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Factors Affecting The Survival and Sporulation of *Eimeria* oocysts of Cattle

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Abstract: A significant effect of temperature, oxygen and salinity on survival and speed of sporulation of different *Eimeria* spp. oocysts was observed while insignificant effect of pH and centrifugation on *Eimeria* oocysts of cattle was recorded. Furthermore, a significant effect of subzero temperature for different periods on various *Eimeria* oocysts was also investigated.

Key words: Factors, Affecting, Sporulation, *Eimeria* , Cattle

Introduction

Climatic factors, such as temperature, moisture and oxygen tension can greatly affect the viability and longevity of sporulated and unsporulated oocysts, as well as the sporulation process itself. Unsporulated oocysts are seen to be less able to survive extreme changes in climatic factors compared with sporulated ones (Horton-Smith and Long, 1954).

Christensen (1939) reported that the presence of oxygen is a very important factor for survival and sporulation of many *Eimeria* species. Furthermore, he commented that without oxygen not a single species would survive and this view was confirmed by Marquardt *et al.* (1960) in their experimental work on *E.zuernii* oocysts in which they reported that at least 10% oxygen was necessary for survival of the oocysts. Freezing temperatures are lethal to many *Eimeria* species. Marquardt *et al.* (1960) observed 37 and 5% survival of species of *E.zuernii* oocysts at -30°C for 6 and 24 hours respectively. On the other hand, Hagan (1958) observed rapid death in *Eimeria* species at or below -10°C. *Eimeria* species (*E. zuernii*) normally sporulate at 13 to 32°C. Below 13°C they require a long time before sporulation occurs (Marquardt *et al.*, 1960).

Nomi (1926) reported that 89 and 100% of *E.zuernii* oocysts sporulate in 35 and 40 days at 11-15°C respectively. Wilson and Morley (1933) observed that the process of sporoblast formation took place within 24 hours at 20-25°C and complete sporulation occurred in 35 and 100% of the oocysts after 48 and 72 hours respectively. Wilson (1930) did not find any effect of centrifugation on sporulation and survival of oocysts at 1000rpm for 10 minutes. Also Marquardt *et al.* (1960) failed to find any effect of centrifugation even as high as 6000xG on presporulated and 14000 X G once sporulation had commenced in *E.zuernii* oocysts.

Marquardt *et al.* (1960) observed that unsporulated oocysts could not survive at low humidity. They recorded 2-12, 10-32 and 21-56 percent sporulation at 25, 50 and 75% humidity respectively. Whereas Duncan (1959) reported that oocysts of *E.labbeana* (from pigeon) which were allowed to dry at room temperature for 3 days, retained their infectivity even though they were badly distorted.

The present study was therefore, planned to investigate the effects of different factors on the survival and sporulation of oocysts of bovine *Eimeria* species.

Materials and Methods

All tests were carried-out on oocysts separated by the McMaster and Clayton Lane methods (MAFF, 1986) from freshly collected (*per rectum*) faeces. The faceal suspension was prepared and then strained through a 0.15mm aperature nylon mesh to form a shallow layer in Petri dish. The oocysts of mixed species were then allowed to sporulate at 30°C for 3-4 days. The criterion of viability was the ability of oocysts to sporulate normally following treatment. The sporulation percentages given, represent the results obtained from the examination of 100 oocysts.

Sporulation of *Eimeria* oocysts at different temperatures: Faecal suspensions obtained from the McMaster technique were pooled and mixed with an equal quantity of 2% potassium dichromate and 2ml of this solution were added to a Petri dish. Forty Petri dishes for each temperature were incubated at 10, 20 and 30°C respectively. They were examined daily until sporulation was complete.

