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The Effects of Temperature and pH Upon the Exogenous Development of *Eimeria tenella* Railliet and Lucet, 1891, and *Eimeria acervulina* Tyzzer, 1929, Coccidian Parasites of the Chicken

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The oocysts of the Coccidia are known to be fairly resistant to chemical disinfectants in general, but relatively susceptible to heat (Chang, 1937). The following investigation was carried out in an attempt to evaluate the combined effects of temperature and pH upon the exogenous development of oocysts of *Eimeria tenella* and *Eimeria acervulina*.

Materials and Methods

The unsporulated oocysts of *E. tenella* and *E. acervulina* were obtained from fresh feces or cecal contents of infected chickens using the zinc sulfate flotation method (Farr, 1941) to concentrate them. Small glass vials containing 2 ml. of either distilled water or buffered solutions of a known pH, measured electrometrically, were suspended in a water bath which maintained a specific temperature within $\pm 0.2^\circ$ C. Sufficient time was allowed to elapse for the solutions in the vials to come to the same temperature as that of the water bath. The unsporulated oocysts, concentrated by flotation, were transferred by means of a wire loop from the surface of the concentrating agent to one of the vials in the water bath. At this time a stopwatch was started. The remaining vials were inoculated similarly, with the time of inoculation being recorded by observing the running watch. Thus, by removing all the vials at the same time, each container had been exposed to the same temperature for successively shorter periods. Immediately upon removal from the water bath, the vials were plunged into ice water for a short time to insure rapid cooling and to prevent continued exposure to the oocysts in the vials. These vials were then plugged with cotton and set aside for two days at room temperature. After this interval, the contents of each vial were again concentrated in a zinc sulfate solution and floated oocysts transferred to a glass slide by means of a wire loop. The oocysts were then examined microscopically. The criterion of the effects of heat was occurrence or failure of sporulation, as well as abnormalities of sporulation. The mortality

(percent kill) was calculated by the formula $\frac{(x-y)\ 100}{x}$ where x= the number of oocysts sporulated in the control and y=the number of oocysts sporulated in the exposed vial.

Concerning percent kill, it was suggested by Reinhardt and Becker (1933) and reiterated by Chang (1937) that the half-kill or three-quarter kill is a more accurate criterion than total kill for time and temperature effects. In this study the half-kill was used.

The data were plotted graphically so that the mortality was plotted against time of exposure. From the points obtained, eye-fitted lines were used in estimating the exposure times for the half-kill.

Results

The effects of temperature upon oocysts of *E. tenella* were as follows:

At a pH of 6.8, the means of the estimated exposures for half-kill at 52° C., 50° C. and 48° C. were 2.4, 15.1 and 66.6 min., respectively. The 48° C. series was repeated five more times, because the time indicated by Chang (1937) at 48° C. was 83 minutes. It was found that the estimated exposure times observed for half-kill ranged between 53 and 74 minutes. At temperatures of 52° C. and 50° C., however, the estimated exposures for half-kill corresponded within several seconds to those indicated by Chang.

At a pH of 2.2, the means of the estimated exposures for half-kill at 52° C., 50°C. and 48° C. were 1.9, 12.1 and 60 min., respectively.

At a pH of 8.4, the means of the estimated exposures for half-kill at 52° C., 50° C. and 48° C. were less than 1 min., 11.3 min. and 52.1 min., respectively.

The effects of temperature upon oocysts of *E. acervulina* were as follows:

At a pH of 6.8, the means of the estimated exposures for half-kill at 52° C., 50° C. and 48° C. were 3.3, 21.9 and 37.3 min., respectively.

At a pH of 2.2, the means of the estimated exposures for half-kill at 52° C., 50° C. and 48° C. were 3.2, 11.4 and 17.8 min., respectively.

At a pH of 8.4, the means of the estimated exposures for half-kill at 52° C., 50° C. and 48° C. were 2.2, 10 and 18.3 min., respectively.

