SEROLOGICAL CROSS REACTIONS AMONG LEPTOSPIRAE OBSERVED WITH SERA FROM ANIMALS INFECTED WITH LEPTOSPIRA POMONA

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SEROLOGICAL CROSS REACTIONS AMONG LEPTOSPIRAE OBSERVED WITH SERA FROM ANIMALS INFECTED WITH *LEPTOSPIRA POMONA*

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INTRODUCTION

Due to the variation in common usage of terminology any discussion of serological reactions necessitates an explanation of terms. Particularly important to this discussion is the denotation of the term cross reaction. Kabat and Meyer's (1948) explanation of the antigen-antibody relation in a cross reaction suits this need well.

While antibodies generally react only with the antigen stimulating the response (the homologous antigen), certain exceptions, termed cross reactions, have been noted in which reactions occur with substances other than the homologous antigen. Chemical studies indicate that cross reactions are due to structural similarities between the heterologous and homologous antigens. The concept cross reaction, then, applies to serological relationships between different <u>single</u> antigens, which presumably possess similar structural groupings within their molecules.[25]

A mixture of antigens (example: a bacterial cell containing more than one antigenic component) when introduced into a living animal system stimulates the production of distinct antibodies to each of the constituents. If this antiserum reacts with another antigen mixture (example: some other bacterial cell), it may be due either to a common identical antigen, or to a chemically-related antigen. If the two

mixtures contain an identical antigenic component this is a homologous reaction, but if they contain only chemically related antigens this is a true cross reaction.[25]

In the genus Leptospira there is a marked serologic heterogeneity. Distinctions by serological procedures have not been sharp enough in some cases to indicate the antigenic composition of leptospiral strains. Also interpretations of the results obtained by these procedures have certain deterrents as cited by Wilson and Miles.[54] Chemical analysis of leptospirae has been hampered by the low yield of organisms obtained when grown in culture.[2, 21] Consequently little chemical analysis has been done. At present little is known of the antigenic structure of these organisms.

In order to establish a classification, leptospiral strains are gathered antigenically into serotypes which share a major antigenic component. Common serotypes constitute a serogroup.[55] Exactly what the difference antigenically is among the serotypes of a serogroup and among different serogroups can only be conjectured at present.

For the sake of clarity in this paper, agglutination-lysis reactions between members of different serogroups will be termed cross reactions. Whether these reactions are true cross reactions or merely homologous reactions between a common identical antigen can only be decided by the analysis of the antigenic structure of Leptospira.

The existence of antigenic cross reactions among leptospiral strains is a fact well known by workers in the field. Commonly, cross agglutination-lysis reactions are encountered with antisera against L. pomona,[1, 4, 11, 15, 18, 56] L. icterohaemorrhagiae,[1, 6, 13, 15, 18, 56] L. autumnalis,[18, 56] and L. canicola.[6, 9, 50, 56]

L. pomona infections constitute the major share of the leptospitoses occurring in domestic livestock.[30, 60] Early diagnosis and definite identification of the etiological agent are essential. However, due to the complication of antigenic cross reactions confusion or error sometimes results in routine serological testing.[10]

It has been the endeavor of this research to establish the antigenic relationships among serotypes as is evidenced by infections observed in various animal species experimentally infected with L. pomona. Further, an attempt was made to determine the degree of cross reactivity throughout the course of infection in a given animal species.

Antisera against L. pomona strain Wickard of bovine origin, and strain Ohio of porcine were employed. Ovine,[29, 36] porcine,[34] caprine,[35] and guinea pig leptospiral antisera were examined using cultures of L. pomona (strain Johnson), L. icterohaemorrhagiae (AB), L. icterohaemorrhagiae (A), L. canicola, L. sejroe, and L. hebdomadis as antigens in a modified microscopic agglutination-lysis tube test.[5]

LITERATURE REVIEW

Pathogenic leptospira were first seen by Stimson[48] in 1907 in sections of kidney. Isolation of the agent was accomplished from cases of Weil's disease by Inada and Ido in 1915,[24] and independently in the same year by Hubener and Reiter,[22] as well as Uhlenhuth and Fromme.[49] During 1916 in Japan Ido et al.[23] discovered L. hebdomadis, while Koshina et al.[28] in 1925 discovered L. autumnalis. Noguchi[37] coined the name Leptospira and with Zuelzer described the morphology of these organisms with great accuracy.[52] Ido and his associates first introduced serological tests for distinguishing pathogenic leptospirae.[6]

Soon numerous strains were recognized and attempts were made to classify leptospirae schematically. It became apparent that morphological characteristics, biochemical reactions, and growth requirements of leptospirae are not sufficiently different to be used as a basis of differentiation.[52, 55] Taxonomic classification became possible only through serological procedures, but this was not quickly accepted by many investigators.

Antigenic analysis of leptospiral isolates divided the organisms into antigenically well-defined groups. Antigenic classification became more complicated, however, as more strains were isolated with serological patterns which

showed overlapping or deviations from the established serogroups. It became more difficult to fit these serologically aberrant strains into a fixed serologic scheme.[56] The question then arose whether the various strains are true species or whether they must be considered as variants of one species.

Baermann[3] in 1927, and Zuelzer,[61] confused by the great diversity of strains and the apparent contradiction of serological tests with the clinical characteristics, abandoned clinical and serological differences and regarded all saprophyte and parasitic leptospira as a unity. They also maintained that the strains continuously changed their serological groupings, a fact which van Thiel[52] and Wolff[56] among others, emphatically deny. This early theory may have been based upon the antigenic phenomenon observed in relapsing fever spirochetal infections.

Schlossberger, Grillo and Scheile,[41] and Schlossberger[39] accepted the existence of different types of leptospira but did not consider the agglutinative characteristics sufficiently different to separate the groups into species. These researchers assume the antigenic make-up to be composed of different "partial antigens" and that differences exist in relation to the distribution of these antigens among the leptospiral strains.

Vaucel[53] and van Riel[51] defend an intermediate view point and assert that a few leptospiral strains can be separated on a serological basis into species.

Starting in 1924 the first real effort to classify the many leptospiral strains into definite serological groups was made by Schuffner and his associates in the Amsterdam Leptospiral Laboratory.[55] These workers compared locally-isolated strains with those of pathogenic leptospirae from other parts of the world, a first grouping was established. Also, a number of well-defined leptospiral entities with a constant antigenic pattern were separated and reported.[55]

The work of this group was paralleled by other workers throughout the world.[55] Castellani in 1902 devised the agglutination absorption procedure as a method of analyzing the antigenic structure of a bacterium. However, Ruys and Schuffner[52] introduced this method into leptospiral research and by use of it Klarenbeck and Schuffner[26] separated L. canicola from L. icterohaemorrhagiae. It was by this procedure that Borg-Petersen[7] in 1938 found that the serotype L. icterohaemorrhagiae could be further divided into two subtypes called biotypes by Gispen and Schuffner.[17] Borg-Petersen[6] did not recommend the use of the term biotypes.

Schuffner[46] in 1938 established a system of classification based upon the following six criteria:

1) the specific antigenic characteristics based upon agglutination-lysis reactions;
2) the clinical picture encountered;
3) the geographical distribution of the organism;
4) the species of the animal host or reservoir;

5) the manner of acquiring infection; and

6) the pathogenicity of the given Leptospira for the guinea pig.

Upon further investigation, only the first criterion for differentiation of leptospiral strains proved to be constant enough for classification of these organisms.[19]

Practically all reported leptospiral research ceased during World War II, but resumed again about 1947.

On purely serological procedures Bernkopf and Olitski[4] postulated the antigenic structure of two leptospiral strains in relation to one another. By the removal of labile antigens by suitable absorptions, they distinguished somatic antigens G and B by cross-absorption experiments with varying quantities of antigens and antibodies.

Gochenour et al.[19] in 1952 distinguished eight leptospiral strains in North America by detection of homologous antibodies in human and animal sera as shown by the complement fixation, agglutination-lysis test, and cross absorption studies.

Wolff and Brcom[58] in 1953 asserted that a classification can be based on the principle that the agglutination-lysis test reveals the stable and specific antigenic characteristics of the members of the genus Leptospira.

Wolff[55] in 1953 proposed a classification based on the above principle and upon the cross-agglutination-lysis reactions and cross absorption test of leptospiral strains. The genus is divided into serogroups, composed of closely

related serotypes which share major antigenic components. By definition, a serotype includes all strains indistinguishable one from the other on the basis of cross absorption procedures. Within the serogroup are not only heterologous serotypes, but complete and incomplete biotypes as well. Antigenic symbols may be used to designate the biotypes, as AB for the complete and A for the incomplete biotype. The incomplete biotype contains part but not all of the antigenic components of the complete biotype.

Wolff provisionally considered[59] two strains to be heterologous if the antiserum of each strain, after absorption by the other strain, retains at least 10 percent of its original titer when retested against the homologous strain.

Alexander et al.,[1] due to the differences in serological techniques, modified the criteria for determination of homologous and heterologous strains as proposed by Wolff. According to Alexander two strains are considered heterologous if the antiserum of each strain following absorption by the other strain retains at least 1/16 of the homologous titer. Similarly, two strains are homologous if the residual titers following absorptions are less than 1/16 of the original homologus titers.[2]

Wolff's[55] proposed classification listed 36 antigenically distinct leptospiral serotypes into 20 serogroups, but Wolff and Broom[59] proposed 34 serotypes and 20 serogroups. This scheme has been tentatively accepted by various leptospiral

typing laboratories as a working basis in the typing of leptospiral strains.[2]

The Report by the Study Group on Leptospirosis of the World Health Organization[27] in 1955 defined criteria and procedures for classification of the genus, Leptospira. The division of the genus into serotypes on the basis of agglutinogen characters, as determined by agglutination-lysis and cross-absorption reactions with immune rabbit sera was recommended. In differentiating one serotype from another, i.e., determining heterologous and homologous serotypes, the standards proposed by Wolff[55] were adopted. The Group recognized that biochemical methods of antigenic fractionation of leptospirae, or other methods, may in the future provide a new and more satisfactory basis of differentiation.

Many workers have sought to gain an insight into the antigen composition of leptospirae by chemical analysis. All of the efforts have been hampered by the difficulty of cultivating adequate amounts of organisms and by the intricacy of the serological relationships which now differentiate some fifty or more serotypes of the genus.[21, 38]

Ezell et al.[12] in 1953 demonstrated the presence of complement-fixation antigens in the supernatants of leptospiral cultures which were serotype-specific.

Schneider[42] in 1953 and in 1954[43, 44] made several types of extracts which in the complement-fixation test were reactive with homologous whole organism antisera and cross

reacted to a lesser extent with antisera prepared against the cells of other leptospiral serotypes.

Hashimoto[38] in 1954 sensitized sheep erythrocytes for immune hemolysis by pretreating them with an extract obtained by boiling concentrated suspensions of leptospirae. The sensitizing antigen was found to be common to the three serotypes studied.

