explain how a single carrier animal whether pig or cow could

initiate infection in a susceptible herd of cattle. Spread of infection from one farm to the other could easily occur by the contamination of a connecting water source by infected urine. The severity of an outbreak in any case largely depends on the virulence of the infective agent and the number of susceptible animals in an infected herd.

Active campaigns for the destruction or elimination of carrier animals from a herd and appropriate hygienic measures should greatly help in reducing the incidence of leptospirosis. Jones et al. (58) reported a successful attempt to control leptospirosis among dogs at the war dog reception and training center by the total elimination of all carrier animals. Each dog was kept in quarantine on arrival for a period of two weeks, agglutination tests carried out and the dog was destroyed if the serum reacted positively. Evidently such drastic measures are neither practicable nor justified when swine are considered as carriers and the large number of subclinical or inapparent carrier states occur in cattle.

Therapy and management of individual infected animals has received considerable attention during the past few years. Treatment as in any other acute or chronic infectious disease depends on the stage of the disease that might be presented. It should aim at the preservation of the life of the patient in the acute stage and to prevent or treat carriers in chronic stages. Treatment with the available antibiotics may be most effective in the early stages and of very little value in the carrier condition. In vitro studies indicate penicillin, streptomycin, aureomycin, terramycin, and oxytetracyclin as active against leptospires, particularly the latter two (59). However, results of treatment in actual cases with any one or a combination of them are variable. None of them have been shown conclusively to influence significantly the course of the disease

when administered after hepatic or renal changes have occurred (106).

Thus, in the absence of any effective therapeutic agent, careful attention to general supportive measures and dietary regulations should be considered as of paramount importance in the treatment of individual animals.

It is well known that a natural attack of leptospirosis confers a degree of immunity in man and other animals. Obviously, active immunization of susceptible cattle in an enzootic area or in an infected herd coupled with appropriate hygienic measures, appears to be the logical approach to the control or elimination of bovine leptospirosis. Since the disease spreads slowly through a herd of cattle, vaccination to stop spread of infection might be looked upon as a form of herd therapy in the control.

Successful active immunization against leptospiral infections was first reported by Inado et al. (55) in 1916. They immunized guinea pigs with emulsions made from the livers of infected animals, the organisms being killed by 0.5 per cent phenol.

Noguchi (81) in 1918 showed that three injections of 0.5 milliliters of killed leptospiral cultures made guinea pigs completely resistant to both homologous and heterologous strains of leptospires. He stated when smaller quantities were given, the immunity established was only often sufficient to protect the animals against the homologous strain, and not against heterologous strains. Active immunity was found to persist for eight weeks.

Berger et al. (5) in 1923 claimed to have successfully immunized guinea pigs with cultures of leptospira killed by treating them with copper foil.

The vaccination of acute jaundice caused by leptospira Icterohaemorrhagiae was used successfully in field trials in England by Dalling and Okell (28). The vaccine was prepared from the livers of infected guinea pigs and was claimed to have produced satisfactory immunity.

Wani (121) in 1933 reported very successful results from the large scale immunization of workers in Japan. He claimed a marked reduction in the incidence of Weil's disease among coal miners vaccinated with killed suspensions of Leptospira Icterohaemorrhagiae.

Smith (102) in 1937 claimed to have set up a solid immunity in guinea pigs, but stated that the animals later became urinary shedders, after challenging with virulent cultures. In this experiment, different groups of guinea pigs were vaccinated with living nonvirulent strains of Leptospira Canicola and Icterohaemorrhagiae. In the other groups, vaccination was carried out by treating them with heat-killed, phenolized, formalized or dettolized vaccines of the same organisms. Among the animals inoculated with living nonvirulent cultures, no carriers were detected. In the other groups, protected by killed cultures, carriers of virulent leptospires were demonstrated after challenge. Again, where formalized emulsions of Leptospira Canicola and living cultures of virulent Leptospira Icterohaemorrhagiae were employed for vaccination, further evidence of carrier condition was obtained. Thus, immunization with chemically killed culture vaccines of virulent strains of leptospira were not successful in preventing the carrier condition. Presumably, he had succeeded in setting up only a partial immunity to suppress obvious clinical symptoms and not the carrier condition.

During the last war, Russian workers claimed excellent results in controlling bovine leptospirosis in Ukrain and Crimea by successful vaccination.

On the other hand, Olitzke et al. (82) in 1949 have conducted immunization experiments in calves with formalized cultures of Leptospira Bovis. Their experiments revealed that the formalized leptospires produced only a moderate but not a solid immunity. They concluded that formalized leptospires

were devoid of antigen which was believed to be present in the living organisms, or that the formalized cultures did not contain the amount of antigen necessary to establish solid immunity. Comparatively better results, however, were obtained in their experiments by repeated inoculations by living cultures of the relatively inocuous Gryphotyphosa or treatment with formalized vaccine followed by inoculation with virulent bovine strain.

In the same year Brunner and Mayer (20) in their efforts to immunize dogs against Leptospira Icterchaemorrhagiae found that some of the immunized dogs though apparently healthy proved to be virulent urinary shedders. The vaccine used by them was prepared by killing the cultures by lyophilization, and concentrated by high-speed centrifugation. This procedure, they believed, was not supposed to reduce the antigenicity of the organisms. In their experiments with guinea pigs immunized with the same vaccine, and challenged later with virulent living cultures, it was found that though the guinea pigs were infected, they did not show any apparent symptoms of infection.

York and Baker (126) and York and Buckner (129) in 1953 in the United States have published accounts of a method of vaccination for which good results have been claimed. The vaccine consisted of suspensions of Leptospira Pomona organisms (Cornell strain T262) that had been cultivated in eggs. They have inactivated the organisms by treating with formalin or by freezing and thawing. The vaccine was initially tested in calves and guinea pigs for development of immunity. Two to six weeks after vaccination, animals were challenged by intraperitoneal inoculation of defibrinated blood that contained virulent leptospires. The response was evaluated by thermal reaction and serological tests. They claimed that all the calves and guinea pigs vaccinated in the experiment developed immunity and resisted challenge,

while the controls in both groups showed some form of clinical infection. In field trials where natural infection would occur, it was claimed that immunity could be produced in a significant number of animals and thus limit the spread of infection in the vaccinated group.

Similar results were obtained by Brown et al. (17) in their experiments with another bacterin. They have immunized guinea pigs with various dilutions of bacterin in sterile physiological saline followed by challenge with virulent organisms. The response was again evaluated by thermal reaction and serological titration. Further evaluation of the response of this bacterin was carried out in cattle. A single dose of 5 milliliters of the bacterin is said to have elicited sufficient serum antibodies to give an initial positive agglutination lysis test at serum dilutions of 1:10 to 1:250 which was considered sufficiently high to indicate good protection lasting for a period of one year.

Hoag in 1955 (49) described a soluble immunogenic agent prepared by the acid heat extraction of living cultures of Leptospira Pomona. The results of its use indicated that it produces a satisfactory immunity in calves against infection with homologous strain. Subcutaneous inoculation of this immunogenic agent seemed to have produced sufficient immunity in two weeks to resist infection with virulent organisms, whereas the control animals all developed clinical symptoms, pathological lesions and leptospiruria.

