THE EFFECTS OF SOME CHEMICAL AND PHYSICAL AGENTS ON THE OOCYSTS OF *ISOSPORA LACAZEI* LABBÉ (PROTOZOA:COCCIDIA)

數種理化因子對等孢球蟲囊孢時期的影響

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中文摘要

等孢球蟲 (*Isospora lacazei* Labbé) 的囊孢時期 (oocyst) 隨寄主糞便排出後，如果環境適宜，內部即分裂而產生兩個孢子 (spore)，這一過程叫做孢子形成 (sporulation)，這時爲其感染時期，本研究以數種理化因子處理等孢球蟲(*Isospora lacazei*)隨寄主糞便排出之囊孢 (oocyst)，觀察此等因子是否能阻遏其孢子形成 (sporulation)，甚或殺死囊孢 (oocyst)，藉以設法減少此種球蟲的感染。

研究結果；等孢球蟲 (*Isospora lacazei*) 的囊孢，對 10 %和 15 %氯化鈉溶液 (sodium chloride solution)，1 %和 2 %福馬林 (formalin) 以及氫游子 (pH 1 或 13)均具抵抗力；但水、稀濃度氯化鈉溶液 (0.85 %) 以及較濃的福馬林 (5% formalin) 均可阻遏其孢子形成 (sporulation)；5°C 以下的低溫，40°C 以上的高溫以及乾燥均可殺死囊孢；氧氣缺少時，可阻遏孢子形成，但不能殺死囊孢，故當氧氣恢復時，內部又迅速形成孢子。

ABSTRACT

The effects of sodium chloride solution, formalin, pH, moderately low and high temperatures, drying and lack of oxygen on the viability and ability of

Isospora lacazzi oocyst to sporulate were investigated. It was found that the oocysts from the bengalee (*Uroloncha domestica*) are considerably resistant to various concentrations of sodium chloride solution, but the very dilute solution of sodium chloride (0.85%) and water are poor media for oocyst sporulation. Oocyst sporulation occurs normally in both 1% and 2% formalin, but is prevented by 5% formalin. As shown by tests in extreme pH values of 1 and 13, it appears that hydrogen ion has no effect on the oocyst sporulation. Temperatures as low as 4° to 5°C and as high as 40°C are lethal to the oocysts. The oocysts are severely distorted by drying. Lack of oxygen inhibits the oocyst sporulation temporarily, upon airing, oocysts sporulate rapidly again.

INTRODUCTION

The coccidia are protozoa belonging to subphylum Sporozoa, class Telosporea, subclass Coccidia, order Eucoccidia, and suborder Eimeriina. Members of this suborder have been found in various vertebrates and invertebrates, and their presence are usually the cause of the disease known as coccidiosis of the hosts.

The best known stage of the coccidia life history is the resistant cyst, the oocyst. The oocysts are passed out in the host feces. At this time they contain a single cell known as the sporant (Fig. 1). In the presence of oxygen outside of the host body, the oocysts develope to the infective stage (Fig. 2) by a process known as sporulation.

Isospora lacazei Labbé is commonly present in sparrows, finches, blackbirds, crows and other passerine birds. The present study of the effects of various agents on the oocysts of *Isospora lacazei* is aimed at the determination of (1) which agent will prevent oocyst sporulation and (2) which one will kill the oocysts. In either case a method will be suggested to prevent oocyst from infecting, only in case (1), sporulaton will occur if the agent is removed.

A number of investigations of the resistance of oocysts have been done dealing with the coccidian parasites of poultry (Beach and Corl, 1925; Herrick, 1925; Fish, 1931; Chang, 1937; Ellis, 1938; Becker and Crouch, 1939; Brotherston, 1948; and Edgar, 1954). The resistance of pigeon coccidium (*Eimeria labbeana*) and bovine coccidium (*Eimreia zurnii*) have been worked out by Duncan (1959) and Marquardt et al (1960) respectively. But most of the other

coccidia, including *Isospora lacazei*, have been neglected.

MATERIALS AND METHODS

Feces from the bengalee (*Uroloncha domestica*) infected with *Isospora lacazei* were collected and transferred to petri dishes where they were spread out in a test or contol solution which is of sufficient quantity to cover them.

A solution of 2% $K_2Cr_2O_7$ is usually used as sporulation medium for coccidian oocysts. The oocysts immersed in this solution at a room temperature from 20° to 26°C was utilized as control in the pressnt investigation. Within this room temperature range (20° to 26°C) all tests of oocyst resistance except those dealing with the effect of temperatures were carried out. Test substances used included distilled water, sodium chloride, hydrochloric acid, sodium hydroxide and formalin.

In test for the effects of moderately high and low temperatures, feces in 2% $K_2Cr_2O_7$ solution were placed in refrigerstors or ovens for varying length of time. The resistance to disiccation was tested by placing fresh feces containing oocysts on filter paper in a room where relative humidity was not higher than 40%.

Feces in 2% $K_2Cr_2O_7$ solution were placed in a jar. The air from the jar is evacuated to a tension of 13% of the normal by a vacuum pump, and subsquently replaced by nitrogen gas.