Storage of *Eimeria* oocysts at subzero temperatures: Four mls of pooled faecal suspensions mixed with 2% potassium dichromate were added to test tubes, sealed and placed at subzero temperature for the required period. Before subjecting each tube to the subzero temperatures concerned, the tubes were kept at 4°C for 30 minutes to reduce the possible effects of sudden shock of low temperature on the oocysts. Fifteen tubes were used at -20°C for 1 and 24 hours respectively. Other tubes of the same number were stored for 1 week, 2 weeks, 1 month and 2 months respectively. Fifteen tubes for each time period were also subjected to -70°C for 5 and 10 minutes, 1 and 24 hours, 1 week, 1 month and 2 months respectively. After the recommended time period, these tubes were removed from the deep freeze and stored in a refrigerator for 30 minutes, then transferred to an incubator at 30°C and examined the following week for evidence of sporulation. The sporulated oocysts were identified and the percentage of sporulated to unsporulated oocysts counted.

Storage of *Eimeria* oocysts at low temperature (4°C): Faecal suspensions with 2% potassium dichromate were stored in Petri dishes at 4°C for 2 weeks, 1, 2 and 3 months respectively.

Survival and sporulation of oocysts at high temperature: Oocysts were kept at 38, 45 and 55°C in an incubator for 4, 8 and 24 hours respectively and then transferred into another incubator at 30°C and examined seven days later for evidence of sporulation.

Centrifugation of *Eimeria* oocysts at different speeds: Faecal suspensions remaining from the McMaster technique were subjected to the Clayton Lane method and the oocysts obtained were added to tap water and again centrifuged at 1000, 1500, and 2000rpm for 10, 20 and 30 minutes

respectively according to the procedures outlined by Wilson, (1930) and Marquardt *et al.* (1960). Following incubation at 30°C, the samples were examined for evidence of sporulation and the effects of different centrifugation speeds determined.

Storage of oocysts in saturated saline (NaCl): Faecal suspensions were prepared in tap water and filtered using a mesh size of 0.15mm. The test tubes were centrifuged at 1500rpm for 2 minutes, the supernatant discarded and an equal volume of saturated sodium chloride added and the tubes stored at 10°C for 2, 4, 6 and 8 days, as described by Ryley and Ryley, 1978. Following the recommended periods, the oocysts were washed with tap water and potassium dichromate added, incubated at 30°C and examined seven days later for evidence of sporulation.

***Eimeria* oocysts in the absence of oxygen:** Oocysts were separated as described earlier and maintained in potassium dichromate at 30°C on slides (groved slide) with coverslips sealed at the edges with glyceel (Searl ltd). They were examined seven days later for evidence of sporulation.

Statistical Methods: Data were analyzed by analysis of variance (one way ANOVA), using minitab package 1993/94 to test the significant difference of effects of any treatments on survival and sporulation of the oocysts of different *Eimeria* species.

Results

Effects of temperature on speed of sporulation of *Eimeria* oocysts: Table 1 shows that the highest percentage of sporulation was recorded in all species at 30°C. Also all species showed a significant increase in the percentage sporulation at 30°C. Depending on species sporulation ranged from approximately 50-77% over a range of temperature of 10-30°C. Generally, all species showed a trend of increasing % sporulation with increasing temperature. Furthermore, a trend of increasing speed of sporulation was evident with increased temperature.

Effects of storage at low temperature (4°C) on the sporulation and survival of *Eimeria* oocysts: Table 2 shows that the mean percentage of oocysts which sporulated in all *Eimeria* species showed a significant decrease with time. A significant decline in the % of oocysts which sporulated over the 3 months was seen in the following species, *E.bovis, E.zuernii, E.brasiliensis* and *E.ellipsoidalis/ alabamensis* and their sporulation at 2 weeks and 3 months were recorded as 77.44, 81.18, 76.14, 72.92 and 55.10, 50.77, 49.16 and 55.26% respectively. However, no significant effects of storage for 3 months were observed in the case of *E.auburnensis* and *E.canadensis*.