Chang and McComb[10] in 1949 described an erythrocyte-sensitizing substance (ESS) which exhibited a uniform level of reactivity in hemagglutination tests with rabbit or human antisera against all leptospiral serotypes tested.

Cox[11] in 1955 showed that ESS, as prepared by Chang and McComb[9] was capable of sensitizing erythrocytes for lysis as well as for agglutination.

Schneider[45] in 1955 isolated in two cell-free preparations virtually all of the leptospiral cell's antigenic principle which is reactive in the complement fixation test with hyperimmune rabbit serum. Two serotypes were studied. The aqueous extract contained two immunologically distinct antigens namely a genus specific complement fixation principle and a serogroup specific agglutinogen. The alcoholic extract reacts as a partial antigen or haptene in rabbits. The antibodies of homologous hyperimmune serum reacts well in the complement fixation test with the latter antigen and are sharply serotype specific. The aqueous cell-free extract, compared to the viable whole leptospiral organism, possesses

the property of stimulating the formation of a specific serogroup agglutinin without a detectable genus reactive agglutinin. An obvious application of this property in the classification of leptospira is that strains that group cross react can be separated without recourse to absorption studies.

Rothstein and Hiatt[38] in 1956 prepared serologically reactive extracts from 25 leptospiral cultures, representing 20 different serotypes and studied their immunological, chemical, and physical properties. An ethanol extract of leptospiral cells was divided into two portions on the basis of solubility in distilled water. The soluble portion when introduced into rabbits elicited genus-specific precipitins and type-specific agglutinins indicating the presence of two antigenic components. By absorption with the homologous organism the agglutinins could be removed from the antiserum. Rothstein and Hiatt[38] postulate from these findings that leptospirae contain two major antigenic components: a P antigen, which is a peripheral type-specific principle, and an S antigen, which is a somatic, genus-specific principle. The S antigen appears to be a lipo-polysaccharide; the chemical nature of the P antigen was not determined.

In the early years of leptospiral research many workers were seeking the source or carriers of the pathogenic leptospirae. Zuelzer (1922) observed that saprophytic spirochetes resembled in morphology and habitat the pathogenic leptospirae and postulated from this and other investigations

that these saprophytes may change into pathogenic Weil's disease organisms by adaptation to animal protein as a source of nutrient. Many workers[52] since 1922 have tried to support Zuelzer's hypothesis by observation and research but instead have conclusively disproved it.

In view of the evidence such a drastic change in the structure and metabolism of a microorganism seems unlikely. However, lesser changes in an organism with a corresponding change in the antigenic structure have recently been investigated.

The degree of antigenic relationship between different serotypes varies greatly in regard to the number of serotypes which inter-react and the degree of the cross-titers.[8] Some common serotypes are so closely related that in human infections it may be impossible to decide by cross-absorption tests on the patient's serum, which of two serotypes caused the illness.[8] L. hyos has practically no cross reactivity with any other serotype whereas other serotypes share a serogroup with as many as four other closely-related serotypes.[56]

Gsell[20] in 1949 offers an explanation for the observed variations in antigenic relationship and suggests that adaptation to an unnatural carrier-host may lead to the development of a new serotype, if the proteins of the two hosts differ in chemical constitution.

Broom[8] conjects that in time the leptospira would become so completely adapted to its new environment that its

metabolism and hence its antigenic constitution would gradually alter and would produce an antigenically distinct serotype.

Evidence in support of these speculations may be found in the experiments of Bacon, Burrows, and Yates[8] who caused a biochemical mutation of a pathogenic bacterium and found a corresponding change in virulence which these workers attributed to the change in growth requirements. The investigators made no mention of an alteration in antigenicity between the parent cells and the mutants.

A case of induced mutation of a leptospiral strain is related by Schlossberger.[40] A new serotype, highly virulent leptospiral isolate, designated as Strain St. was heat-killed and mixed with a non-virulent living strain of L. icterohaemorrhagiae and then inoculated interperitoneally into guinea pigs. From a sample of the peritoneal fluid was isolated Strain St. Other combinations of various strains were treated similarly but failed to show mutation. In no case was there infection.

Seppilli (1950), considering the fact that the generation time of leptospires is short, compares these organisms with other bacteria and concludes that one can expect a wide variety of leptospiral mutants to occur within a measurable period of time. If mutants of a serotype are produced in the tissue of an unnatural host, one of the mutants may, by chance, be better adapted to the fresh environment than the parent strain. Here the mutant would tend to establish

itself at the expense of the parent cells, whereas in the natural host a reverse situation would occur.

If new serotypes are constantly being formed due to mutation of leptospiral strains in nature, with a corresponding change in antigenic structure, then the classification process will be an endless one. Nevertheless, classification, i.e., identification of the etiological agent, will ever be essential to the servalence of the leptospiroses.

MATERIALS AND METHODS

The sources of the leptospiral strains used as antigens and inocula were as follows:

L. pomona (strain Wickard) was isolated[32] from an infected dairy cow in Wisconsin by Dr. E. V. Morse and Miss Virginia Allen. The microorganism has been maintained continuously in young guinea pigs for approximately three years.

L. pomona (strain Johnson) and L. icterohaemorrhagiae, AB, were obtained in 1952 from K. R. Reinhard, United States Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana.

L. pomona (strain Ohio) was obtained from the Ohio Agricultural Experiment Station at Wooster, Ohio, and has been maintained in continual hamster or guinea pig passage since it was isolated from an infected hog.

L. icterohaemorrhagiae, A, (strain Kantorwicz) and L. hebdomadis were obtained in 1956 from Mrs. Mildred Galton, Communicable Disease Center, United States Public Health Service, Chamblee, Georgia.

L. sejroe was obtained in 1956 from Lt. Colonel L. C. Murphy, Division of Veterinary Medicine, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C.

L. canicola was furnished by Dr. J. P. Newman, Department of Microbiology and Public Health, Michigan State University.

Six antigens were used in the serological survey: (1) L. pomona (strain Johnson); (2) L. icterohaemorrhagiae, AB; (3) L. icterohaemorrhagiae, A, Kantorowicz; (4) L. canicola; (5) L. sejroe; (6) L. hebdomadis. These strains were carried in stock cultures with weekly transfers in modified Chang's fluid medium,[9] (hereafter called Chang's medium) containing 0.01 percent hemoglobin (Difco). Sterile rabbit serum, which was added to give a final concentration of 10 percent of the medium, did not contain agglutinins for any of the serotypes used in these tests. All cultures were incubated at 30 C.

Antigens for the agglutination-lysis test consisted of 4 to 8 day old cultures which were grown in "antigen jars" (2 inches in diameter and 4 inches high) containing 20 to 40 ml. of Chang's medium. Estimation of antigen density was made by darkfield microscopy. Density was adjusted to approximately 10^8 organisms per milliliter. The necessary dilutions were made with sterile Chang's buffer. This buffer (pH 7.0-7.2) consisted of 4.0 g $Na_2HPO_4 \cdot 7H_2O$, 0.8 g KH_2PO_4 and 8.0 g NaCl in two liters of distilled water. Sterilization was effected by autoclaving at 15 pounds for 20 minutes. Adjustment of antigen density usually resulted in at least a 1:2 dilution and occasionally was as high as 1:5. Dilution of antigen in the agglutination-lysis test has been recommended as a routine

procedure. The antigens were centrifuged for 10 minutes in an International Clinical Centrifuge model C. L. at 2,400 rpm (1,500 x g).

The modified microscopic agglutination-lysis tube test was employed,[33] using each of the living leptospiral antigens. Sterile buffer solution was employed to dilute the sera from the various animals which had been experimentally infected. Ten-fold serum dilutions were used starting with a 1:10 dilution. The dilution factor created by adding antigen to each tube was not included in the serum dilutions as expressed throughout this thesis. The tests were incubated at room temperature (±22 C) for 12 to 18 hours, before being read using a modified darkfield microscope with 100x optical magnification.[33] A modified darkfield type illumination was produced by fitting a star diaphragm into an Abbe condenser and mounting this assembly on a conventional microscope.

A reaction of 25 percent agglutination, lysis or both was considered significant at serum dilutions of 10^{-2} or higher. At the 10^{-1} serum dilution 50 percent antigen-antibody reaction was considered to be significant. All titers mentioned in this thesis, unless otherwise stated, are arithmetric averages of the negative exponents of the end point serum dilutions calculated separately for each day of infection for each species of animal in the respective group.

Pre-exposure serum samples from individual sheep, swine and goats as well as a representative number of guinea

pigs were examined. Serum antibodies for the six leptospiral serotypes used as antigens were not demonstrable. In every case the serum samples were kept in the frozen (-10 C) state prior to examination.

The serum absorption procedure was that employed and recommended by workers in this field.[1, 2, 4, 5, 10] Leptospirae were grown in Roux bottles containing approximately 100 ml of Chang's medium. A 10 percent inoculum consisting of a seven-day-old stock culture was used. Sufficient growth occurred with 7 to 9 days at 30 C. A 5 percent solution of commercial formaldehyde, C. P. (approximately 37 percent solution) was made and 0.5 ml of this was added to each Roux bottle culture, mixed thoroughly, and allowed to stand at room temperature for two hours. The cultures were then centrifuged in 40 ml aliquots for 30 minutes at 10,500 rpm (14,100 x g) in a Servall Superspeed Centrifuge, type SS-1, and were resuspended with more of the culture (approximately 20 ml) and centrifuged as before. Following centrifugation the supernatant was decanted, except for about 1 ml, and to this was added enough sterile Chang's buffer to give a cell mixture of approximately 2 percent of the original culture volume. A homogeneous cell suspension was made by mixing thoroughly with a 2 ml pipette. The volume was adjusted to 2 ml and thus resulted in a 50-fold concentration of leptospiral cells.

The serum to be absorbed was diluted 1:10 in sterile Chang's buffer. One part diluted serum (.5 ml) was mixed with 4 parts (2 ml) of the cell suspension resulting in a final serum dilution of 1:50. The serum-antigen mixture was incubated at 30 C for 18-20 hours with occasional mixing during this period. Following absorption the cells were separated from the serum using a Servall Small type A centrifuge at 5,000 rpm (3100 x g) for 30 minutes. The serum was decanted and again centrifuged for 90 minutes at the same speed to remove remaining clumps of leptospirae. The clear diluted absorbed serum was removed with a pipette to be used for the respective agglutination-lysis tests. Serum dilutions employed were 1:50, 1:100, 1:500, 1:1000, 1:5000, etc.

Sera were obtained from sheep, swine, goats and guinea pigs which were experimentally infected with L. pomona.[29, 34, 35, 36]

Some of the animals had been exposed to L. pomona, strain Wickard, while others were infected with strain Ohio.