Webster and Reynalds in 1955 (123) from New Zealand have also published satisfactory results of vaccination in guinea pigs and sheep with Leptospira Pomona bacterin. All the vaccinated animals resisted challenge with 1 milliliter of a seven-day old culture of Leptospira Pomona, three weeks after vaccination and again eleven months later. The criteria of infection was

thermal reaction, leptospiruria and differences in weight gains in both groups. They claimed complete protection of the vaccinated sheep and guinea pigs against infection.

While the present study was in progress, Phillips (83) in evaluating three different leptospiral bacterins concluded that all the products proved to be exceptionally potent immunogenic agents. In hamsters, protective immunity could be conferred at a dilution of antigens as high as 1:512 against an extremely heavy challenge with Leptospira Pomona. Similar evaluations were carried out with calves between 600 to 800 pounds. The calves were seronegetive to agglutination-lysis tests at the beginning of the experiment. Twenty days after vaccination in three different lots of calves, all of them including controls were challenged with 2 milliliters of 10 per cent virulent hamster tissue consisting of liver, kidney and spleen. The challenge dose was estimated by dark field microscopy to contain 1,000,000 plus hamster M:L.Ds. The results of vaccination were evaluated by thermal reaction and serological titres. All the controls reacted to challenge with fever while the vaccinees did not. All the vaccinees and controls, however, showed a sharp rise in serum titres. In the vaccinees, there was a gradual but low increase in titres for a period of fifteen days (recorded) from the lowest prechallenge titre of 1:250 to a low postchallenge titre of 1:1250. The highest titre obtained was 1:781,250. Similar titres in controls rose sharply from almost nil to 1:1,000,000.

During the same period Gillespie and Kensy (38) (39) (37) published accounts of their evaluation of three different bacterins in calves of different ages. They have concluded from the results of a series of tests that:

1. Leptospira Pomona bacterins appear to induce in 6 to 8 month old

heifers a high degree of immunity which persists for at least 7 to 8 months.

2. Vaccinated cattle that become infected on challenge often appear to experience a mild form of disease.

3. The vaccination resulted in an apparent reduction of severity of the disease with suppression of "shedding" even though infection may occur.

4. The calves that received the bacterin when three to five months of age acquired relatively effective resistance.

They have stated that "Either the appearance of the agglutinins in high titre or a clearly defined increase (100 fold or more) over the vaccination titre was found to be reliable criterion of infection in vaccinated cattle." This procedure supplemented by thermal reaction, animal inoculations "sometimes" dark field examination of urine, was the basis for the above experiments. Their results further indicate that the serum titres six weeks after challenge in the 6 to 8 month old vaccinees were almost negative, although these animals have resisted challenge. In one of the vaccinee's in this group, the only indication of infection was a post challenge titre of 10-4. There was no thermal reaction and the animal was found to be a urinary shedder. One of the controls showed a post challenge titre of 10-3. There was no fever and no evidence of infection could be detected. From these results it appears that there is no unequivocal evidence of protection against infection.

MATERIALS AND METHODS

Preliminary work carried out in the laboratories of the Department of Pathology, Kansas State University, prior to the beginning of this work indicated chinchilla (Chinchilla Laniger) as a suitable experimental animal to leptospiral infections. This prompted further trials in this direction

with a view to determine the suitability or otherwise of the various experimental animals available for this work. Rengen and Okazaki (91) compared guinea pigs, Syrian hamsters, white mice and two-day old chicks to their susceptibility to infection with Leptospira Pomona. They found that the guinea pigs and white mice are about equally susceptible, the hamster significantly more resistant and the chicks highly resistant. Fisher et al. (33) and Howarth and Reina-Guerra (51) have also found that the chicks are more resistant than hamsters to different species of leptospiral infections. Early experiments in this work confirmed that the hamsters and chick embryos are more resistant than guinea pigs and that the chinchilla is the least resistant of all to experimental infection with Leptospira Pomona. In hamsters and guinea pigs, intraperitoneal inoculation of virulent organisms produced only a mild thermal reaction without any apparent clinical symptoms and lasted only several hours, while the same dose in chinchillas resulted in a prompt thermal reaction, clinical symptoms of fever followed by death with typical pathological lesions. To date, this has proved to be the only susceptible animal tested to leptospiral infection, since in no other animal death could be produced by comparatively larger doses of organisms. Eighteen chinchillas, 58 guinea pigs, 12 hamsters and a number of embryonated eggs were used in these trials. While these experiments were progressing, Roberts and Turner (93) published similar findings. Later in this study, it was found that while death could be produced in chinchillas with as low as 250 organisms per dose, some of the guinea pigs may not even show thermal reaction with the same number of organisms.

With these findings in this laboratory and similar experiences of other workers (93) it was decided to use chinchilla for this study along with guinea pigs for comparative results.

Animals

All the experimental animals in this work were drawn from the breeding stock maintained in the small animal laboratories of this department. The experiment was carried out in two stages: preliminary and confirmatory. In the preliminary work, 19 healthy chinchillas of different ages and 21 young, active guinea pigs weighing between 150 and 290 grams were selected. One-half of each species were used as controls. Chinchillas were kept in small cages, two for each, and the guinea pigs in large cages, eleven in each, one of them marked as controls.

In the confirmatory test 24 chinchillas as vaccinees and 8 controls, 12 guinea pigs in each of vaccinees and controls were used. Body weights of the guinea pigs were recorded at regular intervals before and after challenge to observe differences if any in daily weight gains. All the experimental animals were kept under similar conditions throughout the period and were closely observed daily. Temperatures were recorded and any animal not appearing normal was replaced before each experiment was commenced.

Bacterin

One of the Leptospira Pomona bacterin which was reported to produce satisfactory immunity on vaccination in natural outbreaks in the field, was selected for use in this study. Two-tenths (0.2 milliliters) of a minimal bovine immunogenic dose of similar vaccines was generally considered adequate to produce satisfactory immunity against a heavy challenge in guinea pigs and other experimental animals. Dilutions of 1:16 to 1:512 of this dose of these bacterins was reported to have given sufficient immunity in guinea pigs and hamsters (83). One-fourth part of a recommended dose for cattle was therefore

considered adequate to produce the necessary immunity in chinchillas and guinea pigs in this study. Hence, 0.5 milliliters of the test vaccine was used for vaccination throughout this study. In the preliminary work, 0.5 milliliters of the vaccine was injected intramuscularly in the gluteal muscle of the right hind limb of the vaccinees in each group while the controls were untreated. In the confirmatory test, one half of the same dose was injected similarly in each limb. In addition, one half of the vaccinees in this group were given a second injection with the same dose 15 days after the initial vaccination. Temperatures were recorded following vaccination and the site of injection was observed for any general or local reaction.

Strain of Leptospira Pomona Used

Leptospira Pomona (Schelecty strain) which was isolated in this laboratory from the kidneys of a calf diagnosed on necropsy was used in this study for challenge material. The calf came from Alma, Kansas and the isolation was made using guinea pigs. The organism was later typed at the Walter Reed Army Medical Center, Washington, D. C. The strain has since been maintained by cultivation in Stuart's modified medium (110) containing 7 to 10 per cent rabbit serum.

Virulence

The virulence of the organism has been maintained by routine passage through chinchillas. Thus the organism has been through 18 serial passages. The virulence of the strain at the time of challenge was such that thermal reaction and clinical symptoms were consistently produced as early as third day of inoculation resulting in death of the animal by fifth or sixth day

with typical pathological changes in many organs described later. The organism was recovered from the chinchilla by the cardiac puncture on the 18th passage and cultivated in Stuart's medium and was used for challenge in the preliminary experiment. The strain was however, passed through another passage for the second half of the experiment.