Effects of subzero temperature on survival and sporulation of *Eimeria* oocysts: When unsporulated oocysts of different species of *Eimeria* were kept at -20°C for 1 and 24 hours respectively, only five species survived (Table 3a). These species were *E.bovis, E.ellipsoidalis/alabamensis, E.canadensis, E.zuernii* and *E.subspherica* and the mean sporulation was 70.8, 66.0, 56.0,76.4 and 50.6% respectively. Only *E.bovis* and *E.zuernii* survived for 24 hours the sporulation recorded were 60.9 and 70.9% respectively. Beyond this period no species survived for 1 week, 2 weeks, 1 month and 2 months. *E.cylindrica and E.pellita* were not observed. However, when unsporulated oocysts of the different species of *Eimeria* were subjected to -70°C for 5 and 10 minutes respectively, all survived (Table 3b). The species which survived for 5 and 10 minutes exposure were *E.bovis, E.auburnensis, E.subspherica, E.brasiliensis, E.canadensis, E.zuernii, E.wyomingensis, E.ellipsoidalis/ alabamensis* and *E.cylindrica* respectively. However, in line with the survival figures, *E.bovis* and *E.zuernii* both survived -70°C when exposed for 1 hour.

Effects of high temperature on survival and sporulation of *Eimeria* oocysts: When unsporulated oocysts of different species of *Eimeria* were kept at 38, 45 and 55°C for 4, 8 and 24 hours respectively, most species survived at 38 °C for 4, 8, 24 hours, but none survived at 45 and 55°C. However, *E.zuernii* did not survive at 38°C for any time period. The mean percentage of the species which survived at high temperature are given in Table 4. Statistically, no significant effects of temperature at 38°C for the different time periods were observed in the case of *E.bovis, E.aubumensis,* and *E.wyomingensis*. However, a significant effect of 38°C was recorded at 24 hours with a decrease in sporulation in *E.brasiliensis, E.ellipsoidalis/ alabamensis* and *E.canadensis*.

Effects of pH on survival and sporulation of *Eimeria* oocysts: No effect of various hydrogen ion concentrations occurred since sporulation took place at all hydrogen ion concentrations. The mean percentage of oocysts which sporulated for all species is summarized in Table 5. No significant decline in sporulation at any pH was recorded for any species of *Eimeria*.

Effects of saturated saline solution (NaCl) on survival and sporulation of *Eimeria* oocysts: Unsporulated oocysts of various *Eimeria* species were kept for 2, 4, 6, and 8 consecutive days in saturated salt solution and their survival is given in Table 6. *E.bovis* produced 67.64% sporulation after 2 days of treatment while by day eight sporulation had significantly reduced to 55.58%. A significant decrease in sporulation was also recorded for *E.zuernii* and *E.ellipsoidalis/alabamensis*. However, no significant effects of exposure to saturated salt solutions were observed in the case of *E.cylindrica, E.wyomingensis, E.brasiliensis* and *E.canadensis*. It is clear that *E.zuernii* is the most sensitive to salt solutions and only survived up to four days exposure.

Effects of centrifugation on the survival and sporulation of the oocysts of *Eimeria* species: When unsporulated oocysts of *Eimeria* species were centrifuged at different speeds for different periods of time, no effects were recorded (Table 7).

Effects of oxygen on survival and sporulation of *Eimeria* oocysts: When unsporulated oocysts of *Eimeria* species were exposed to reduced oxygen at 30°C for one week and sporulation examined it was seen that no sporulation occurred in any species.

Discussion

A significant increase in the mean percentage sporulation of each species of bovine *Eimeria* at the different temperature studied. This indicates that there is close relationship between temperature and sporulation of oocysts of *Eimeria* species. All ten species recognized in this study had completed their sporulation within 10-14 days at 10°C. While the same species completed their sporulation at 20 and 30°C after 5-9 and 2-5 days respectively. Parker (1991) observed similar trends in sporulation of *E.brasiliensis* and *E.alabamensis*, both

Table 1: The speed and mean percentage sporulation of the oocysts of *Eimeria* species at various temperatures