Part I: Serum samples from five sheep showing a titer for L. pomona were heat deactivated by incubation in a waterbath at 56 C for 30 minutes. The agglutination-lysis test results were then compared with those obtained for the unheated sample.

Part II: L. pomona antisera from 17 sheep, 9 swine, and 4 goats were tested for antibodies against the following antigens: L. pomona (Johnson); L. icterohaemorrhagiae, AB;

L. icterohaemorrhagiae, A; L. canicola; L. sejroe; and L. hebdomadis.

Part III: Pre-inoculation serum samples from a representative number of guinea pigs were tested and found not to contain demonstrable antibodies. Fifteen guinea pigs were inoculated interperitoneally with 1.0 cc of blood, containing L. pomona (Wickard). This blood was obtained by exsanguination of guinea pigs which were in the leptospiremic stage of infection. Another group of 15 guinea pigs were inoculated in a like manner with blood containing viable L. pomona (Ohio) leptospirae. Five guinea pigs were kept as uninoculated controls. Each guinea pig was bled from the heart at approximately five-day intervals for a period of two months following inoculation. Sera from each animal were examined for the presence of antibodies for the six leptospiral antigens listed above (Part I).

Approximately two months following primary inoculation, each of the two groups was divided into three subgroups. Some subgroups contain four animals while others contain three animals (Tables 7 and 8). Each subgroup received three inoculations at four-day intervals of one of the leptospirae. One group received L. icterohaemorrhagiae, AB, another received L. icterohaemorrhagiae, A, while the third received L. canicola. The inocula consisted of 1.0 cc of a 7-day old culture of the respective leptospirae and were administered subcutaneously.

Part IV: Serum absorption tests were conducted on sera from two sheep and one pig. Portions of each serum were absorbed with L. pomona (Wickard), L. pomona (Johnson), L. icterohaemorrhagiae, AB, L. icterohaemorrhagiae, A, and L. canicola. The antibody level of each serum prior to and following absorption was ascertained using antigens of the above five leptospirae.

RESULTS

Part I

Heat deactivation (56 C for 30 min.) of L. pomona antiserum samples from five sheep showed no significant difference in homologous (L. pomona) or heterologous (L. icterohaemorrhagiae, AB) end point titers as compared to the original untreated samples (Table 1).

Part II

Cross reactions for L. sejroe or L. hebdomadis were not unequivocally demonstrated with sera from L. pomona infected sheep, swine, goats and guinea pigs. Agglutination was observed with some at the 10^{-1} serum dilution; however these reactions were no greater than 25 percent agglutination and were considered to be non-specific.

The pertinent results relative to sheep, group I, is expressed in Table 2 and Figure 1, and is as follows. L. icterohaemorrhagiae, AB, L. icterohaemorrhagiae, A and L. canicola antigens were agglutinated by L. pomona antisera of ovine origin (Table 2). Generally, serum reactions occurred simultaneously with the homologous (L. pomona) and the three above mentioned antigens. An increase in the three heterologous titers was coincident with a rise in homologous (L. pomona)

serum antibodies. (Figure 1). All titers mentioned in this thesis, unless otherwise stated, are arithmetric averages of the negative exponents of the end point serum dilutions calculated separately for each day of infection for each species of animal in the respective group. From the 10th to the 30th day of L. pomona ovine infection each of the three heterologous serotype reactions exceeded the 10^{-2} level. During this period of definite cross reaction, a homologous antibody titer greater than 10^{-6} prevailed. The maximum L. canicola cross reaction titer for these 9 sheep was 10^{-5} whereas L. icterohaemorrhagiae, AB and L. icterohaemorrhagiae, A were 10^{-4}. These maximum heterologous titers occurred on the 17th day of infection as did the maximum homologous titer of 10^{-9}. In general, the cross reactions between L. pomona sera and L. canicola antigen exceeded that of the other heterologous antigens. The degree of cross reactivity of L. icterohaemorrhagiae, AB and L. icterohaemorrhagiae, A was approximately equal. Residual heterologous titers of at least 10^{-2} remained as long as the 88th day following exposure.

Six L. pomona (Wickard) infected sheep,[36] Group II, presented similar serological results (Table 3). The same three heterologous serotypes were reactive. Cross agglutination with the three heterologous serotypes occurred to a titer of 10^{-2} or greater from approximately the 11th to about the 22nd day following exposure. The homologous titer for this period exceeded 10^{-5}. The maximum L. pomona (Wickard) serum

antibody titer was 10^{-8}. The maximum cross reaction titer for L. icterohaemorrhagiae, AB was 10^{-4} which occurred on the 11th, 14th and 18th day. The titer on the 11th day represented the result of the examination of one serum sample. L. canicola cross reacted to a maximum titer of 10^{-4} on the 14th and 18th day. L. icterohaemorrhagiae, A had a maximum titer of 10^{-3} on the 14th and 18th day. The L. icterohaemorrhagiae, AB, titer generally exceeded the titer of L. canicola and L. icterohaemorrhagiae, A. The latter two cross reactions closely approximated one another. Residual heterologous titers of 10^{-1} to 10^{-2} remain from the 23rd day through the 53rd day following exposure.

Sera from two sheep (group II) infected with strain Ohio showed less cross reactivity and homologous titers developed sooner than was observed for sera from sheep infected with strain Wickard (Table 3). Heterologous titers for L. icterohaemorrhagiae, A and L. canicola were the same and neverexceeded 10^{-1}. From the 8th to about the 53rd day cross reaction with L. icterohaemorrhagiae, AB was 10^{-2} or greater. A maximum titer of 10^{-3} with this serotype occurred on the 8th and 14th day while the homologous titer was 10^{-6} and $10^{-5.5}$, respectively. On the 7th, 8th and 9th day of exposure, the homologous titers of strain Ohio infected sheep was $10^{-4.5}$, 10^{-5}, 10^{-6}, respectively. On these same days strain Wickard exposed sheep, group I, showed homologous serum

titers of $10^{-2.8}$, $10^{-4.5}$, $10^{-5.2}$ and for sheep, group II, titers of 10^{-0}, $10^{-2.2}$, $10^{-5.2}$ were observed.

Sera from swine[34] experimentally infected with L. pomona strain Wickard agglutinated antigens of each of the three heterologous serotypes (Table 4). About the 24th day of infection the heterologous titers had reached a level of 10^{-2}; thereafter the titers exceeded this value for 151 days. The average maximum degree of cross reactivity for L. icterohaemorrhagiae, AB, was $10^{-3.2}$, for L. icterohaemorrhagiae, A and L. canicola it was 10^{-3}. A maximum homologous titer of 10^{-9} was observed (Figure 3). High homologous (10^{-8}) and heterologous titers (approximately 10^{-3}) were not observed until about the 38th day of infection. A slight distortion of the curve (Figure 3) showing changes in the amount of demonstrable serum antibody may have resulted from the fact that blood samples were not obtained from the 9th to the 24th day following exposure. During this period a characteristic serum antibody "peak" usually occurred as seen in Figure 4. L. icterohaemorrhagiae, AB cross reacted to a greater degree than either of the other two heterologous reactive serotypes. L. icterohaemorrhagiae, A and L. canicola had approximately the same levels of cross reactivity throughout the course of infection.

L. pomona strain Ohio infected pigs were found to have serum antibody levels above 10^{-2} for the three heterologous serotypes from approximately the 11th day of infection to about

the 83rd day. A corresponding homologous titer greater than 10^{-6} prevailed for this period (Table 4). A maximum cross reaction of $10^{-3.7}$ with L. icterohaemorrhagiae, AB appeared on the 14th day. On the 13th and 21st days L. icterohaemorrhagiae, A had a maximum cross reaction of 10^{-3}, while L. canicola had a maximum titer of $10^{-2.8}$ on the 83rd day. These maximum titers are only slightly more than the average titer observed for the respective serotypes (Figure 4). In general, L. icterohaemorrhagiae, AB antigens reacted to a higher degree than L. icterohaemorrhagiae, A antigens which were intermediate in position. L. canicola antigens were least reactive.

Sera from four goats infected with L. pomona (Wickard) similarly demonstrated a cross reaction with L. icterohaemorrhagiae, AB, L. icterohaemorrhagiae, A and L. canicola antigens (Table 5). Approximately the 13th day following exposure and continuing until about the 32nd day, each of the three heterologous titers exceeded 10^{-2} while the homologous titer exceeded 10^{-6} for the same period. On the 26th day following exposure a maximum titer for L. canicola (10^{-4}) and L. icterohaemorrhagiae, AB (10^{-3}) was observed. L. icterohemorrhagiae, A showed a maximum cross reaction of 10^{-2} from the 14th to the 33rd day. The maximum homologous titer of 10^{-8} occurred on the 26th day of infection. L. canicola antigens reacted with the antisera to a greater degree than did either of the other two heterologous serotypes. L. icterohaemorrhagiae, A was least reactive of the three heterologous serotypes. A decided

decrease in homologous serum antibody level after 26 days was coincident with a marked decrease in cross reactivity for L. canicola and L. icterohaemorrhagiae, AB.

Part III

There were no significant differences in cross reactivity between sera of guinea pigs infected with L. pomona strain Wickard and strain Ohio. Sera of guinea pigs infected with either strain agglutinated L. pomona (Johnson), L. icterohaemorrhagiae, A and L. canicola antigens (Tables 6 and 7). Definite serum reactions were not observed with L. icterohaemorrhagiae, AB. Initially, L. canicola was slightly more reactive than L. icterohaemorrhagiae, A but the reverse situation prevailed after 30 days. (Figures 6 and 7). The degree of serum cross reactivity was low. In general L. icterohaemorrhagiae, A and L. canicola reacted with sera from guinea pigs infected either strain to a titer of approximately 10^{-2}. L. canicola cross reacted with strain Ohio antisera to a slightly higher degree and showed a maximum titer $10^{-2.6}$ on the 24th day.

A second inoculation of these same animals with L. icterohaemorrhagiae, AB, L. icterohaemorrhagiae, A and L. canicola respectively caused an increase in serum reaction for each of these serotypes (Tables 8 and 9). The inoculation of these leptospirae did not produce an anamnestic response

(increase in L. pomona titer) in guinea pigs infected with L. pomona strain Wickard but did so in guinea pigs infected with strain Ohio. (Figures 6 and 7). A rise in the homologous titer for each of the three serotypes used as a second inoculum was observed.

Part IV

All leptospiral antibodies were removed from the serum of a sheep infected with L. pomona (Wickard) by absorption with either L. pomona strain Wickard or strain Johnson. (Table 10) The homologous serum titer was reduced by absorption with any of the three heterologous serotypes. Serum absorbed with L. icterohaemorrhagiae, AB did not agglutinate the other two heterologous antigens. Reactions with L. icterohaemorrhagiae, AB was reduced following serum absorption with L. canicola and agglutination did not occur with L. icterohaemorrhagiae, A. Serum absorption with L. icterohaemorrhagiae, A reduced the degree of reaction with the other two heterologous serotypes.

