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Changes in the Differential Leukocyte Count of Chicks Inoculated with *Salmonella*[1]

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Studies were conducted to determine the effects of *Salmonella anatum* and *S. heidelberg* infections on the differential leukocyte counts of baby chickens. Newly hatched broiler-type chicks were inoculated in the yolk sac with suspensions of either *S. anatum* or *S. heidelberg*. At 0, 24, 48, and 72 hr after inoculation, blood was taken by heart puncture from chicks of both inoculated groups and from a group of uninoculated chicks. The averages of the leukocyte counts of three or four chicks from each group were used as the blood values for specific time intervals. The six classes of leukocytes counted were lymphocytes, monocytes, juveniles, heterophils, eosinophils, and basophils. The leukocytes classified as juveniles were immature or degenerate heterophils and were found almost exclusively in the infected chicks. Changes in heterophil, juvenile, and lymphocyte counts were affected by both the number of cells in the inoculum (300 versus 3 million cells of *S. anatum*) and species (*S. anatum* and *S. heidelberg*). Infection with either *Salmonella* species resulted in the development of a severe heterophilic leukopenia, and a significant increase in the percentage of both juveniles and lymphocytes by 48 hr postinoculation. Mortality rate was higher in groups of chicks inoculated with *S. heidelberg* than in groups given *S. anatum*.

The differential leukocyte count is an important aid in the diagnosis of human diseases. The technique is also important to avian pathologists, but the field of avian hematology is not nearly as well developed as in human hematology. Before differential leukocyte counts can be used extensively as a diagnostic tool by poultry pathologists, a large amount of basic information must be accumulated relative to the blood pictures of diseased birds as well as those of healthy birds.

The immunological and hematological characteristics of newly hatched chicks differ greatly from those of birds only two or three weeks older. In spite of this fact, workers in avian hematology frequently refer in their reports to the ages of chicks used as either young or adult.

Reports of earlier investigations indicate that *Salmonella* infections cause a heterophilic leukocytosis in chickens and turkeys (5, 10), but these studies involved birds considerably older than those used in the present investigation.

Vestal and Stephens (9) demonstrated variation among *Salmonella* species in degree of pathogenicity for baby chicks. Subsequently, it was reported (8) that these differences in species could not be correlated with growth rate of the *Salmonella* in the chicks' yolk sacs. Considerable changes in the blood picture of chicks infected with *S. pullorum* were reported by Kelly and Dearstyne (2). Since *S. pullorum* is highly pathogenic for baby chicks, it was thought that changes in the blood picture of chicks inoculated with various species of *Salmonella* might be related to the degree of pathogenicity of the species.

The objectives of this study were to gain information on the effects of *S. anatum* and *S. heidelberg* infections on the differential leukocyte counts of newly hatched chicks, and to determine whether differences in differential leukocyte counts of infected chicks could be related to the degree of pathogenicity of the *Salmonella* species.

MATERIALS AND METHODS

Four trials were conducted to compare the effects of *S. anatum*, a mildly pathogenic serotype, and *S. heidelberg*, a highly pathogenic serotype (9), on the differential leukocyte count of baby chicks. A second study consisting of two trials was conducted to determine whether a less severe infection, initiated by administering relatively small amounts of cells of *S. anatum*, would invoke a different type of response

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from that observed when chicks were given a larger amount of *Salmonella* cells.

Treatments. Three groups of newly hatched broiler-type chicks were used in both studies. In the first trial involving a total of 357 chicks, each chick in one group was inoculated in the yolk sac with 1 ml of a suspension containing approximately 3 million cells of *S. anatum*. Chicks in the second group were similarly inoculated with *S. heidelberg*, and a third group served as uninoculated controls.

In the second study involving a total of 141 chicks, one group was inoculated with approximately 30,000 cells of *S. anatum*, the second group with approximately 300 cells of *S. anatum*, and the third served as an uninoculated control group.

In both studies, chicks were kept in chick boxes at 31 C without access to feed and water for the 72-hr duration of each trial. All chicks given the same treatment were kept together in one box. Mortality records were kept in all trials, and the approximate time of death of each chick was recorded.

The cultures of *Salmonella* used in these studies were originally isolated from shells of eggs laid by healthy breeder flocks and were maintained in the lyophilized state until shortly before their use. The inocula were prepared by washing cells from 48-hr tryptose agar cultures with sterile 0.1% proteose peptone no. 3 solution. The suspensions were further diluted in the sterile proteose peptone solution to a reading of 55% light transmittance at 525 nm in a spectrophotometer (Bausch and Lomb model 340). This suspension, found by plate counts to contain approximately 300 million cells of *Salmonella* per ml, was diluted 1:100 to provide about 3 million cells per ml. A 2-ml syringe fitted with a 25-gauge needle was used to inject 1 ml of the suspension, through the navel, into the yolk sac of each chick in the inoculated groups. The navel area was first cleaned with 70% ethanol. All chicks were less than 24-hr old when the trial was initiated.