Method of Challenge

Normal temperature of all animals was recorded daily starting four days prior to the day of challenge and continued to completion of the experiment. The animals were inoculated intraperitoneally.

Considering the results of the preliminary study, it was concluded that the challenge dose was too large to withstand the immunity, if any, and that the period following vaccination might be too short for the development of complete immunity. In order to rectify these defects, a method has been devised to determine the LD-50, and the time between vaccination and challenge had been increased, and serum samples before vaccination and challenge were collected and titrated by agglutination lysis tests.

The method followed for the determination of LD-50 was essentially the same in principle as described by other workers (88) (25), except the number of animals used in each dose level was limited. A number of media tubes were inoculated with the organism and incubated at 29° C. for 48 hours. The cultures were examined by dark field illumination and a culture which did not show any clumping of organisms was selected. Quantities of 0.0005 milliliters of the culture were placed on a clean glass slide by using a microsyringe fitted with a 27 gauge needle and were allowed to dry. They were fixed by gentle application of heat and stained by Fontana's silver method

(63). Total number of organisms in each quantity was counted under oil-immersion and the number per milliliter of the original culture was determined by simple multiplication. The culture was then diluted with stock medium as to have a known number of organisms per inoculum. A number of chinchillas were inoculated with graded doses starting from 10,000 to 250 organisms per dose. LD-50 was determined by calculating the number of organisms which might cause death in 50 per cent of the animals.

Thus, in the second part of the experiment, both the vaccinated and control animals were challenged with the above dose on the 70th day following vaccination.

Following challenge in both experiments, temperatures were recorded on each day and an average of 3° F. rise in any animal was considered a thermal reaction. Cultures were made at the initial rise of temperature from the heart blood. Each animal was anesthetized by ether, and an area was clipped close of about one inch on the ventral thoracic region anterior to the xyphoid cartilage. The clipped area was treated with rocal antiseptic solution. With a 5 milliliter syringe fitted with a 24 gauge needle, cardiac puncture was made by introducing the needle in between the ribs on the left lateral aspect nearest to the heart. One milliliter of blood was drawn, and one to two drops were inoculated aseptically into tubes of Stuart's modified medium. The tubes were incubated at 29° C. Every effort was made to prevent contamination during these bleedings. The cultures were examined on the fifth day of inoculation and at weekly intervals thereafter for a period of 30 days.

Animals were observed closely following the thermal reaction, clinical symptoms recorded, and a thorough necropsy was conducted on each animal immediately after death. Tissues were saved for histopathological studies.

Criteria of Infection

Clinical symptoms, bacteriological evidence and pathological lesions were depended upon as evidence of infection in vaccinated as well as control animals. Agglutination-lysis test was made use of only in the determination of immunity following vaccination.

RESULTS

Serological

Serum samples taken from chinchillas and guinea pigs prior to vaccination and challenge were titrated by agglutination-lysis tests and the results were tabulated (Tables 7-11). Majority of the samples showed negative titres both before and after vaccination. Only 10 chinchillas and 8 guinea pigs gave slight reactions in dilution of 1:10. These results did not indicate any appreciable increase in serum titres following vaccination in chinchillas and guinea pigs.

Clinical

In the preliminary experiment, both vaccinated and control chinchillas developed thermal reaction following challenge with virulent culture. Clinical signs exhibited are fever, lassitude, depression or weakness, partial or complete anorexia and occasional bloody diarrhea. Thermal response was observed on the third or the fourth day of inoculation. There was an average increase of 3.8° F., the maximum in some animals reaching 104° F. During this period of pyrexia, the animals were very dull, depressed and did not eat or drink. They started crouching up in corners, refused to move and gradually became very weak. (Plates I-II.) By the fifth day, all of them

appeared very apathetic and took very little interest in their surroundings. The temperature became subnormal and they became very weak, lying down and prostrate. In the next few hours they were moribund and died. By the sixth day, all the animals were dead (Tables 1-2). Deaths occurred in the vaccinated animals first and the controls survived longer than in the vaccinated group. Generally, the thermal reaction did not persist longer than 36 hours.

All the above symptoms were observed in the confirmatory experiment with the following additional or modified reactions. Fever began only on the 4th day in both vaccinees and controls and persisted for the same length of time. The reaction was not as severe, the maximum recorded temperature being only 103.4° F. Deaths occurred only from the 7th day, and in many animals, the period of survival was much longer. All the vaccinees in the single dose group, nine in the double dose group and three in the control group died of the infection. Three in the double treated group and five in the control group survived infection by the 16th day, and thereafter continued to live apparently healthy. After this date they started going down in condition, appeared sick and on the 49th day one in the remaining three of the double dose vaccinees and another in the control died of the disease. Thermal reactions and deaths started earlier in the vaccinees than in the control animals. One hundred per cent of the single dose vaccinees, 83 per cent in the double dose group and 37 per cent in the control group died in the acute stage of infection (Tables 12-14).

Differential counts indicate a distinct leucocytosis, the percentage of neutrophils varying from 58 to 74 per cent and a shift to the left (Tables 17-18).

In guinea pigs, febrile reaction developed both in vaccinees and

controls between the fourth and the seventh day of inoculation. Fever reached a maximum of 106.8° F. in some animals, with an average increase of 4° F. The febrile period persisted for two to three days in some animals and then returned to normal. No other clinical evidence of infection was noticeable in these animals (Tables 3-4). All apparently looked bright and normal in appearance and activity.

Both vaccinees and control guinea pigs were gaining an average of 4.5 grams per day prior to challenge inoculation. Following challenge the average daily gain per animal had dropped by 0.5 grams for the first ten days and thereafter, all of them started regaining the average preinoculation gains (Tables 5-6). Throughout the period under observation, the animals were under similar conditions of feeding and management.

Similar clinical picture in guinea pigs was noticed in the confirmatory experiment. Thermal reaction was very mild. Fever appeared only on the 8th day, reaching a maximum of 105.2° only with an average of 103.3° F. Five animals in the control group and three in the vaccinees did not show a temperature response (Tables 15-16). Body weights were not recorded.

Bacteriology

In the preliminary experiment, leptospires were isolated from 8 of 10 vaccinated and 7 of 9 control chinchillas. In guinea pigs, 5 of 10 vaccinated and 2 of 11 controls the cultures were positive (Tables 1-4). The negative results were thought to be due to the use of contaminated serum in the media.

In the second experiment, 11 of 12 single dose vaccinees, 10 of 12 double dose vaccinees and 7 of 8 control chinchillas gave positive blood cultures. Two of six double dose vaccinees, three of six single dose

vaccinees and four of twelve control guinea pigs did not show a thermal reaction. The rest of the guinea pigs gave positive blood cultures (Tables 12-16).

Pathology

Review of literature. Bovine leptospirosis occurs in an acute septicemic form or a chronic nephritic form. The septicemic form is generally hemolytic and fatal. Such cases present generalized icteric or anemic discoloration of the mucous membranes, hemoglobinuria, and hemoglobinemia. Petechial hemorrhages may be found on the submucosa or subserosa of many organs and tissues. The liver may be swollen, yellowish or mottled. The spleen was usually normal in appearance. The kidneys were of normal color, the stripped surface presenting numerous reddish brown spots, about 1 to 2 millimeters in size (115). The lungs were normal or slightly edematous.