With one exception, comparable results were obtained using the same absorption procedure for the serum of a pig infected with L. pomona (Wickard). Contrary to the findings in the previous absorption, L. canicola absorbed serum gave no reaction with L. icterohaemorrhagiae, AB (Table 11).

Absorption with either L. pomona strain Wickard or strain Ohio removed all leptospiral antibodies from the serum of a pig infected with L. pomona (Ohio). (Table 12). Absorption with any of the three heterologous leptospirae reduced the serum reaction against both L. pomona antigens as well as the other heterologous serotypes. Serum absorption with L. canicola or L. icterohaemorrhagiae, A did not remove all of the agglutinin-lysins for L. icterohaemorrhagiae, AB. Absorption with any of the serotypes removed reactivity for L. canicola and L. icterohaemorrhagiae, A.

DISCUSSION

Variations and similarities in serum antibody reactions were observed among the various animal species studied. All of these animals were infected experimentally, therefore, these results may not correlate directly with the situations found in natural infections. An unequivocal comparison of the homologous titers in the various animal groups is not possible since the exposure inocula and the conditions of the experiments varied.

Heat deactivation (56 C for 30 min.) of L. pomona antisera from sheep did not reduce the heterologous titers observed in the unheated sera. It appears that the serum factor responsible for the leptospiral cross reactions is not heat labile. Apparently the heterolcgous titers are the result of a specific reaction.

L. pomona antisera from sheep, swine and goats cross reacted with L. icterohaemorrhagiae, AB, L. icterohaemorrhagiae, A, and L. canicola but definite reactions with L. sejroe and L. hebdomadis were not observed. It would appear that there is no antigenic relationship between L. pomona, L. sejroe or L. hebdomadis. The supposition that animals which have L. pomona titers are reactors due to L. sejroe infection is nct indicated. Antisera obtained from guinea pigs reacted with all heterologous antigens except L. icterohaemorrhagiae, AB.

This finding in guinea pigs is contrary to that observed in another report[31] in which L. icterohaemorrhagiae, AB cross reactions were found to be due to L. pomona infection. The fact that several different strains of L. pomona were used to infect the guinea pigs and a different stock of guinea pigs was used, may account for this variance in results.

A rise in the homologous titer was reflected by an increase in the three heterologous titers in all animal species with the above exceptions. During the first days (5-7 days) of demonstrable serum antibody, cross reaction titers greater than 50 percent of the homologous titers, were observed in all the animal species. The heterologous titers were never observed to exceed the homologous titers. The reverse has been observed during the course of both natural and experimental leptospiral infections.[14,17]

L. pomona (Wickard) antisera from the sheep and from the goats had significant heterologous titers (10^{-2} or greater) during the 11th day to the 2nd or 3rd week following exposure. L. pomona (Ohio) antisera from sheep had comparable L. icterohaemorrhagiae, AB titers. The heterologous titers with L. icterohaemorrhagiae, A and L. canicola, however, did not ever rise to such a significant level. Homologous titers observed in both strain Ohio and strain Wickard infected sheep were essentially equal. Apparently, strain Ohio does not elicit antibodies of quite the same composition or completeness since

L. icterohaemorrhagiae, A and L.canicola titers were considerably less with these sera.

Sera from pigs infected with either strain of L. pomona had significant heterologous titers from approximately the 12th day to the final day of examination (83 days and 151 days). Approximately the same degree of cross reactivity was observed with sera from pigs infected with either strain. The antibodies elicited in pigs by strain Wickard and strain Ohio are seemingly the same. Similarly, sera from guinea pigs show the same degree of cross reactivity for both strain Ohio and strain Wickard. The differences observed in antibody response to strains Ohio and Wickard in sheep would indicate that these animals do not react to some antigenic component or determinate group of the strain Ohio cell. On the other hand, swine and guinea pigs are capable of producing antibody against this "masked" portion of the Ohio organism.

It is difficult to interpret a comparison of the degree of cross reactivity of the three heterologous serotypes in relation to the infecting strains of L. pomona and the animal species. During the first 3 weeks of infection L. canicola had a greater average cross reaction than did the other heterologous leptospirae. This was observed with sera from sheep, group I, and with goats, both infected with L. pomona (Wickard). Sera from sheep, group II, and from pigs infected with strain Wickard and some with strain Ohio gave

a greater reaction with L. icterohaemorrhagiae, AB. The average L. icterohaemorrhagiae, A titer was the same as the average L. canicola titer in sheep infected with Ohio and pigs infected with Wickard. Moreover, with sera from pigs exposed to strain Ohio, L. icterohaemorrhagiae, A was intermediate in cross reactivity and exceeded the L. canicola titers. In every other group of sera, however, L. icterohaemorrhagiae, A was least reactive. The general trend in reactivity among heterologous serotypes was L. icterohaemorrhagiae, AB which was greater than L. canicola, which in turn was greater than L. icterohaemorrhagiae, A.

Except for the results cited below, the titers for L. icterohaemorrhagiae, AB, L. icterohaemorrhagiae, A, and L. canicola were approximately equal throughout the course of infection. A difference greater than one tube (10-fold) dilution was considered to be significant. Several examples of heterologous titer differences greater than one tube dilution were observed in sheep infected with strain Wickard. (Tables 2 and 3) With sera from sheep infected with strain Ohio, however, L. icterohaemorrhagiae, AB had an average cross reaction titer 48 percent of the homologous titer while L. canicola and L. icterohaemorrhagiae, A had titers which were only 15 percent of the homologous titers. Sera of goats infected with strain Wickard had a L. canicola cross reaction which was 45 percent of the average homologous titer, while

L. icterohaemorrhagiae, AB and L. icterohaemorrhagiae, A reacted to 35 percent of the homologous titers.

In the following discussion the three heterologous leptospirae will be treated as a group, unless a serotype is specifically mentioned. A comparison of the degree of cross reactivity for the various antisera must be considered in relation to the L. pomona titer. With sera of sheep infected with strain Wickard the averages of the titers of the three cross reactive serotypes were approximately 40 percent of the average homologous titer. (Figures 1 and 2) L. pomona (Ohio) antisera from sheep showed an average heterologous titer which was 24 percent of the homologous titer. As stated above however, L. icterohaemorrhagiae, AB reacted to 48 percent and L. icterohaemorrhagiae, A and L. canicola reacted to 15 percent. (Table 3) L. pomona (Wickard) and strain Ohio antisera of swine had average cross reactive titers approximately 34 percent of the homologous titers. (Figures 3 and 4) Sera from goats infected with L. pomona (Wickard) have average cross reactions which were about 36 percent of the homologous titer. Again, as stated above, L. canicola reacted to 45 percent and L. icterohaemorrhagiae, AB and L. icterohaemorrhagiae, A reacted to 35 percent. Sera from guinea pigs infected with either strain Wickard or strain Ohio reacted with L. icterohaemorrhagiae and L. canicola to approximately 35 percent of the homologous titer.

In general the degree of cross reactivity for the sera of various animals is as follows (the greater being listed first):

(1) sera from sheep infected with L. pomona (Wickard)

(2) sera from goats infected with L. pomona (Wickard)

(3) sera from pigs infected with either strain Wickard or strain Ohio,

(4) sera from guinea pigs infected with either strain Wickard or strain Ohio,

(5) sera from sheep infected with strain Ohio.

The antibodies produced in response to a given leptospira are apparently different in various animal species. The differences in antibody composition are evidenced by the varying degrees of cross reactivity observed in different animal species infected with the same serotype.

The antibodies produced in response to the L. pomona strains in a single given animal species may differ in composition. This is illustrated by the differences in heterologous titers observed with sera from a group of sheep infected with strain Ohio, and another group infected with strain Wickard.

The serum absorption studies indicate a close antigenic relationship between L. pomona strain Wickard, strain Johnson and strain Ohio, and supports the inclusion of the three strains into a single serotype. The differences in degrees of agglutination with the same serum, however, indicate some slight antigenic differences among these three L. pomona

strains. For two of the three sera the original homologous titer was reduced the same amount by absorption with any of the three heterologous serotypes. The third serum, that of sheep 777, following absorption with L. icterohaemorrhagiae, A did not reduce the L. pomona strain Johnson titer to the expected level. Absorption of the three sera with L. icterohaemorrhagiae, AB removed all the antibody reactive for L. icterohaemorrhagiae, A and L. canicola, even though the L. icterohaemorrhagiae, AB unabsorbed serum titer was less than either of the other serotypes. This indicates that the L. pomona antibody component or components in sheep and pig sera responsible for cross reactions are more closely related to the antigenic structure of L. icteronaemorrhagiae, AB, than the other two heterologous serotypes. Absorption with L. canicola always removed all the antibody reactive with L. icterohaemorrhagiae, A regardless of the relative degrees of cross reaction. Perhaps a closer relationship exists between L. pomona and L. canicola than exists between L. pomona and icterohaemorrhagiae A.

In the early stages of experimental infection (5 to 7 days) heterologous and homologous titers are approximately the same. Some reports[14, 17] state that early in natural infections, before homologous antibodies are fully developed, heterologous titers exceed those produced by the infecting organism. During this period the identification of the

etiological agent may be difficult or impossible to ascertain by routine serological procedures. Serum absorptions with reacting leptospirae do not always result in definite identification.[7] Positive identification of the causative agent may be made only by serological examination at a later stage of infection.

SUMMARY

(1) Essentially the same homologous and heterologous agglutination titers were observed with heated (56 C for 30 minutes) and unheated L. pomona antisera from sheep.

(2) Sera from sheep, goats, swine and guinea pigs infected with L. pomona were examined for cross agglutination reactions with L. icterohaemorrhagiae, AB, L. icterohaemorrhagiae, A, L. canicola, L. sejroe, and L. hebdomadis antigens.

(a) Reactions for L. sejroe or L. hebdomadis were not unequivocally demonstrated with sera from L. pomona infected animals.

(b) A comparison of the titers obtained for the antisera of various species indicated differences in the degree of antibody response for reactive heterologous serotypes.

(c) Marked differences in the degree of cross reaction for L. icterohaemorrhagiae, A and L. canicola were observed between sera from sheep infected with L. pomona strain Wickard and ovine sera containing antibodies against strain Ohio.

(d) Guinea pig sera containing antibodies for either strain Ohio or strain Wickard did not agglutinate L. icterohaemorrhagiae, AB to an observable level.

(3) An anamnestic response was obtained in guinea

pigs infected with L. pomona (Ohio) upon challenge with any of the three cross reactive serotypes.

(4) Serum absorptions were conducted on one sheep serum and two pig sera. Homologous titers were reduced by absorption with any of the three reactive heterologous serotypes. Absorption with L. icterohaemorrhagiae, AB always removed all of the antibody for L. canicola and L. icterohaemorrhagiae, A.