Hematology. Blood was obtained for the differential leukocyte counts by heart puncture (4). Differential leukocyte counts were made on four chicks from each of the three groups at 0, 24, 48, and 72 hr after inoculation in the first series of trials, except in a few instances where mortality reduced the number of chicks in a group so that only two or three were available for sampling. Only one blood sample was taken from each chick, since it was thought that blood loss resulting from the heart puncture might affect later differential counts.

After withdrawal of blood from a chick, several drops were discarded from the syringe and then one drop was placed on the end of a clean glass slide for preparation of the blood smear. The blood films were stained with Wright's stain for 5 min; a buffer (*p*H 6.4) was then added for an additional 5 min. The counts were subsequently made under oil immersion at a magnification of 1,000 diameters. Preliminary studies indicated that six classes of leukocytes would be sufficient to measure changes occurring in the blood picture of the baby chicks. The identification of the lymphocytes, monocytes, heterophils, eosinophils, and basophils was based on the descriptions given by Lucas and Jamroz (3). The cells classified as juveniles were leukocytes that resembled heterophils, but they were grossly different in appearance from heterophils found in the uninoculated chicks. The most obvious difference between the heterophils and the juveniles was that the juveniles were either void of or deficient in the eosinophilic staining rods seen in normal heterophils (3). Cells that contained at least half the normal complement of rods were classified as heterophils. Another morphological characteristic of the leukocytes classified as juveniles was the rodlike, dumbbell, pince-nez appearance (6) of the nuclei. A few of the leukocytes placed in the juvenile class were obviously immature granulocytes, but the majority were degenerate and atypical heterophils and were seen only in the blood of chicks inoculated with *Salmonella*.

In the second study, differential counts were made on only two chicks from each treatment group at each time interval. Two hundred leukocytes from each chick were classified, and the average of the counts from two birds in each treatment group was taken as the value for a given time interval. Other procedures were identical to those used in the first study.

Pertinent data were analyzed by analysis of variance according to Snedecor (7).

RESULTS AND DISCUSSION

The percentages of heterophils, juveniles, and lymphocytes in young chicks were significantly altered by infection of either *S. anatum* or *S. heidelberg*. During the 72-hr period immediately after inoculation of the chicks with approximately 3 million cells of either species of *Salmonella*, the lymphocytes and juveniles increased in number, whereas the heterophils decreased. Percentages of eosinophils, basophils, and monocytes were altered by infection with *Salmonella*, but not as markedly as were the other three classes of leukocytes. The number of eosinophils varied greatly between individual chicks and in relation to uninoculated chicks, whereas the percentages of basophils and monocytes remained about the same or increased slightly in relation to

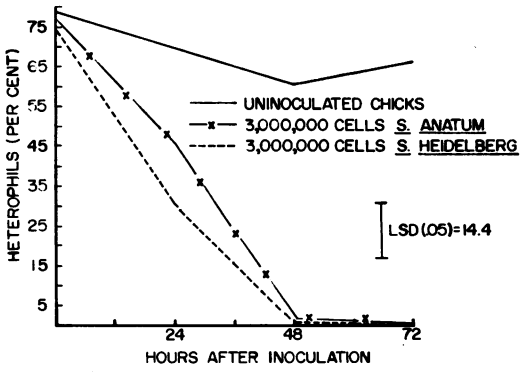

FIG. 1. *Effect of Salmonella infections on concentration of heterophils in blood of broiler-type chicks.*

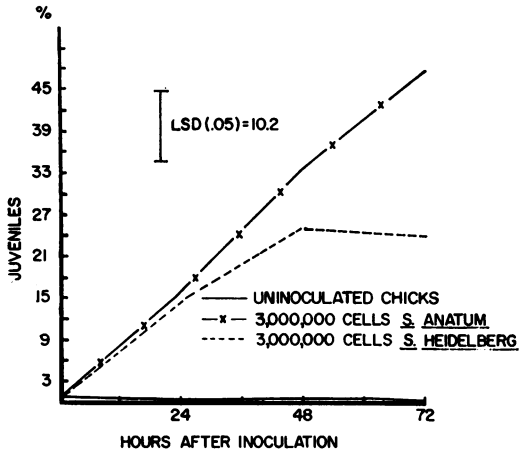

FIG. 2. *Effect of Salmonella infection on concentration of juveniles in blood of broiler-type chicks.*