Microscopic lesions were consistent in the liver and kidneys, varying only in the degree among individuals. The kidney changes consisted of tubular degeneration, cloudy swelling or necrosis and may be distended by granular debris, protein casts, and leucocytic casts. Thickening of the Bowman's capsule and congestion of the glomerular tufts was reported (89). Diffuse and focal infiltration of lymphocytes, plasma cells and macrophages were found throughout the stroma of the renal cortex.

In the liver, disorganization of the hepatic cords, diffuse and focal infiltration of leucocytes, areas of focal necrosis with cytoplasmic degeneration and occasional hemosiderosis were reported. Hemorrhagic foci and ulcerations in the abomasal mucosa were observed (115). Occasionally, pericorpuscular congestion and hemosiderosis in the spleen were reported.

Lungs were not involved in most of the cases.

In chronic cases, the lesions consisted of focal interstitial nephritis characterized by infiltration of large masses of lymphocytes in the cortical stroma.

Present Study. Gross and microscopic changes observed in both groups of experimental animals were very similar. Of the total chinchillas in both experiments, 84.3 per cent died in the acute stage. Two guinea pigs from each experiment were sacrificed on the 179th day for observations.

In chinchillas, characteristic gross lesions were jaundice, petechial and or ecchymotic hemorrhages in most of the organs and tissues, enlarged liver, hemorrhages and free blood in the stomach and gastroenteritis. In addition, the lungs and psoas muscles presented many ecchymotic and large hemorrhages.

Jaundice. Jaundice varied from mild to severe according to the period of survival of the animals. This condition was observed in 17 of the 44 chinchillas evidenced by the icteric discoloration of the subcutaneous tissues, peritoneum and liver. The serum collected at the time of death was typically yellowish in color.

Hamorrhages. Petechial and ecchymotic hemorrhages in the tissues and organs were present in all the animals that died during the acute stage. These hemorrhages were observed in the serous surfaces, visceral pleura and occasionally on the mucous membranes of the stomach and intestines. In the majority of the animals, the surface of the lungs was studded with hemorrhages. In the rest of the animals, the lungs were emphysematous. Free bleeding in the stomach occurred in almost all the animals and was a constant lesion. Hemorrhages were also observed on the visceral surface of the stomach and intestines. In a few animals, petachiation was marked in

the kidneys. Extensive hemorrhages were seen in the psoas muscles in many of the chinchillas that died. Petechiation was also constantly found in the cortex of the adrenals.

Kidneys. The kidneys were somewhat swollen and pale in appearance, and some of them presented minute petechiation on the surface of the cortex, which was particularly visible when the kidney was stripped. Greyish white foci on the surface of the cortex, described in other animals were not observed.

Liver. The liver was enlarged and mottled and somewhat congested. Varied tints of yellowish green were present on the surface. In animals without marked icterus, minute petechial hemorrhages in the substance of the liver could be observed. The gall bladder appeared normal.

Spleen and lymph nodes were generally congested and enlarged.

Lungs. The most striking lesions in the lungs were ecchymotic hemorrhages on the surface. In some, these hemorrhages were only a few while in others, they were extensive and numerous. In a few animals the lungs were emphysematous.

Microscopically, characteristic changes observed were focal interstitial nephritis accompanied by degenerative changes in the tubules, cloudy swelling and dissociation of liver cord cells and focal hemorrhages in the lung perenchyma. Spleen was generally normal except for slight to moderate increase in the hemosiderin pigment.

Kidneys. Kidney lesions were always consistent, though variable. The tubular epithelium of the convoluted tubules revealed cloudy swelling and various stages of degeneration and also regeneration. The cytoplasm was very granular in some instances. The lumens of the tubules were filled with granular debris and dead cells. An increased cellularity in the

collecting tubules with lymphocytes and nutrophils was especially striking in some. Fatty degenerative changes in the corticomedullary area and less changes in the distal portion of the calyces were observed. The glomerular alterations were striking. A slight to moderate thickening of the basement membrane, cloudy swelling and apparently hyalinization of the capsular epithelium were observed. Varying degrees of dilation of the space within the Bowman's capsule and varying amounts of albuminous precipitate in the capsular spaces were observed.

Leptospires were numerous and typical and were found in the kidney sections stained by silver impregnation technic.

The liver also presented evidence of degenerative changes. Marked cloudy swelling and fatty infiltration were consistently observed. Dissociation of liver cells was observed in many instances. Generalized cellular infiltration or focal infiltration around the triads and large vessels was significant.

Large number of leptospires were seen in silver impregnated sections of the liver. These were observed between the liver cord cells.

Spleen. Except for considerable hemosiderin pigment in the spleen, no other pathological lesions were seen in this organ. No organisms were seen in sections stained by levaditi's method.

Leptospires were also observed in the lung sections.

Other organs and tissues did not present any significant pathological lesions.

DISCUSSION

While these serological tests show contact with leptospira, either acute or chronic, they do not evaluate the ability of the animal to resist infection, especially a low descending or vaccine titre which could be confused with a nonspecific reaction.

So remarked Stoenner (107) regarding the serological tests in leptospirosis. His statement could very well be appreciated from the innumerable variations in titres of both vaccinated and control animals reported by many workers (83) (38) (39) (37) in leptospiral immunology. Stoenner attributed these variations to be mainly due to the bacterial cell concentration in the antigen, the variability of dilution technic, growth time and strain variation in the agglutination-lysis technic. It has been demonstrated many a time in the study of other diseases too, that the amount of circulating, agglutinating, precipitating or complement fixing antibodies are not true measures of protection or immunity. The immunity which develops in a syphilitic human patient is a unique example. It is maintained at high level by continuous antigenic stimulation by a small number of organisms present in obscure areas of the body. It is well known at present that animals can be infected with leptospirosis, experience leptospiremia, develop renal foci and leptospiruria, all without showing a marked febrile response or other clinical evidence. Clinical experience with leptospiral vaccines has failed to give convincing results in some cases. There was other evidence traced earlier that formalinized or other chemically killed leptospiral vaccines have failed to prevent renal carrier states in experimental animals (102) (28).

The relation of demonstrable antibody and effective immunity is far from clear. There seems to be no corelation between serological titres and effective immunity. The titres should not be taken too strictly as evidence of immunity until it is clearly shown that such a co-relation exists.

Therefore, any evaluation of vaccine should necessarily include adequate bacteriological demonstration of protection against infection in the vaccinated animals, and susceptibility to infection in control animals.

Failure to demonstrate organisms in the urine under microscope, or even failure to isolate from the urine by animal inoculation from vaccinated or control animals after challenge may not necessarily mean that the vaccinated ones are protected. This is particularly true when it is realized that quite often leptospiruria is intermittent or uncertain, and that the knowledge of the carrier condition at present is inadequate.

To consider death or survival as a true measure of infection or protection by vaccination, none of the experimental animals were found to be highly susceptible to leptospiral infection until recently. It is not always easy to demonstrate infection or protection by other available methods because of the possibility of the variations in technic and the peculiarities of the disease as had been pointed out earlier.

Guinea pigs, hamsters, other experimental animals and cattle frequently have a certain amount of natural resistance and survive inoculation with virulent strains of leptospira, although the organisms might establish foci of infection in certain areas of the body. The results of vaccination and challenge in these animals therefore might be irregular and confusing.