TABLE 1

ANTIBODY LEVEL COMPARISON BETWEEN UNHEATED AND HEAT DEACTIVATED* L. POMONA ANTISERA**

Sheep	Antibody Levels† Using																			
	L. pomona (Johnson) Antigen												L. icterohaemorrhagiae, AB Antigen							
	Serum Samples:																			
	1	2	3	4	5	6	7	8	9	10	11	12	A	B	C	D	E	F	G	H	I	J
901																				
Unheated[x]	0	0	3	3	4	6	6	6	5				0	1	1	2	3	2	2			
Deact.	0	0	3	3	3	6	7	6	5				0	0	1	2	3	3	2			
921																				
Unheated	2	4	5	5	5	4	4	3	4				0	1	1	3	3	3	1	1	2	1
Deact.	2	3	5	5	5	4	5	3	3				1	1	2	3	2	2	2	2	2	2
926																				
Unheated	3	6	7	6	6	6	6	5	5	5	5	5	0	2	3	2	1	0	0	0	0	0
Deact.	3	6	6	7	5	6	6	6	5	5	5	5	0	1	3	2	2	0	0	0	0	0
951																				
Unheated	5	4	4	4	3	3	4	4					3	3	2	3	2	3	3	3		
Deact.	4	4	3	3	4	4	4	5					3	3	2	2	2	3	3	3		
975																				
Unheated	2	4	5	7									1	2	2	4						
Deact.	2	3	5	7									1	2	2	4						

*Heat deactivation was accomplished by water incubation at 56° C for 30 minutes.

**Sheep 901 and 921 infected with strain Ohio and sheep 926, 951 and 975 infected with strain Wickard.

†The titers are expressed as the negative exponent of the highest serum dilution showing agglutination, lysis or both. Reactivation of 25 percent or greater is considered as end point. Ten-fold serum dilutions were used.

[x]No evidence of agglutination or lysis at 1:10 serum dilution.

TABLE 1 a

Explanation of the System Used in Expressing Titers for Tables 2 through 9 Inclusive

Titers for a single serum sample are arranged in a group of four. The position of a number in the group of four numbers, signifies the antigen used in testing the serum sample. The titers are expressed as the negative exponent of the highest serum dilution showing agglutination or lysis, or both.

Example: $\begin{matrix} 53 \\ 21 \end{matrix}$

signifies that for this serum the following titers were observed:

L. pomona (Johnson) titer	10^{-5}
L. icterohaemorrhagiae, AB	10^{-3}
L. icterohaemorrhagiae, A	10^{-2}
L. canicola titer	10^{-1}

Ten-fold serum dilutions were used. A reaction of 25 percent or greater was considered as end point.

TABLE 2

SEROLOGICAL CROSS REACTIONS OBTAINED WITH OVINE SERA,* Group I

Sheep		Titers on Days Following Exposure																			
		5	6	7	8	9	10	11	12	14	17	18	22	25	31	36	59	63	69	75	82	88
608	**	10	10	20	30		40			51					61							
	†	00	00	00	01		01			12					21							
737				11	21	51	62	62	73					52				41			41	
				12	12	13	13	23	34					22				12			12	
761		00	30	40	61					61					82	62						
		00	10	10	11					21					31	22						
764		10	41	61	73					83					72	62						
		21	21	21	22					33					22	22						
777		42	52	73	84					94	94	94			63	63						
		22	24	34	45					45	45	35			34	34						
781		21	42	52	73					94			84		73	62						
		20	21	21	32					43			32		32	32						
786				01	42	64	84	95	94					64			53			63		63
				00	11	32	33	44	44					33			32			32		32
832				00	22	52	84	104	95					103			62		51			51
				01	12	22	33	34	44					23			22		22			22
836				00	31	51	62	73	103				73									
				00	11	11	23	34	45				34									

*The antisera were furnished by Dr. Kaare Lindqvist, Michigan State University. The infecting strain was L. pomona Wickard.

**For an explanation of the system used in expressing titers, refer to table 1 a.

†No evidence of agglutination or lysis at 1:10 serum dilution.

TABLE 3

SEROLOGICAL CROSS REACTIONS OBTAINED WITH OVINE SERA*, Group II

Sheep	Titers on Days Following Exposure																			
	6	7	8	9	10	11	13	14	15	16	17	18	20	25	26	32	35	37	41	53
905	**	00 00	22 10		53 21		53 13				53 12			52 11		52 11		42 11	42 11	
906		00 00	31 00	52 00	63 12		63 12			63 23	63 12			52 11		42 11		42 11		
907			11 11	32 11	42 12		64 24	64 34	64 34		73 34	54 34								
925†				42 21						53 22						43 22		42 12	42 11	42 11
926			30 00		62 10		73 20							62 10		61 10		60 00		50 00
975			21 20	42 20	52 20	84 20														
901	20 00	51 00	51 00	62 00				63 00					52 00	52 00						
921	20 00	42 11	53 22	64 22				53 22					43 11		42 11		42 11		42 11	

*The antisera were furnished by Dr. E. V. Morse, Michigan State University. Sheep 905, 906, 907, 925, 926 and 975 infected with L. pomona (Wickard), while 901 and 921 infected with L. pomona (Ohio).

**For an explanation of the system used in expressing titers refer to Table 1 a. "0" indicates no evidence of agglutination or lysis at 1:10 serum dilution.

†The exact date of infection is unknown; therefore, the days following infection showing a titer are only approximate.

TABLE 4

SEROLOGICAL CROSS REACTIONS OBTAINED WITH PORCINE SERA*

Pig	Titers on Days Following Exposure																					
	7	8	9	10	11	12	13	14	17	21	24	31	38	59	83	87	101	102	122	129	151	216
4921		**63									74		84	103		93	93			83	73	
		21									34		33	43		32	32			32	32	
4922	21		63								94		94	94		104	93		83			
	21		40†								42		32	32		32	32		22			
4927	10	51		62		83		74	83	83		63			52			42				
	10	11		11		31		32	31	31		21			11			11				
4928	11	31		42		63		63	83			63			71							
	00	00		11		22		12	12			13			13							
4929	11	32	42	53	73	83	74	84	84	83		74			94							
	11	11	12	32	32	33	43	43	43	43		43			35							
4961		20	51								92		82	82		93	93		93			
		10	21								22		23	34		34	34		34			
4963		31	52								63		63	73		82				63	63	
		11	12								22		23	23		32				23	23	
4972	21		52								73		83	93		94	63			83		82
	11		11								22		33	33		32	32			22		12
4973	10	40	41		52		93		83	73		83			82							
	00	12	11		21		22		22	22		22			22							

*The antisera were furnished by Dr. E. V. Morse, of Michigan State University. Pig 4921, 4922, 4961, 4963 and 4972 infected with L. pomona (Wickard), while 4927, 4928, 4929, and 4973 infected with L. pomona (Ohio).

**For an explanation of the system used in expressing titers refer to Table 1 a.

†No evidence of agglutination or lysis at 1:10 serum dilution.

TABLE 5

SEROLOGICAL CROSS REACTIONS OBTAINED WITH CAPRINE SERA*

Goat	Titers on Days Following Exposure								
	7	8	10	13	14	26	32	33	39
820	**00	11	52	83	62				
	00	11	23	24	23				
889	00		40	51			52		
	00		11	12			22		
988	20		51	52				41	41
	00		11	12				22	12
995	00		52	83		83			
	00		11	34		24			

*The antisera were furnished by Dr. E. V. Morse, Michigan State University. Infection with L. pomona (Wickard).

**For an explanation of the system used in expressing titers refer to Table 1 a. "0" indicates no evidence of agglutination or lysis at 1:10 serum dilution.

TABLE 6

SEROLOGICAL CROSS REACTIONS OBSERVED WITH SERA OF GUINEA PIGS EXPERIMENTALLY INFECTED WITH L. POMONA (WICKARD)

Days Following Exposure	Guinea Pig														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
							Titers*								
8								00	100	00	10	00	00	00	00
								00	00	00	00	00	00	00	00
11	40**	41	31	40	41	41	41								
	11	12	22	22	22	22	22								
14								40	40	30	40	40	40	40	30
								22	12	21	12	22	22	12	11
17	41	41	31	41	41	41	40								
	11	13	12	22	22	21	11								
21								51	51	51	51	61	51	51	51
								22	21	22	22	23	21	22	21
24	41	40	31	40	41	30	41								
	12	23	12	22	22	21	21								
31								50	40	50	40	40	40	50	50
								22	21	22	12	11	21	12	21
38	50	50	50	50	50	40	50								
	20	20	20	20	20	20	10								
45								50	60	60	50	50	40	50	50
								11	21	21	21	11	11	11	21
52	50	50	50	50	50	50	50								
	10	20	10	20	10	10	10								
59								40	40	40	30	40	40	30	40
								00	00	00	00	11	00	00	10

*For an explanation of the system used in expressing titers refer to Table 1 a.

**No evidence of agglutination or lysis at 1:10 serum dilution.

TABLE 7

SEROLOGICAL CROSS REACTIONS OBSERVED WITH SERA OF GUINEA PIGS EXPERIMENTALLY INFECTED WITH L. POMONA (OHIO)

Days Following Exposure	Guinea Pig														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Titers*														
8	10	10	10	10	10	20	10								
	**00	00	00	00	00	00	00								
11								31	40	31	31	31	40	31	31
								22	12	11	12	11	11	11	12
14	40	41	40	40	40	40	40								
	12	12	11	12	12	22	22								
17								40	41	--	40	51	41	41	41
								22	22	--	12	12	12	22	22
21	40	41	40	51	--	40	51								
	12	23	12	22	--	12	22								
24								40	40	--	51	51	40	51	40
								22	22	--	23	23	22	23	23
32	50	50	--	50	--	50	50								
	22	12	--	22	--	21	22								
39								60	50	--	50	50	60	50	50
								21	11	--	12	12	12	11	21
46	60	50	--	50	--	60	50								
	10	11	--	11	--	11	10								
53								60	60	--	60	60	60	60	60
								22	23	--	23	22	22	21	22
60	50	50	--	50	--	50	50								
	20	10	--	10	--	00	10								

*For an explanation of the system used in expressing titers refer to Table 1 a.

**No evidence of agglutination or lysis at 1:10 serum dilution.

- No blood sample obtainable due to the death of the animal.