The viability of unsporulated forms was tested by returning the oocysts to control condition for as long as 10 days and counted the percentage of sporulation.

For determinaton of the percentage of sporulation, one hundred oocyst were counted at a magnification of 400X. A fully sporulated oocyst (Fig. 2) possesses two spores which in turn contains four sporozoites.

RESULTS

(a) Control condition 100% sporulation was never seen either in control or test media or at various temperaturs. Charkarvarty and Kar (1946) reported that all of the *Eimeria labbaena* oocysts in 2.5% $K_2Cr_2O_7$ solution at 22.2°C were sporulated. In the present study, the highest sporulation observed was 90% with an averag of 80%.

(b) Distilled water Distilled water was proved to be a poor medium for

oocyst sporulation as have been pointed out for a number of other coccidia. In the present study about 40% oocysts sporulated.

(c) Sodium chloride Sporulation of oocysts proceeded rapidly in 10% and 15% NaCl solution, but was slightly reduced in 20% NaCl solution. Although 0.85% NaCl solution is commonly used as physiological saline solution for birds, it was proved to be a poor medium for oocyst sporulation, in which only 30% oocysts sporulated.

(d) Hydrogen ion concentration Sporulation occurred normally in both 0.1 N HCl and 0.1 N NaOH solution with a pH approximately 1 and 13 respectively. It is evident that the hydrogen ion had no effect on oocyst sporulation.

(e) Formalin Sporulation of oocysts was slightly reduced in both 1% and 2% formalin, but it was almost inhibited by 5% formalin in which only 10% oocysts were sporulated.

(f) Temperature

(1) Low temperature Oocysts did not sporulate at 4° to 5°C and at −4° to −5°C. Upon return to room temperature after 30 days subjected to a temperature of 4° to 5°C, only 4% oocysts sporulated. None of the oocysts that had been kept at −4° to −5°C for 30 days sporulated upon return to room temperature.

(2) High temperature Sporulation of oocysts was tested at 30°, 35°, and 40°C respectively. Sporulation was slightly reduced at both 30° and 35°C, but no sporulation occurred at 40°C. Oocysts did not sporulate when they were placed under room temperature after subjecting to a temperature of 40°C for 3 days.

(g) Disiccation The ooccysts were severely distorted by drying, it was impossible to determine whether they sporulated or not.

(h) Oxygen In the present investigation, oocysts were kept in a jar with the air evacuated to a tension of 13% of the normal for 4 days. Sporulation took place but was greatly reduced, the sporulation percentage was 13%. After returning to normal air condition, these samples sporulated rapidly. The sporulation percentage was as high as 70%.

DISCUSSION

The percentage of sporulation of *Isospora lacazei* oocysts in water at room temperature was small. Most likely the bacteria in the water prevent sporulation,

since the addition of a bactericidal agent such as potassium dichromate to water containing oocysts results in sporulation commencing immediately.

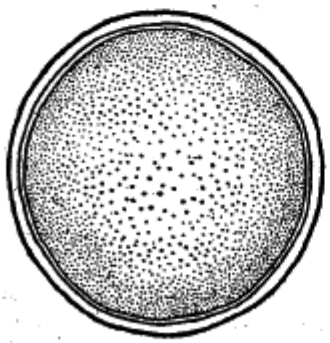
In the present study, the oocysts were found to be resistant to the effect of sodium chloride, it is similar to that observed by Duncan (1959) with the oocysts of *Eimeria labbeana*. Since water is a poor medium for oocyst sporulation that the oocysts treated with dilute sodium chloride solution (0.85%) could not sporulate well.

Hydrogen ion was found to be harmless to the oocysts sporulation, this agrees on the observation by Marquardt (1960) with *Eimeria zurnii*.

Sporulation occurred normally in both 1% and 2% formalin, but was reduced greatly in 5% formalin. Perhaps the high concentration of formalin killed the oocysts.

The observation that the oocysts of *Isospora lacazei* did not sporulate at moderately low temperature agrees on what is known of many species of coccidia. In the present investigation, it was found that no sporulation occurred after returned to room temperature following their exposure to a temperature of 4° to 5°C for 30 days. This differed from that was observed by Duncan (1959) with *Eimeria labbaena*. He reported that sporulation occurred upon return to room temperature after the oocysts have been treated with 0° to 2.5°C for 30 days. From this result it appears that the oocysts of *Isospora lacazei* may be less resistant to cold than *Eimeria labbaena*.

Since no sporulation occurred at 40°C, it may be assumed that the high body temperature of the host pervents oocyst sporulation within the host's intestine.



1.

2.

Fig. 1. Unsporulated oocyst of *Isospora lacazei* Labbé from bengalee.

Fig. 2. Sporulated oocyst of *Isospora lacazei* Labbé from bengalee which contains two spores.

The oocysts were found to be badly distorted after treated with drying. This is the same as that reported by many authors (Duncan, 1959; Ellis, 1938; and Farre, 1949), according to their conclusions that drying is an important factor in killing oocysts.

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