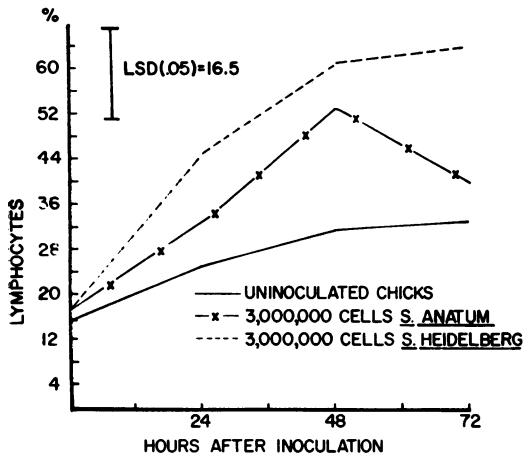

FIG. 3. *Effect of Salmonella infections on concentration of lymphocytes in blood of broiler-type chicks.*

the uninoculated chicks during the 72-hr period. The differential leukocyte counts for uninoculated chicks in these studies compare favorably with figures reported by Kelly and Dearstyne (2) and Burmester, Severens, and Roberts (1).

At 48 hr after inoculation of the chicks with 3 million cells of either *S. anatum* or *S. heidelberg*, a severe heterophilic leukopenia was detected (Fig. 1). A 25% decrease in the number of heterophils in the blood of these chicks was observed as early as 24 hr after inoculation. In uninoculated chicks, the percentage of heterophils never dropped below 60% of the total leukocyte count. Many of the heterophils from birds inoculated with *Salmonella* differed from heterophils found in uninoculated chicks. The eosinophilic-staining rods were often swollen, deficient in number, and indistinct. These cells, however, were classified as heterophils instead of juveniles on the basis stated above, i.e., they contained at least half the normal complement of rods (3).

The juveniles made up approximately 1% of the leukocytes in the uninoculated chicks throughout the studies; however, in the inoculated chicks, the percentage of juveniles increased to approximately 24% at 72 hr postinoculation in chicks inoculated with 3 million cells of *S. heidelberg* and to about 48% in those inoculated with 3 million cells of *S. anatum* (Fig. 2). At 72 hr postinoculation, the percentage of juveniles in the blood of chicks inoculated with *S. anatum* was significantly higher ($P < 0.05$) than that of the blood from chicks given 3 million cells of *S. heidelberg* (Fig. 2). The plateauing of the juvenile count after 48 hr in chicks inoculated with *S. heidelberg* was apparently because of the extreme

TABLE 1. *Mortality after inoculation of day-old chicks with Salmonella species*

Time after inoculation (hr)	Averages of trials 1 to 4[a]		Averages of trials 5 and 6[a]	
	S. anatum (3,000,000 cells)	*S. heildelberg* (3,000,000 cells)	*S. anatum* (30,000 cells)	*S. anatum* (300 cells)
48	4	42	2	2
72	20	24	19	14
Total[b]	24/115	66/150	21/55	16/46
Per cent	20.86	44.0	38.18	34.78

[a] No deaths occurred in the uninfected chicks.
[b] Denominator indicates total number of chicks inoculated; numerator indicates number that died.

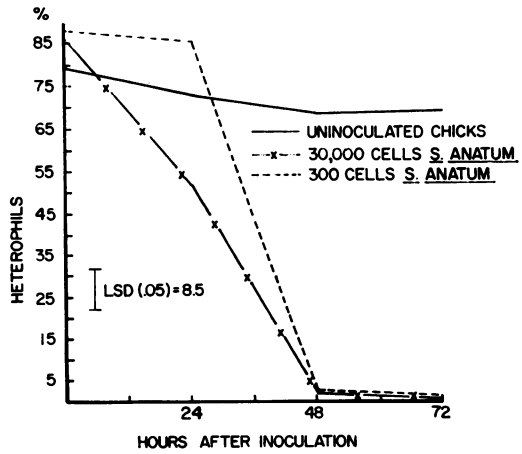

FIG. 4. *Effect of Salmonella anatum infection on concentration of heterophils in blood of broiler-type chicks.*