The results of the present experiments have failed to indicate any protection in the vaccinated chinchillas. The maximum titre at the time of challenge was only 1:10 in a few animals and none at all in the majority of them. The low titres recorded may be nonspecific and may not be due to vaccination. Clinically, all the vaccinees and controls had reacted to the challenge similarly with typical clinical symptoms, and characteristic gross and microscopic lesions.

As pointed earlier, it was interesting to observe more severe reactions and early deaths in vaccinated than in control animals. This condition was observed in both the experiments. In the second part, 87.5 per cent of the

vaccinated animals died, while only 37.5 per cent of the controls died of infection. The thermal reaction was more severe in the vaccinated than in the control chinchillas and guinea pigs as well. Such a phenomena has long been recognized in other diseases too. It was postulated earlier that bacterial cell substances may act as sensitizing as well as immunizing agents. This type of sensitivity to leptospiral bacterin has not been reported previously. Evidently, the bacterin injected in an attempt to immunize chinchillas had sensitized them to a subsequent infection. As could be expected, an animal hypersensitive to an antigen, either bacteria or its cell substance, would be more susceptible to infection than a normal animal on account of the enhanced toxic action of the bacterial antigen (114). The question arises then, whether the exaggerated response of the tissues of the hypersensitive chinchilla to the infective organism had any protective action. For, it is known that such specific responses to the antigens of the pathogens are sometimes mediated by detectable antibodies. From the results of the present experiment, it is evident that this sensitized state in chinchillas seems to be independent of the immune response.

In the determination of the LD 50, it was realized that the number of animals used at the various dose levels was limited. However, looking at the results in the control group of animals (37.5 per cent deaths, 62.5 per cent survivals) the method of determination was believed to be fairly satisfactory.

The minimum infective dose (MID) of organisms in guinea pigs as reported by Rengen and Okazaki (91) was 350 per inoculum. The results in this study with guinea pigs indicate similar findings. Thermal reaction could not be elicited in 33.3 per cent of the guinea pigs in the second experiment with 250 organisms per dose (LD 50 for chinchilla). This indicates that the

technic adopted for determination of the number of organisms per milliliter compares well with those methods using bacterial counting chambers.

No significant changes in average daily weight gains in vaccinated and control guinea pigs before and after challenge were observed (Tables 5-6).

Histopathological alterations consistently observed were hepatic changes and nephritis. What appears as hyaline degeneration of Bowman's capsule was observed in this study that was not described previously in leptospirosis in domestic animals.

The pathogenesis of leptospirosis is not definitely known. The mechanism of the bleeding tendency in this disease has not been clarified. The probable factors as suggested by other workers (53) seem to be (a) direct mechanical action of leptospires, (b) the effect of hepatic damage and (c) a toxin or a hemolysin produced by the organisms in the tissues.

SUMMARY

This study indicated that chinchilla (Chinchilla Laniger) is a highly susceptible animal to leptospirosis and the most suitable for the laboratory studies of the disease.

The results of serological titrations did not indicate any appreciable increase in serum titres in chinchilla and guinea pig following vaccination.

Clinical, bacteriological and pathological observations brought out that vaccination with a leptospira pomona bacterin in chinchilla and guinea pig did not afford adequate protection against an LD 50 challenge with the organism.

Constant pathological lesions observed were hepatic, renal and splenic alterations, significant of these being the glomerular nephritis in some animals leading to what appears as hyaline degeneration, which has not often

been described in domestic animals.

The chinchilla and guinea pig were found to become hypersensitive following vaccination to subsequent infection with Leptospira Pomona. This type of hypersensitivity in animals due to leptospiral bacterin has not been previously reported.

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APPENDICES

APPENDIX I

Experiment #1

Table 1. Results of challenging immunized chinchillas with Leptospira Pomona. 18 days post inoculation. Single dose bacterin.

Chinchilla number	Thermal reaction *	Clinical symptoms **	Cultural examination	Died on the day post inoculation	presence of leptospira in tissue sections
1171	102.2	-	contd.	5	-
1172	102.4	-	-	5	-
1173	102.2	-	-	5	-
1194	102.8	-	-	6	-
1195	102.8	-	-	5	-
1196	104.2	-	contd.	4	-
1197	102.0	-	-	5	-
1198	102.2	-	-	4	-
1199	103.0	-	-	5	-
1203	101.8	-	-	4	-

* Highest temperature recorded in degrees F.

** Fever, lassitude and partial or complete anorexia.

Experiment #1

Table 2. Results of challenging control chinchillas with Leptospira Pomona. 18 days post inoculation. Controls.

Chinchilla number	Thermal reaction *	Clinical symptoms **	Cultural examination	Died on the day post inoculation	presence of leptospira in tissue sections
1175	103.0	+	+	4	+
1204	101.8	+	contd.	5	+
1205	104.0	+	contd.	4	+
1206	102.0	+	+	5	N.E.
1207	101.0	+	+	5	+
1208	102.0	+	+	6	-
1209	101.4	+	+	6	+
1210	102.4	+	+	6	?
1211	102.2	+	+	5	+

* Highest temperature recorded in degrees F.

** Fever, lassitude and partial or complete anorexia.

N.E. Not examined.

Experiment #1

Table 3. Results of challenging immunized guinea pigs with Leptospira Pomona. 18 days post inoculation. Single dose Bacterin.

Guinea pig number	Thermal reaction *	Clinical symptoms	Cultural examination **
7	106.0	-	✓
1175	105.6	-	Contaminated
1176	103.8	-	✓
1177	105.0	-	Contaminated
1178	104.6	-	Contaminated
1180	105.8	-	Contaminated
1181	104.6	-	✓
1182	105.4	-	✓
1183	105.0	-	Contaminated
1184	106.0	-	✓

* Highest temperature recorded in degrees F.

** Contamination was due to serum used.

Experiment #1

Table 4. Results of challenging control guinea pigs with Leptospira Pomona. 18 days post inoculation. Controls.

Guinea pig number	Thermal reaction *	Clinical symptoms	Cultural examination **
8	103.8	-	Contaminated
1185	104.4	-	✓
1186	105.8	-	Contaminated
1187	104.6	-	Contaminated
1188	104.2	-	Contaminated
1189	106.0	-	✓
1190	104.6	-	Contaminated
1191	105.2	-	Contaminated
1192	104.8	-	Contaminated
1193	105.0	-	Contaminated
1199	103.8	-	Contaminated

* Highest temperature recorded in degrees F.

** Contamination was due to serum used.

Experiment #1

Table 5. Average gain per day in vaccinated guinea pigs (in grams).

Number of animal	Before challenge	After challenge		
	20 days	10 days	20 days	30 days
7	3.9	6.2	3.0	4.7
1175	6.2	1.6	5.2	3.0
1176	4.7	5.8	7.5	6.0
1177	3.9	5.8	3.8	5.0
1178	4.7	2.5	3.8	2.5
1180	5.7	3.5	2.6	5.2
1181	4.2	2.5	5.0	2.5
1182	2.9	2.5	2.8	2.5
1183	4.1	1.7	2.6	3.8
1184	4.7	2.9	4.4	3.0
Average	4.5	3.5	4.0	3.8

Experiment #1

Table 6. Average gain per day in control guinea pigs (in grams).