TABLE 8

L. POMONA ANTISERA (GUINEA PIG) SHOWING ANAMNESTIC CROSS REACTIVITY

First Exposure	Second Exposure	Guinea Pig	Titers* Days Following Exposure*** 0	14	21
Strain: (Wickard)		1	51 11	43 22	43 30
		2	51 21	33 22	43 30
	L. icterohaemorrhagiae, AB	3	50 21	43 22	53 30
		4	51 11	43 22	53 20
		5	31 11	33 23	53 31
	L. icterohaemorrhagiae, A	6	40 12	44 23	44 31
		7	40 12	33 22	43 30
		8	51 10	43 21	43 31
		9	41 11	44 24	53 34
	L. canicola	10	40 11	33 24	42 30
		11	40 11	43 24	42 34
		12	51 11	33 24	42 33
***Positive Controls		13	41 11	31 11	41 21
		14	41 11	41 11	52 21
†Negative Control		15	00 00	00 00	00 00

*For an explanation of the system used in expressing titers refer to Table 1 a. "0" indicates no evidence of agglutination or lysis at 1:10 serum dilution.

**Refers to days following second exposure. Duration of L. pomona infection was 60 days.

***Only exposed to L. pomona (Wickard).

†Unexposed to infection with leptospirae.

TABLE 9

L. POMONA ANTISERA (GUINEA PIG) SHOWING ANAMNESTIC CROSS REACTIVITY

First Exposure	Second Exposure	Guinea Pig	Titers* Days Following Exposure** 0	14	21
Strain:					
(Ohio)		1	51 11	44 24	54 21
	L. icterohaemorrhagiae AB	2	41 21	44 24	54 31
		3	50 11	44 24	54 31
		4	51 11	43 24	64 41
	L. icterohaemorrhagiae, A	5	40 11	43 24	53 31
		6	50 10	44 24	53 31
		7	41 10	43 24	53 34
	L. canicola	8	41 11	44 24	54 34
		9	50 11	44 24	64 34
		10	41 11	43 24	64 34
***Positive Control		11	61 12	51 11	41 11
†Negative Control		12	00 00	00 00	00 00

*For an explanation of the system used in expressing titers refer to Table 1 a. "0" indicates no evidence of agglutination or lysis at 1:10 serum dilution.

**Refers to days following second exposure. Duration of L. pomona infection was 60 days.

***Only exposed to L. pomona (Ohio).

†Unexposed to infection with leptospirae.

TABLE 10

AGGLUTININ-ABSORPTION RESULTS CONDUCTED WITH SERUM* FROM A SHEEP EXPERIMENTALLY INFECTED WITH L. POMONA (WICKARD)

Serum	Absorbing Serotype	Titers** against Antigens				
		L. pomona (Wickard)	L. pomona (Johnson)	L. icterohaemorrhagiae (AB)	L. icterohaemorrhagiae (A)	L. canicola
Sheep 777 17 days following exposure	None	1,000,000	100,000	500	1,000	5,000
	L. pomona					
	(Wickard)	0	0	0	0	0
	(Johnson)	0	0	0	0	0
	L. icterohaemorrhagiae					
	(AB)	50,000	5,000	0	0	0
	(A)	50,000	10,000	50	0	1,000
	L. canicola	50,000	5,000	50	0	0

*The antisera was furnished by Dr. Kaare Lindqvist, Michigan State University.

**Titers are expressed as the reciprocal of the highest serum dilution showing agglutination-lysis.
"0" indicates complete absorption as demonstrated by no evidence of agglutination or lysis at 1:10 serum dilution.
A reaction of 25 percent or greater was considered as end point.

Information Sheet to Figures

These data refer to Figures 1 through 5:

1) To avoid confusion in reading the figure, L. icterohaemorrhagiae, A titers are not plotted since these titers vary little from the other cross reactive serotypes.

2) An arithmetic average of the negative exponents of the end point titers are plotted for each day that positive reactions were obtained.

3) Designation of reaction with Leptospiral serotypes

L. pomona (Johnson) antigen

L. icterohaemorrhagiae, AB antigen

L. canicola antigen

These data refer to Figures 6 and 7:

1) No observable reaction was obtained with L. icterohaemorrhagiae, AB.

2) An arithmetic average of the negative exponents of the end point titers are plotted for each day that positive reactions were obtained.

3) Designation of reaction with Leptospiral serotypes

L. pomona (Johnson) antigen

L. icterohaemorrhagiae, AB antigen

L. canicola antigen

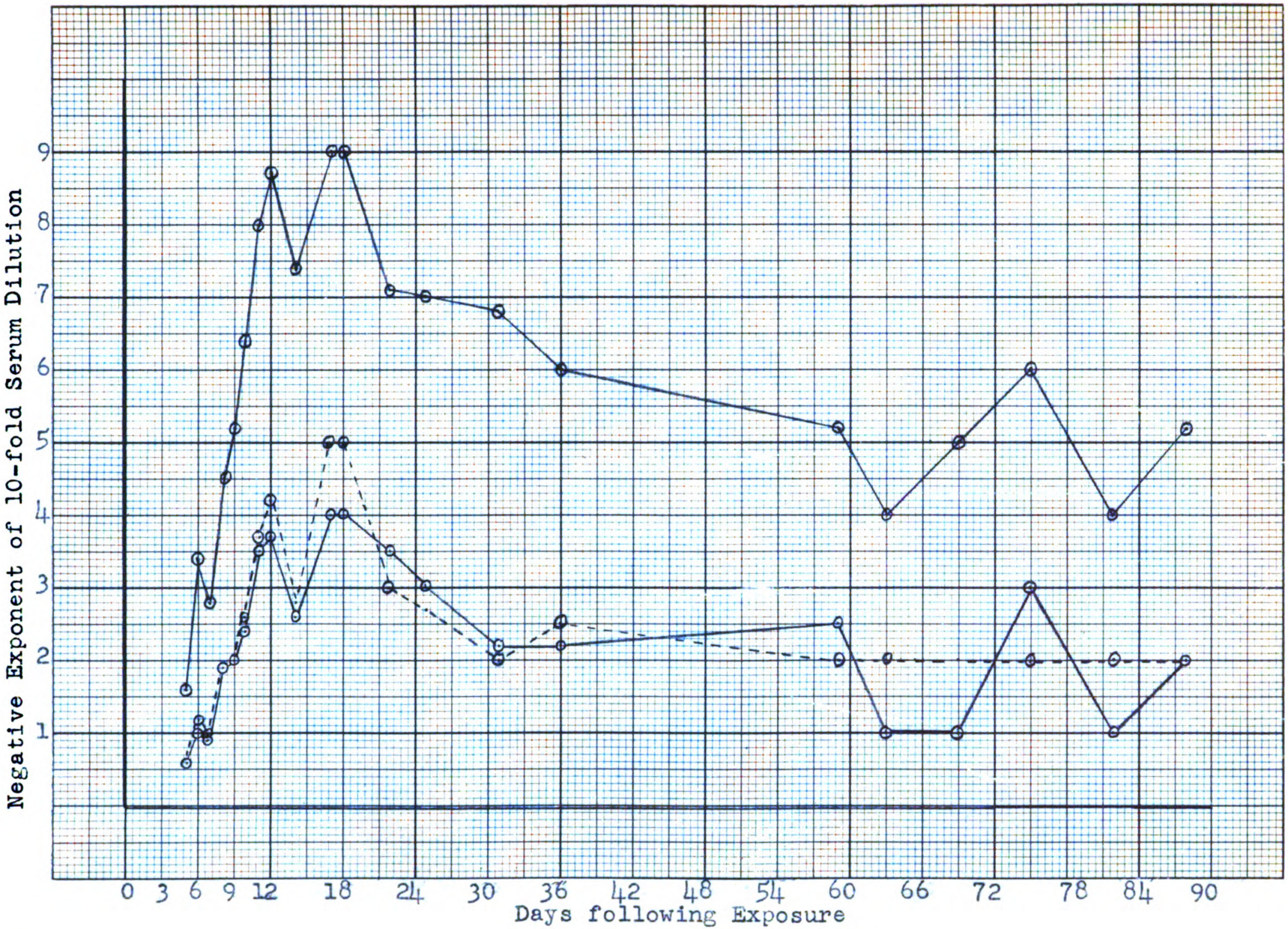

Fig. 1. Average homologous and heterologous serum antibody levels observed in ovine L. pomona (Wickard) infection, Group I. The antisera were furnished by Dr. Kaare Lindqvist, Michigan State University. Nine sheep are represented.

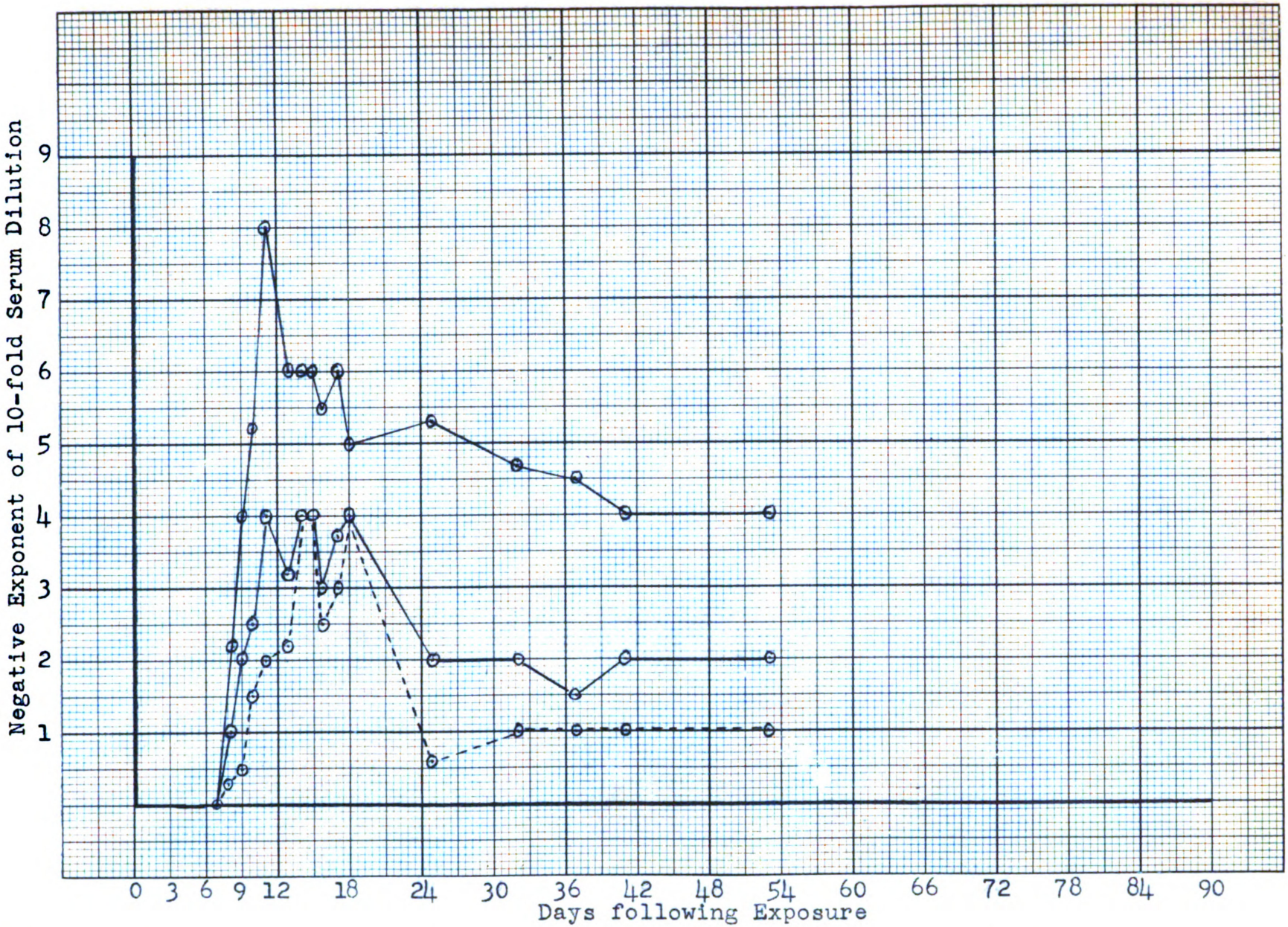

Fig. 2. Average homologous and heterologous serum antibody levels observed in ovine L. pomona infection, Group II. The antisera were furnished by Dr. E. V. Morse, Michigan State University. Six strain Wickard and two strain Ohio infected sheep are represented.