Discussion

The results indicate that at lower temperatures, pH of the aqueous medium in which the oocysts are suspended must be considered as a

function of temperature in producing mortality. Oocysts in a solution at a pH of 6.8 were more resistant to the effects of temperature than those exposed in the buffered solutions. At higher temperatures, the functions of pH appeared to be masked by the action of

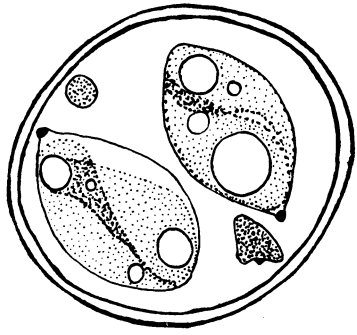

FIG. 1

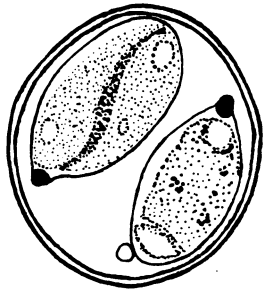

FIG. 2

10 MICRONS

Camera-lucida illustrations of *Eimeria acervulina* oocysts having only two sporocysts.

Figure 1. A sporulated oocyst of *E. acervulina* having only two sporocysts, each having two sporozoites. Observed in a culture exposed to 48° C. for 30 minutes.

Figure 2. A sporulated oocyst of *E. acervulina* having only two sporocysts, one with two sporozoites, the other undeveloped. Observed in a culture exposed to 48° C. for 70 minutes.

heat alone, since the susceptibilities of oocysts at all three pH's were somewhat similar.

From the results of the 48° C. series, it appears as if *E. tenella* is the more resistant species. At 52° C., however, oocysts of *E. acervulina* are apparently more resistant. Field studies have shown oocysts of *E. acervulina,* exposed in both shade and sunlight, survived longer than oocysts of *E. tenella* (Koutz, 1950). As pointed out previously, variations were noted in the estimated exposures for half-kill of oocysts of *E. tenella* during the 48° C. series. Such variations may account for the apparently greater resistance exhibited by *E. tenella* at this temperature.

Of special interest during this investigation was the observance of sporulated oocysts that differed from the normal with four sporocysts in that they had only two or three sporocysts. Fully developed sporozoites were observed in a number of these abnormal forms, in which case each spore had two. (Figs. 1 and 2.)

The abnormal oocysts were rarely seen in *E. tenella* cultures, but were more common in *E. acervulina* cultures, ranging between one and six percent. In fact, these forms could be produced fairly easily by exposing unsporulated oocysts of *E. acervulina* to a temperature of 48° C. They were noted in cultures exposed to this temperature for as long as 70 minutes, but the optimum length of exposure at this temperature for their production appeared to be between 30 and 40 minutes. To determine their viability, several of the two spore forms were isolated using the method described by Becker (1934). These were fed to uninfected chickens and were considered non-viable when oocysts were not recovered from these birds.

E. acervulina cultures observed to contain these two and three spore forms were fed to uninfected chickens. Some of the unsporulated oocysts recovered from these birds were allowed to sporulate normally and then examined for the presence of abnormal oocysts, with negative results. The remaining unsporulated oocysts from the same birds were exposed to a temperature of 48° C. for 30 minutes. After two days, these were examined and the presence of two and three spore forms was noted, but not in greater numbers than previously recorded.

The size of these abnormal oocysts did not deviate from the normal to any great extent. The individual sporocysts in the two spore forms, however, were definitely larger than those of normal oocysts. The former were found to average 13.6μ x 8.9μ compared to 10.9μ x 6.0μ for the latter.

SUMMARY

1. Unsporulated oocysts of *E. tenella* and *E. acervulina* suspended in solutions having a pH of either 2.2, 6.8 or 8.4 were exposed to temperatures of 52° C., 50° C. and 48° C.

2. It was found that at lower temperatures, the pH of the aqueous medium in which the oocysts are suspended must be considered as a function of temperature in producing mortality.

3. It appears that oocysts of *E. tenella* are more resistant than those of *E. acervulina* at 48° C., but oocysts of *E. acervulina* are more resistant at 52° C.

4. During the course of this investigation sporulated oocysts that differed from the normal of four sporocysts were observed. These abnormal forms were seen to have two or three sporocysts. Fully developed sporozoites were observed in a number of these abnormal forms, in which case each had two.

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