Number of animal	Before challenge	After challenge		
	20 days	10 days	20 days	30 days
1185	4.2	2.6	4.4	1.7
1186	3.5	0.1	5.4	1.6
1187	5.6	5.4	3.3	5.2
1188	4.6	4.1	4.0	4.2
1189	3.1	4.4	2.7	3.5
1190	5.4	5.8	5.5	5.5
1191	2.8	2.0	4.5	3.3
1193	5.8	5.4	3.6	5.7
1199	5.7	7.2	2.3	6.2
Average	4.4	4.1	3.9	4.1

Experiment #2

Table 7. Summary of serological titrations in vaccinated chinchillas. Single dose bacterin.

Number of Chinchilla	Preimmunization titre 1:10	1:50	1:250	Prechallenge titre 1:10	1:50	1:250
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	±	±	-	-	-	-
8	-	-	-	-	-	-
9	-	-	-	±	-	-
11	-	-	-	±	-	-
12	-	-	-	-	-	-
13	-	-	-	-	-	-
14	-	-	-	-	-	-
15	-	-	-	-	-	-

Experiment #2

Table 8. Summary of serological titrations in vaccinated chinchillas. Double dose bacterin.

Number of Chinchilla	Preimmunization titre 1:10	1:50	1:250	Prechallenge titre 1:10	1:50	1:250
16	±	-	-	±	±	-
17	+	-	-	+	-	-
18	±	-	-	+	±	-
19	±	-	-	+	-	-
20	-	-	-	-	-	-
21	+	-	-	±	-	-
22	-	-	-	+	±	-
23	+	-	-	+	-	-
24	±	±	-	±	-	-
25	-	-	-	-	-	-
26	-	-	-	+	-	-
27	±	-	-	±	-	-

Experiment #2

Table 9. Summary of serological titrations in control chinchillas.

Number of Chinchilla	Preimmunization titre 1:10	1:50	1:250	Prechallenge titre 1:10	1:50	1:250
9402	+	-	-	+	-	-
9403	+	-	-	+	-	-
9404	-	-	-	+	+	-
9405	+	+	-	+	-	-
9406	-	-	-	-	-	-
9407	-	-	-	-	-	-
9408	-	-	-	+	-	-
9409	-	-	-	-	-	-

Experiment #2

Table 10. Summary of serological titrations in vaccinated guinea pigs.

Guinea pig number	Preimmunization titre			Prechallenge titre		
	1:10	1:50	1:250	1:10	1:50	1:250
181*	-	-	-	≠	-	-
182	-	-	-	≠	-	-
183	-	-	-	≠	-	-
184*	-	-	-	-	-	-
187*	-	-	-	≠	≠	-
188	≠	≠	-	-	-	-
189*	≠	-	-	≠	-	-
190*	≠	-	-	≠	-	-
191	-	-	-	≠	-	-
192	-	-	-	-	-	-
193	-	-	-	≠	-	-
194	-	-	-	≠	-	-

* Double dose bacterin.

Experiment #2

Table 11. Summary of serological titrations in control guinea pigs.

Guinea pig number	Preimmunization titre 1:10	1:50	1:250	Prechallenge titre 1:10	1:50	1:250
171	-	-	-	+	-	-
172	±	-	-	-	-	-
173	+	-	-	±	-	-
175	±	-	-	-	-	-
176	-	-	-	-	-	-
177	-	-	-	-	-	-
178	±	-	-	+	-	-
179	+	-	-	±	-	-
180	-	-	-	±	-	-
195	-	-	-	-	-	-
196	-	-	-	±	-	-
198	-	-	-	+	-	-

Experiment #2

Table 12. Results of challenging immunized chinchillas with Leptospira Pomona. 70 days post inoculation. Single dose bacterin.

Chinchilla number	Thermal reaction *	Clinical symptoms **	Cultural examination	Died on the day post inoculation	Presence of leptospira in tissue sections
3	103.4	-	-	9	-
4	102.8	-	Contd.	10	-
5	102.6	-	-	8	-
6	101.6	-	-	8	-
7	102.4	-	-	11	N.E.
8	102.0	-	-	8	-
9	102.6	-	-	9	-
11	102.2	-	-	9	-
12	101.4	-	-	8	-
13	102.4	-	-	10	N.E.
14	101.0	-	-	8	-
15	101.6	-	-	8	-

* Highest temperature recorded in degrees F.

** Fever, lassitude and partial or complete anorexia.

N.E. Not examined.

Experiment #2

Table 13. Results of challenging immunized chinchillas with Leptospira Pomona. 70 days post inoculation. Double dose bacterin.

Chinchilla number	Thermal reaction *	Clinical symptoms **	Cultural examination	Died on the day post inoculation	Presence of leptospira in tissue sections
16	103.0	-	-	8	-
17	102.8	-	Contd.	8	-
18	101.4	-	-	9	-
19	102.0	-	-	8	-
20	101.2	-	-	9	-
21	102.6	-	-	49	N.E.
22	101.8	-	-	Alive	N.E.
23	102.4	-	-	11	-
24	100.8	-	Contd.	Alive	N.E.
25	101.8	-	-	9	-
26	102.2	-	-	8	N.E.
27	102.4	-	-	7	-

* Highest temperature recorded in degrees F.

** Fever, lassitude and partial or complete anorexia.

N.E. Not examined.

Experiment #2

Table 14. Results of challenging control chinchillas with Leptospira Pomona. 70 days post inoculation.

Chinchilla number	Thermal reaction *	Clinical symptoms **	Cultural examination	Died on the day post inoculation	Presence of leptospira in tissue sections
9402	102.4	-	-	Alive	N.E.
9403	102.4	-	-	Alive	N.E.
9404	101.0	-	Contd.	Alive	N.E.
9405	102.2	-	-	13	N.E.
9406	102.6	-	-	Alive	N.E.
9407	101.4	-	-	9	-
9408	101.4	-	-	49	-
9409	102.2	-	-	16	?

* Highest temperature recorded in degrees F.

** Fever, lassitude and complete or partial anorexia.

N.E. Not examined.

Experiment #2

Table 15. Results of challenging immunized guinea pigs with Leptospira Pomona. 70 days post inoculation.

Guinea pig number	Thermal reaction *	Clinical symptoms	Cultural examination
181*	103.2	-	+
182	102.4	-	N.D.
183	104.8	-	+
184*	103.2	-	+
187*	102.8	-	N.D.
188	102.4	-	N.D.
189*	102.8	-	N.D.
190*	104.4	-	+
191	104.2	-	+
192	104.0	-	+
193*	103.8	-	+
194	102.2	-	N.D.

* Guinea pigs received double dose of bacterin.

N.D. Not done.

Experiment #2

Table 16. Results of challenging control guinea pigs with Leptospira Pomona. 70 days post inoculation.

Guinea pig number	Thermal reaction *	Clinical symptoms	Cultural examination
171	103.8	-	+
172	103.4	-	+
173	102.6	-	N.D.
175	103.2	-	N.D.
176	103.8	-	+
177	104.8	-	+
178	103.8	-	+
179	103.6	-	+
180	102.8	-	N.D.
195	103.0	-	N.D.
196	104.0	-	+
198	104.0	-	+

* Highest temperature recorded in degrees F.

N.D. Not done.

Experiment #2

Table 17. Summary of differential blood counts in normal chinchillas.

Chinchilla number	Eosin.	Juv.	Stab.	Seg.	Lymph.	Mono.
9402	2	2	7	39	49	1
9403	-	1	17	38	42	2
9404	1	-	10	48	40	1
9405	1	-	8	52	39	-
9406	-	3	11	45	40	1
9408	1	2	3	50	43	1
9409	2	3	21	42	31	1
Average	1	1.5	10.8	44.8	40.5	1

Experiment #2

Table 18. Summary of differential blood counts in vaccinated and control chinchillas before death.