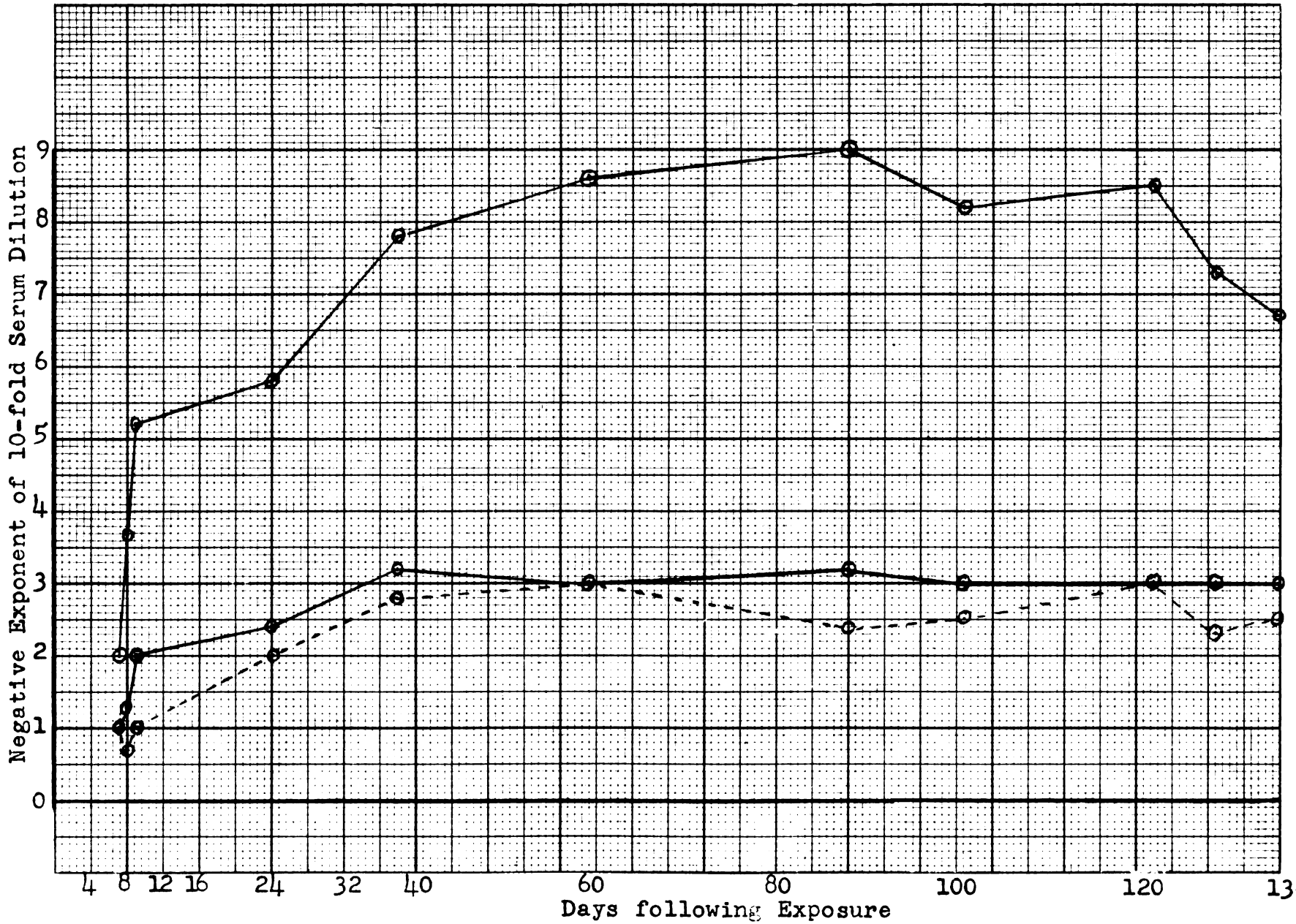

Fig. 3. Average homologous and heterologous serum antibody levels observed in porcine L. pomona (Wickard) infection. The antisera were furnished by Dr. E. V. Morse, Michigan State University. Five pigs are represented.

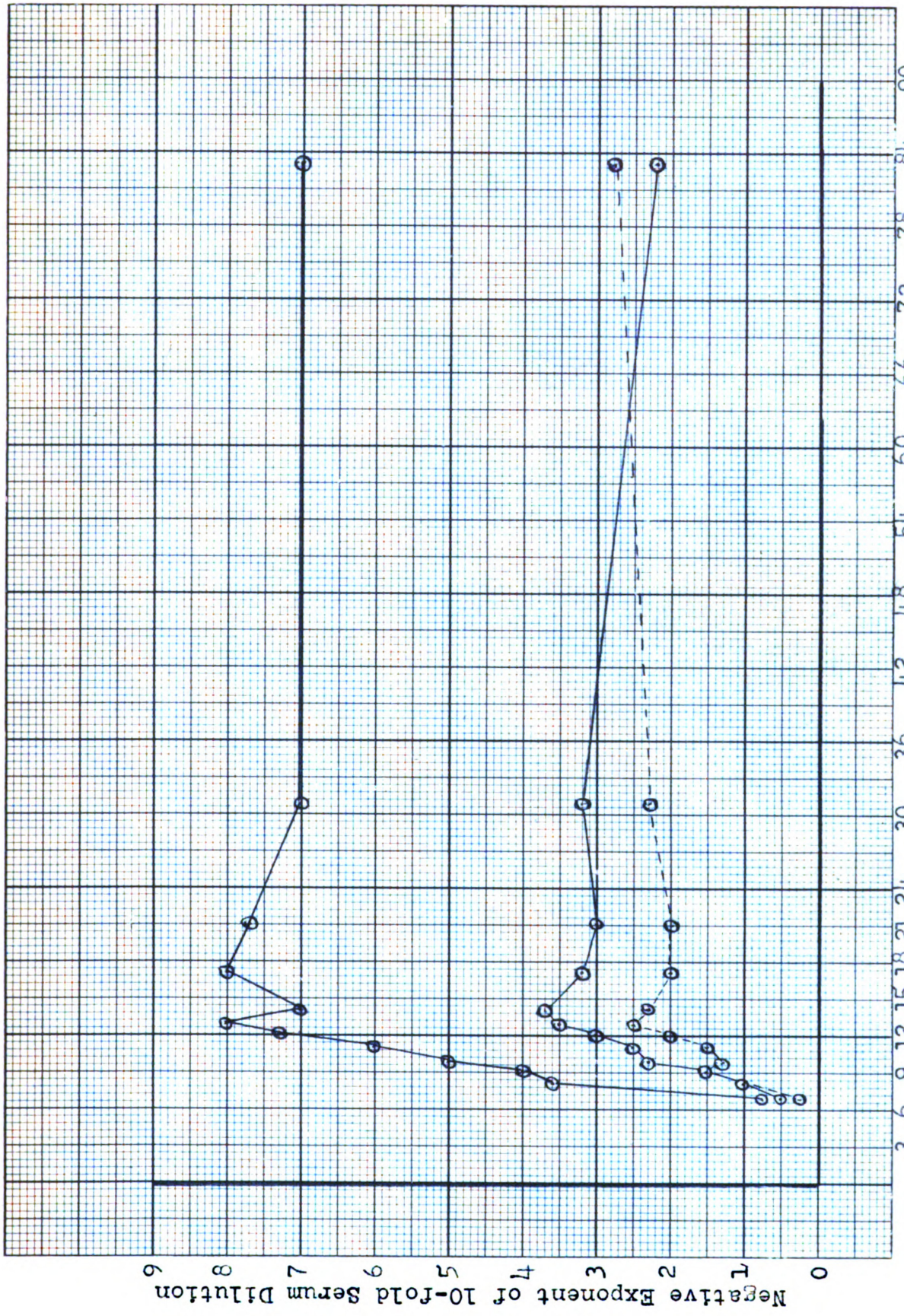

Fig. 4. Average homologous and heterologous serum antibody levels observed in porcine L. pomona (Ohio) infection. The antisera were furnished by Dr. E. V. Morse, Michigan State University. Four pigs are represented.

