Effect of sulphuric acid and formalin on sporulation of *Eimeria bovis* oocysts

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Abstract:

Eimeria bovis oocysts were isolated and identified after obtained the fecal sample from infected calf.

Three different concentrations of sulphuric acid and of formalin were examining to observe their effect on *E.bovis* oocysts sporulation in addition to 2.5% potassium dichromate as a control.

All different concentrations of both chemical solutions (sulphuric acid and formalin) were induced the sporulation of oocyst in addition to the control group (potassium dichromate).

تأثير حامض الكبريتيك والفورمالين على تبوغ أكياس البيضة للـ *Eimeria bovis*

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الخلاصة:

تم عزل وتحديد أكياس البيضة لطفيلي ***Eimeria bovis*** بعد الحصول عليها من عينة براز لعجل مصاب.

درست ثلاثة تركيز مختلفة (M1 ، M3، M5) من حمض الكبريتيك و (1 % ، 3 % ، 5 %) من الفورمالين لملاحظة تأثيرها على تبوغ أكياس البيضة للـ *E. bovis* بالإضافة إلى 2.5 % ثاني كرومات البوتاسيوم المستخدم كمجموعة سيطرة.

وقد حثت جميع التركيز المختلفة لكلا المحلولين الكيميائيين (حمض الكبريتيك والفورمالين) على تبوغ أكياس البيضة بالإضافة إلى مجموعة السيطرة (ثاني كرومات البوتاسيوم).

Introduction:

Nearly most cattle are infected with coccidia, but only a limited number suffer from coccidiosis. The disease occurs mainly in young animals, indicating that immunity may play a role in the protection of older animals. Occasionally, it may occur in calves over 6 months of age or even in adult cattle (1).

Coccidiosis is particularly a problem of confined animals. Intensive animal husbandry practices have increased the problem of coccidiosis. Many cattle are subclinically infected, resulting in considerable economic losses. Subclinically infected animals appear normal outwardly, but suffer

from reduced feed consumption, feed conversion and growth performance. The severity of the infection is related to the number of ingested oocysts. Economic losses from coccidiosis due to mortality, poor performance, cost of treatment and prevention may be considerable, especially in stud farms and calf rearing systems. Coccidiosis costs cattle ranchers more than400 $ million annually in lost profit due to reduced feed efficiency, slower weight gain and increased susceptibility to other diseases. This can set back calves' growth by as much as 2 months (2).

For experimental infection, the oocysts are usually sporulated by treating with 2-3% potassium dichromate at room temperature during two weeks until at least 95% of oocyst have sporulated (3, 4). Sulphuric acid and formalin were used in the embryonated of *Ascaris* spp. Eggs (5, 6). Potassium dichromate is thought to promote the sporulation of oocyst but the sulphuric acid or formalin not used until now.

Therefore, the aim of this study was to prove the effect of sulphuric acid and formalin on the *E. bovis* oocysts sporulation *in vitro*.

Materials and methods:

The diagnosis of *E.bovis* was confirmed by several parameters including long and wide measurements, shape, colour, present or non present of micropyle and polar cap and sporulation time in 2.5% potassium dichromate ($K_2Cr_2O_7$) **(**7).

Oocysts were isolated and identified by the sugar (Sheather's solution) flotation method (8).

Briefly, fresh fecal sample that collected directly from animal's rectum was suspended with distill water at 20g / 20ml (W/V). The fecal suspension was filtered through 60 pore / inch stainless steel strainer to remove the debris. Aliquots of about 10ml of suspension were centrifuged at 3500rpm (1250xg) for five minutes. The fecal pellet of one aliquot was used for identification of oocysts and confirming to the infection throughout re-suspended it in test tube with about 10ml of sheather's solution (500g sucrose + 320ml distilled water + 6.5g phenol) and centrifuged at 2500rpm (700xg) for 2 min., then drops of sheather's solution were added to the tube until reached to the border and covered with cover slide, wait for 2 min. then removed and put on slide and examined **(**7, 9).

After sure that infection was present, the rest aliquots of the same sample were suspended in equal amounts of formalin (1%, 3% and 5%), sulphuric acid (1M, 3M and 5M) and potassium dichromate 2.5% as a control. Each sample was divided into 3 divisions, these were pour into a 32milimeter, 40 ml Putrid dishes and incubated at a depth of 15 mm in 29°C, in a dark incubator and every 24 hrs all Petri dishes were exit and ventilated with manual shaking for 15 min. and

returned to the incubator. The oocyst development was monitored continuously under light microscope. Sporulation was defined as oocyst growth to the four sporocysts stage (9).