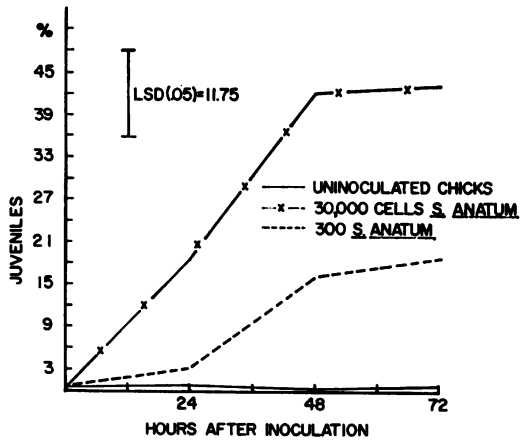

FIG. 5. *Effect of Salmonella anatum on concentration of juveniles in blood of broiler-type chicks.*

pathogenicity of this species, resulting in depression of the hematopoietic system. At 72 hr postinoculation, the chicks given *S. heidelberg* were severely leukopenic; thus, the differential counts may not have been very accurate.

The percentage of lymphocytes in chicks inoculated with 3 million cells of either *S. anatum* or *S. heidelberg* was significantly greater than in uninoculated chicks (Fig. 3). Chicks inoculated with *S. heidelberg* suffered the earliest and greatest mortality during the study (Table 1).

Since differences in differential blood counts of chicks inoculated with *S. heidelberg* and *S. anatum* were not great, attempts were made to determine whether milder infections of *Salmonella* would result in a different type of hematological response in newly hatched chicks. The results of this study, in which chicks were inoculated with either 0, 300, or 30,000 cells of *S. anatum*, are illustrated in Fig. 4–6. Inoculation of chicks with 30,000 cells of *S. anatum* had essentially the same effect on the heterophil counts as inoculation with 3 million cells (Fig. 1, 4). The percentage of heterophils in chicks inoculated with only 300 cells of *S. anatum* differed little from the percentage in the uninoculated chicks at 24 hr postinoculation; however, by 48 hr postinoculation, the percentage of heterophils in chicks inoculated with either 30,000 or 300 cells of *S. anatum* was about the same as that in chicks given 3 million *S. heidelberg* cells (Fig. 1, 4).

The percentage of juveniles in chicks inoculated with 300 cells of *S. anatum* was intermediate between the percentage found in the chicks inoculated with 30,000 cells and that in the uninoculated chicks (Fig. 5). A significant increase in the percentage of juvenile cells was not observed in chicks inoculated with 300 cells of *S. anatum* until 48 hr postinoculation.

The percentage of lymphocytes in chicks inoculated with either 300 or 30,000 cells of *S. anatum* significantly ($P < 0.05$) exceeded that in unioculated chicks at 48 and 72 but not at 24 hr postinoculation (Fig. 6).

Mortality was not reduced by decreasing the number of *S. anatum* cells in the inoculum (Table 1). Much of the mortality occurring during the final 24 hr of each study and some changes in differential leukocyte counts may have resulted from deprivation of feed and water and are not an accurate indication of the pathogenicity of the *Salmonella*.

The results of this study do not provide a clear-cut basis for relating the pathogenicity of *Salmonella* species to changes in differential leukocyte counts; however, basic information illustrating the effects of *Salmonella* infections on the differential leukocyte count is provided. It is possible that the percentages of heterophils and juveniles may be related to the number of *Salmonella* cells in the birds' bodies, since Stephens and Anderson (8) have shown that the number of cells of *Salmonella* injected into the yolk sac has little effect on the total number of cells present in the yolk sac at 48 hr postinoculation.

Our results indicate that heterophils are either rapidly destroyed in chicks infected with *Salmonella* or do not fully develop. Additional studies are needed to determine whether heterophils are destroyed in an inflammatory reaction by the tissues of chicks infected with *Salmonella*.

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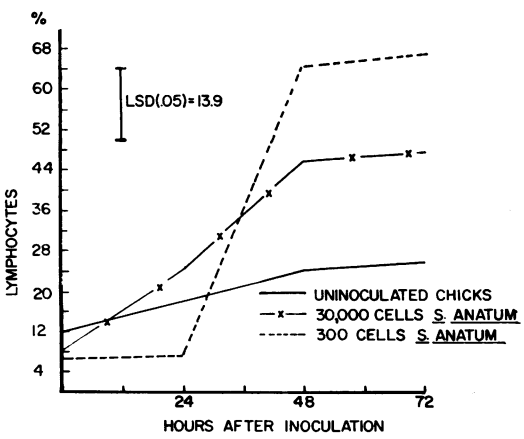

FIG. 6. *Effect of Salmonella anatum on concentration of lymphocytes in blood of broiler-type chicks.*

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