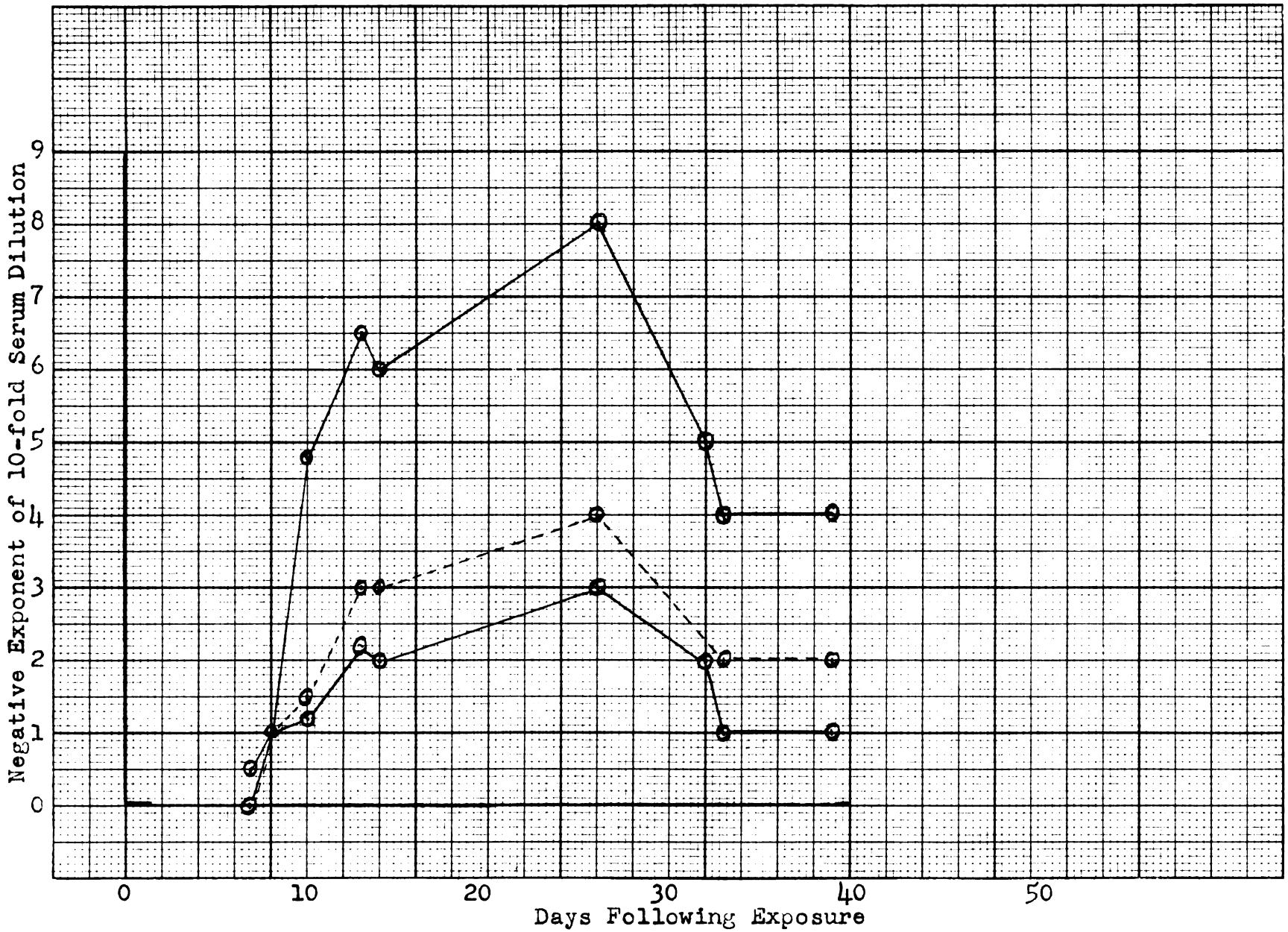

Fig. 5. Average homologous and heterologous serum antibody levels observed in caprine L. pomona infection. The antisera were furnished by Dr. E. V. Morse, Michigan State University. Four goats are represented.

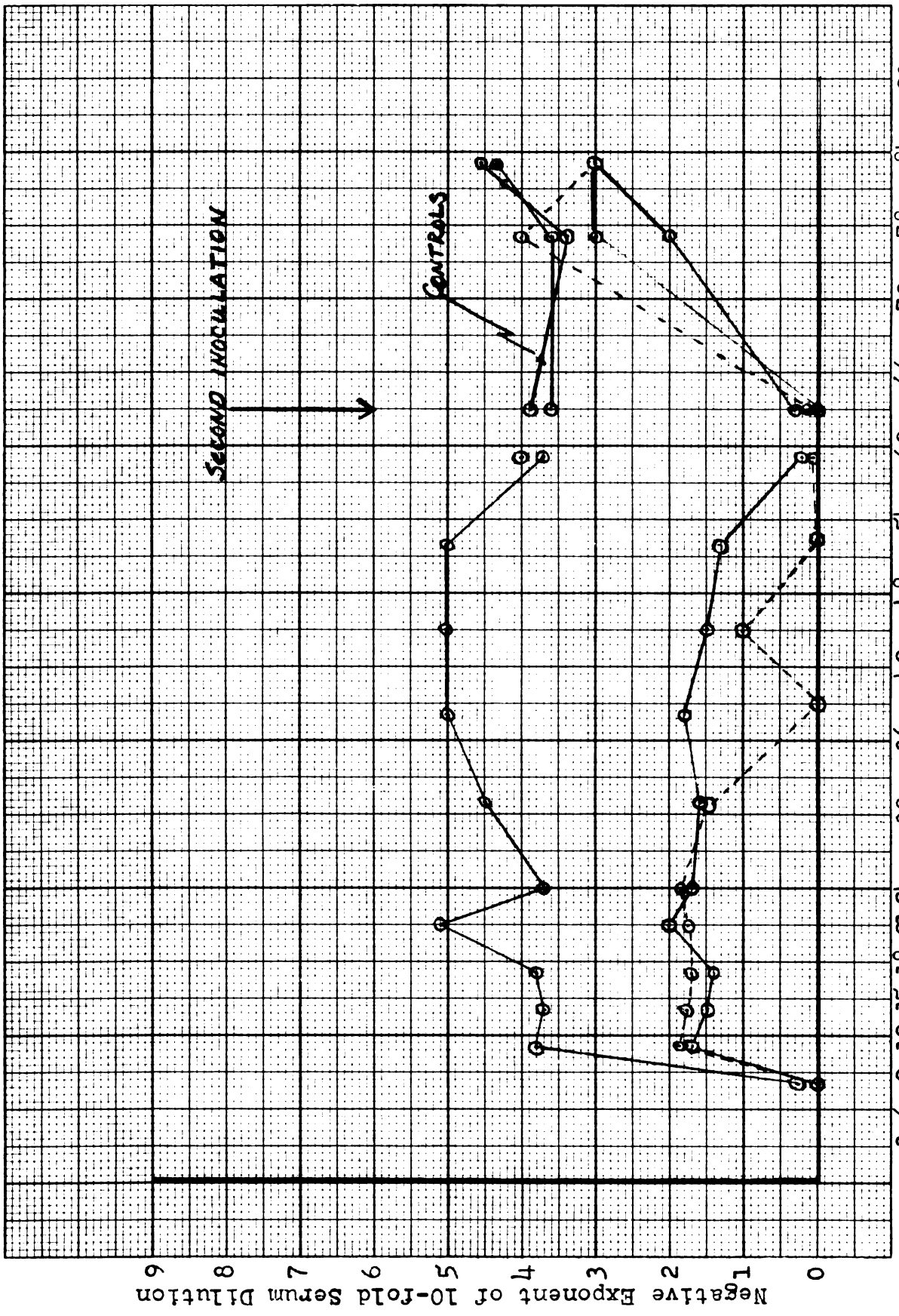

Fig. 6. Average homologous and heterologous serum antibody levels observed in guinea pigs infected with L. pomona (Wickard). L. icterohaemorrhagiae, Ab, L. icterohaemorrhagiae, A and L. canicola used as inocula in separate groups of L. pomona infected guinea pigs.

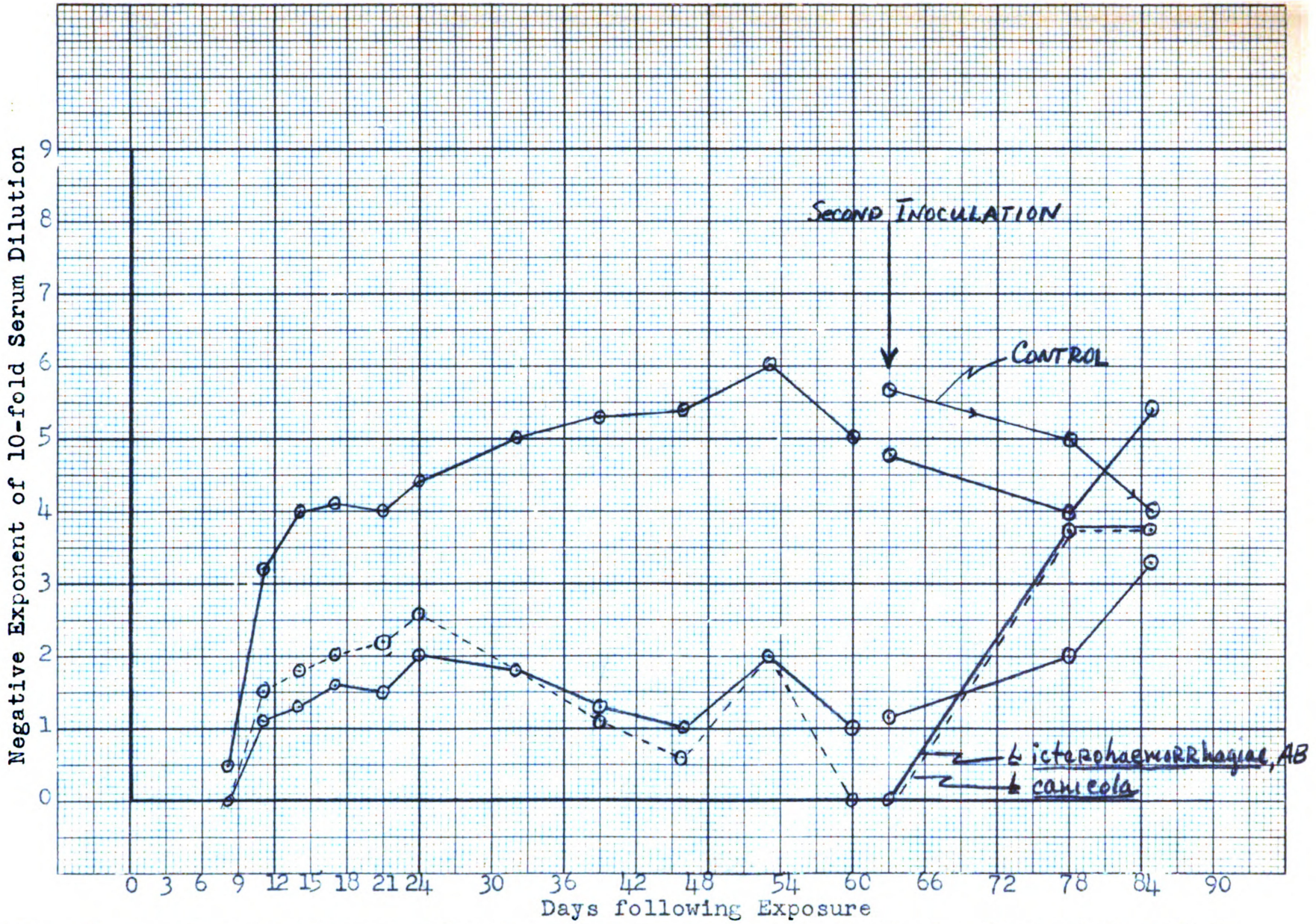

Fig. 7. Average homologous and heterologous serum antibody levels observed in guinea pigs infected with L. pomona (Ohio). L. icterohaemorrhagiae, AB, L. icterohaemorrhagiae, A and L. canicola used as inocula in separate groups of L. pomona infected guinea pigs.

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