Chinchilla number	Myelo	Juv.	Stab.	Seg.	Lymph.	Mono.
6*	-	11	6	52	30	1
9*	2	12	4	42	39	1
11*	1	10	3	51	34	1
23**	1	10	6	45	27	1
9404***	-	8	8	58	28	-
9409***	1	6	11	49	32	1
Average	0.8	9.5	6.3	49.5	31.6	0.8

* Single dose bacterin

** Double dose bacterin

*** Controls

APPENDIX II

EXPLANATION OF PLATE I

Vaccinated Chinchillas in Experiment #2, eight days post inoculation. Observe dullness and glossiness of the eyes.

PLATE I

EXPLANATION OF PLATE II

Vaccinated chinchilla in a moribund state in Experiment #2, nine days post inoculation.

PLATE II

PLATE III

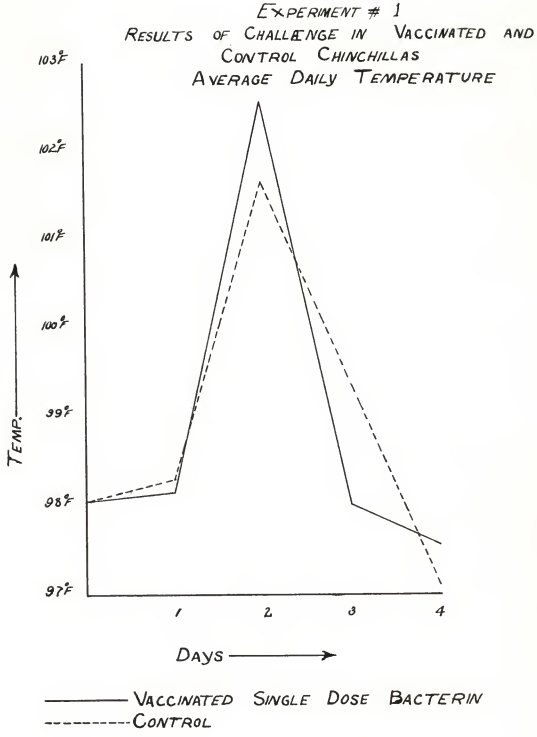

EXPERIMENT # 1
RESULTS OF CHALLENGE IN VACCINATED AND CONTROL CHINCHILLAS
AVERAGE DAILY TEMPERATURE
103°F
102°F
101°F
100°F
99°F
98°F
97°F
TEMP.
1
2
3
4
DAYS
VACCINATED SINGLE DOSE BACTERIN
CONTROL

PLATE IV

EXPERIMENT #1
RESULTS OF CHALLENGE IN VACCINATED AND CONTROL GUINEA PIGS.
AVERAGE DAILY TEMPERATURE

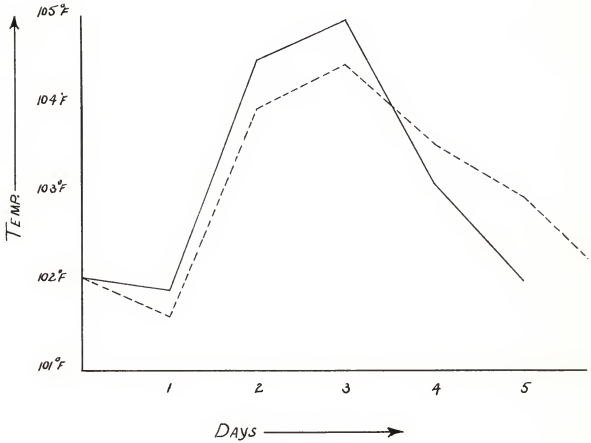


PLATE V

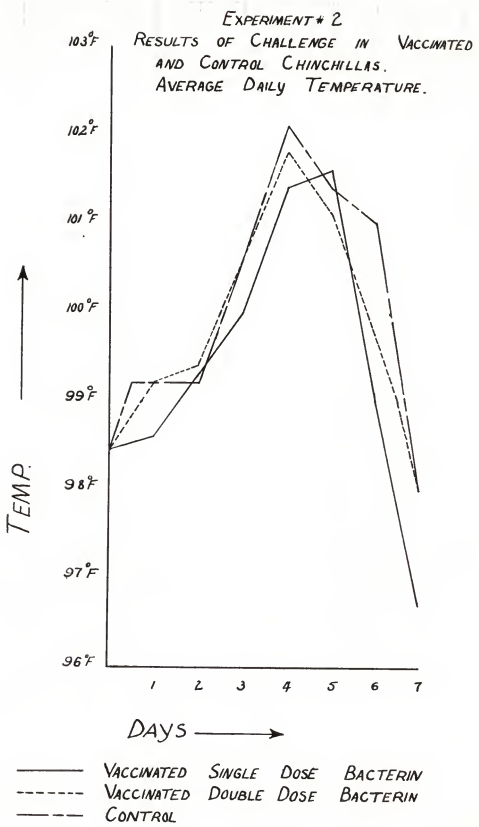
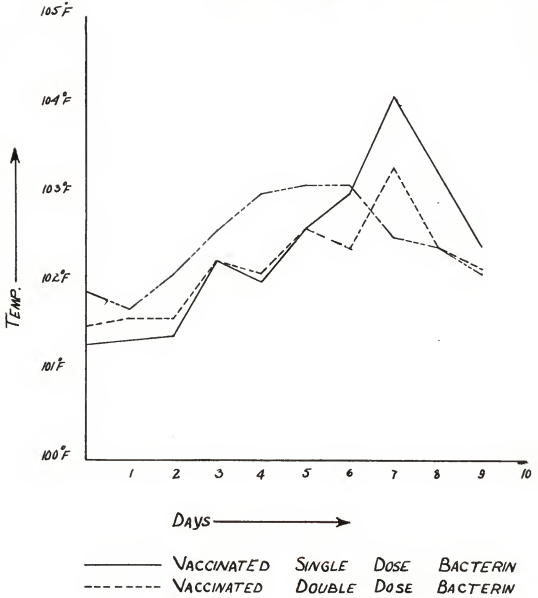

EXPERIMENT # 2
RESULTS OF CHALLENGE IN VACCINATED AND CONTROL CHINCHILLAS.
AVERAGE DAILY TEMPERATURE.
103°F
102°F
101°F
100°F
99°F
98°F
97°F
96°F
TEMP.
1
2
3
4
5
6
7
DAYS
VACCINATED SINGLE DOSE BACTERIN
VACCINATED DOUBLE DOSE BACTERIN
CONTROL

PLATE VI

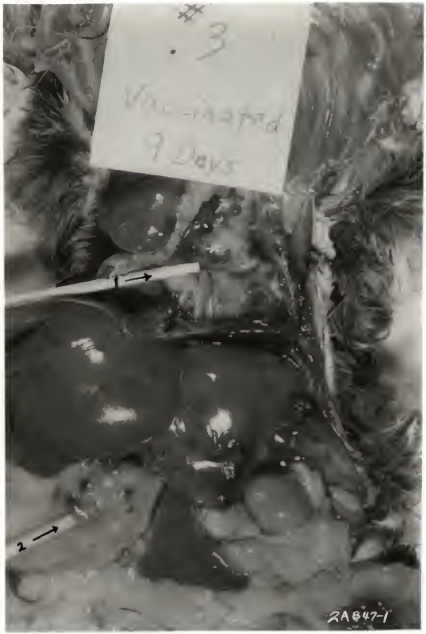
EXPERIMENT #2.
RESULTS OF CHALLENGE IN VACCINATED AND CONTROL GUINEA PIGS.
AVERAGE DAILY TEMPERATURE



EXPLANATION OF PLATE VII

Lesions in chinchilla #3, Experiment #2, single dose bacterin, died on the ninth day post inoculation showing ecchymotic hemorrhages on (1) the surface of the lungs, (2) the visceral surface of the stomach.