Results and Discussion:

The *Eimeria* oocysts which treated with three different concentrations 1%, 3% and 5% of formalin and 1M, 3M, and 5M of sulphuric acid in addition to 2.5% potassium dichromate as a control, were examined every 24 hrs to observed the sporulation occur.

The results of this study was Showed that the *E. bovis* oocysts sporulation was occur at the same time(48hrs after incubation) in all concentrations of both chemicals solutions (formalin and sulphuric acid) in addition to the control (Table 1 and 2; Figure 1 and 2).

Table 1: Effect of sulphuric acid on sporulation of *E.bovis* oocyst.

Chemical solution	Concentration(M)	Sporulation time (hr)
H2SO4	1	48
	3	48
	5	48
K2Cr2O7	2.5	48

Table 2: Effect of formalin on sporulation of *E.bovis* oocyst.

Chemical solution	Concentration (%)	Sporulation time (hr)
Formalin	1	48
	3	48
	5	48
K2Cr2O7	2.5	48

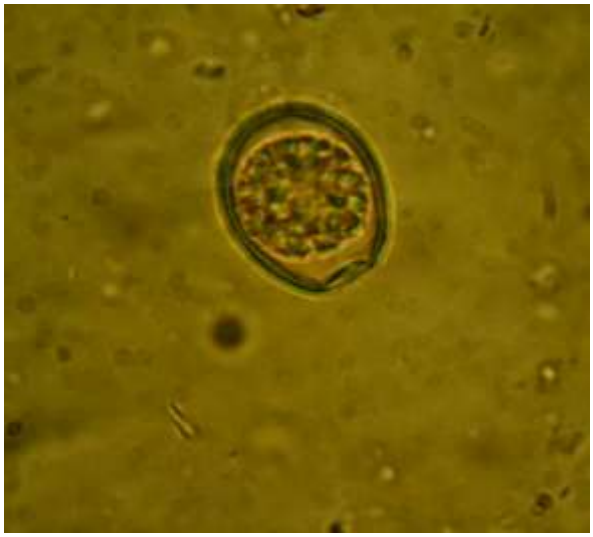

Figure1:Unsporulated E.bovis oocyst Before treated (100X)

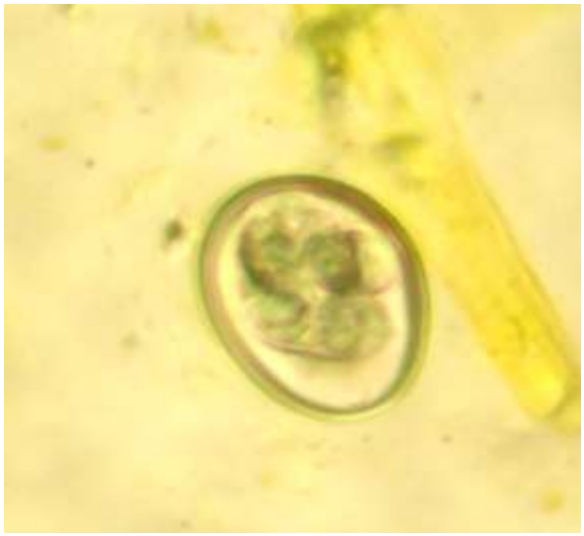

Figure 2: SporulatedE.bovis oocyst after treated (100X)

In the last study (9) referred to that the stronger catalytic of potassium dichromate in the sporulation of the *Eimeria* oocysts, might be due to the production of hydroxyle radicals inducing the sporulation process. Therefore, the sulphuric acid and formalin may play the same role of potassium dichromate in production of radicals that which reflex on the sporulation.

The metallic ions are known to play important role in the metabolic activities of many microorganisms, such as the phosphorylation response of regulated protein in *Bacillus subtillis* (10).

To date, the mechanism of the sporulation of *Eimeria* oocyst is still unknown. (9) speculated that oxygen tention in the incubation fluid might be an important factor in *Eimeria* oocyst sporulation when they observed that sporulation occurred earlier among oocyst shaken at 200rpm than in those that were not subjected to shaking. Shaking the culture raises the oxygen concentration in the culture fluid.

Copper ion like ferrous ion, has been shown to generate oxygen radicals (11). Thus, the ions in the sulphuric acid or formalin might have a similar effect as the generation of oxygen radicals in promoting sporulation.

Most last studies were used the potassium dichromate in the oocysts (*Eimeria*) sporulation (9, 2) and formalin or sulphuric acid in eggs (*Ascaris*) maturation (5, 6, 12), while the current study is the first once that used the formalin and sulphuric acid in oocysts (*Eimeria*) sporulation.

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