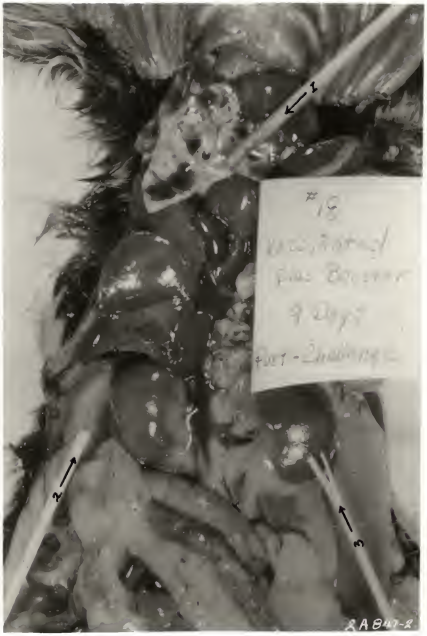
PLATE VII



EXPLANATION OF PLATE VIII

Lesions in chinchilla #18, Experiment #2, double dose bacterin, died on the ninth day post inoculation showing (1) ecchymotic hemorrhages on the surface of lungs, (2) (3) petechial hemorrhages on the cortex of the kidneys.

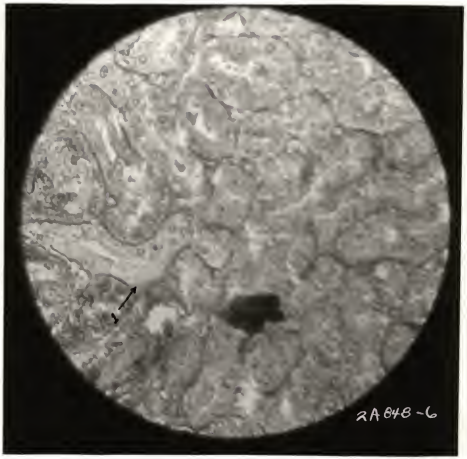
PLATE VIII



EXPLANATION OF PLATE IX

Section of kidney showing degeneration of the epethelium of the tubules. 1 (Hemotoxylin and eosin.)

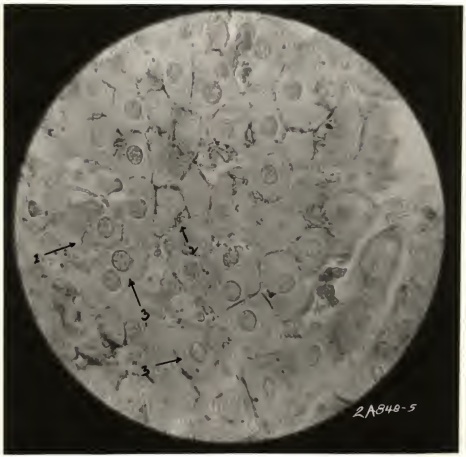
PLATE IX



EXPLANATION OF PLATE X

Section of liver showing (1) (2) Leptospira Pomona,
(3) dissociation of liver cord cells. (Levaditi stain.)

PLATE X



IMMUNOLOGICAL RESPONSE OF *LEPTOSPIRA POMONA* BACTERIN IN LABORATORY ANIMALS

by

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B.V. Sc. Madras University, India, 1951

AN ABSTRACT OF A THESIS

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requirements for the degree

MASTER OF SCIENCE

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OF AGRICULTURE AND APPLIED SCIENCE

1959

Human and animal infections with leptospires have received increasing attention during the past few years. Serological surveys and clinical findings indicate that the disease is widespread in cattle and swine in the United States and nearly 50 per cent of the cattle are estimated to have had either clinical or subclinical infection with Leptospira Pomona. A morbidity rate of 80 per cent and an annual loss of about one hundred million dollars due to leptospirosis in cattle alone was estimated by the United States Department of Agriculture in 1954. This has resulted in continued research advocating various prophylactic programs and commercial bacterins for the eradication or prevention of the spread of infection. These bacterins have been claimed to produce satisfactory immunity although field experiences and laboratory findings many times have not been convincing. Consequently, this study was undertaken to evaluate the immunological response of a widely used commercial Leptospira Pomona bacterin in laboratory animals.

Weil (124) was credited with having first described cases of 'leptospirosis' in 1886 followed by isolation of the etiologic agent by Inado (54) in 1915. Studies by various European, Australian and Japanese workers followed by investigations in United States in recent years have resulted in the present recognition of leptospirosis as a world wide public health and livestock sanitary problem of great concern.

Epidemiologically, although rodents and swine are considered to be the primary animal carriers, recent investigations have revealed a variety of wild mammal hosts (1) (119) and many sylvatic leptospiral carriers.

Diagnosis and prophylaxis of leptospirosis present innumerable problems due to the peculiarities of the disease and the fastidious nature of the organisms.

Successful active immunization against leptospiral infections was reported by various workers (54) (81) (126) (49) (123), while others have indicated a very low grade or no immunity to leptospiral bacterins in both laboratory animals and field outbreaks, resulting in apparent clinical infections or inapparent carrier states (102) (83) (20).

Finding the susceptibility of chinchilla (Chinchilla Laniger) as the most suitable experimental animal tested to leptospirosis, these and guinea pigs were used for the present study for comparative results. Groups of animals were vaccinated with Leptospira Pomona bacterin and a similar number of them were kept as controls. After allowing sufficient time for the development of immunity, if any, the vaccinees and controls were challenged with a known number of organisms from a virulent culture. In the confirmatory test Median Lethal Dose (LD_{50}) for chinchilla was estimated by a simple method and used for challenge. Besides, serum samples collected before vaccination and challenge were titrated by agglutination-lysis test. The virulence of the organism at the time of challenge was such that clinical symptoms were consistently produced on inoculation in normal animals on the third day resulting in death by the fifth day.

Thermal reaction, clinical symptoms, bacteriological evidence and pathological lesions were considered as evidence of infection in vaccinated as well as control animals in these experiments. Agglutination-lysis test was made use of in the determination of immunity following vaccination.

This study indicated that chinchilla (Chinchilla Laniger) is the only susceptible animal tested to leptospiral infections and suitable for laboratory studies of this disease.

The results of serological titrations, clinical, bacteriological and pathological observations indicated that vaccination with Leptospira Pomona bacterin in chinchilla and guinea pig did not afford adequate protection against an LD_{50} challenge.

Pathological lesions consistently observed in chinchilla were hepatic, renal and spleenic, significant of these being the glomerular changes leading to what appeared to be hyaline degeneration which has not often been described in domestic animals.

The hypersensitivity following vaccination of chinchilla and guinea pig to subsequent infection with Leptospira Pomona has not been reported previously.