Species	Temp. °C	Time (days)	Mean (%)	SED	P
E.bovis	10	10-12	57.21	1.63	0.000
	20	5-7	69.87		
	30	3-4	77.32		
E.auburnensis	10	10-14	56.00	3.75	0.000
	20	5-7	67.94		
	30	3-4	72.96		
E.canadensis	10	10-13	54.13	2.02	0.000
	20	5-6	68.10		
	30	3-4	75.92		
E.ellipsoidalis/alabamensis	10	11-14	53.83	2.19	0.000
	20	5-8	66.87		
	30	3-5	71.84		
E.brasiliensis	10	13-14	50.88	3.34	0.000
	20	7-9	67.80		
	30	3-5	71.84		
E.cylindrica	10	10-14	49.97	2.87	0.000
	20	5-8	60.15		
	30	3-5	67.36		
E.zuernii	10	10-13	53.01	2.87	0.000
	20	4-5	65.24		
	30	2-3	77.23		
E.wyomingensis	10	12-14	57.02	2.23	0.000
	20	6-7	72.57		
	30	3-5	75.51		
E.subspherica	10	12-14	51.00	2.47	0.000
	20	6-7	56.36		
	30	3-5	64.54		

Table 2: The mean percentage sporulation of *Eimeria* oocysts at low temperature (4°C) for different time periods

Eimeria spp.	2 week Mean (%)	1 mth Mean (%)	2mth Mean (%)	3mth Mean (%)	SED	P
E.bovis	77.44	67.55	65.82	55.10	2.96	0.000
E.auburnensis	70.22	71.0	62.32	52.28	6.74	0.041
E.canadensis	73.03	69.50	62.26	53.79	6.72	0.036
E.ellipsoidalis/alabamensis	72.92	66.42	59.36	55.26	3.76	0.001
E.brasiliensis	76.14	67.96	65.10	49.16	3.82	0.000
E.zuernii	81.18	77.51	68.41	50.77	3.90	0.000

Table 3a: The mean percentage of survival and spoulation of *Eimeria* oocysts at -20°C

Eimeria spp.	1hr	24hr	1wk	2wk	1mth	2mth	SED	P
E.bovis	70.8	60.9	0	0	0	0	2.14	0.000
E.auburnensis	0	0	0	0	0	0		
E.canadensis	56.0	0	0	0	0	0	1.83	0.000
E.ellipsoidalis/alabamensis	66.3	0	0	0	0	0	2.10	0.000
E.brasiliensis	0	0	0	0	0	0		
E.zuernii	76.4	70.9	0	0	0	0	1.56	0.002
E.wyomingensis	0	0	0	0	0	0		
E.subspherica	50.6	0	0	0	0	0	2.72	0.000

Table 3b: The mean percentage survival of *Eimeria* oocysts at -70 °C for different time peroids

Time Period	5min	10min	1hr	24hr	1wk	1mth	2mth	SED	P
Eimeria species									
E.bovis	75.04	57.99	56.45	0.0	0.0	0.0	0.0	3.40	0.000
E.auburnensis	62.50	55.16	00.00	0.0	0.0	0.0	0.0	3.93	0.073
E.canadensis	75.46	62.05	00.00	0.0	0.0	0.0	0.0	5.24	0.016
E.ellipsoidalis/alabamensis	73.95	62.05	00.00	0.0	0.0	0.0	0.0	3.79	0.030
E.brasiliensis	61.71	57.94	00.00	0.0	0.0	0.0	0.0	4.57	0.416
E.cylindrica	57.57	52.26	00.00	0.0	0.0	0.0	0.0	3.29	0.119
E.zuernii	74.49	67.98	62.83	0.0	0.0	0.0	0.0	2.18	0.000
E.wyomingensis	69.05	62.35	00.00	0.0	0.0	0.0	0.0	3.37	0.048
E.subspherica	58.90	52.10	00.00	0.0	0.0	0.0	0.0	3.49	0.060

Table 4: The mean percentage survial of *Eimeria* oocysts at high temperature for different time periods

Temp. Time	38 °C			45 °C			55 °C			SED	P
	4hr	8hr	24hr	4hr	8hr	24hr	4hr	8hr	24hr		
Eimeria species											
E.bovis	73.61	66.70	75.06	0.0	0.0	0.0	0.0	0.0	0.0	3.91	0.102
E.auburnensis	71.18	65.90	64.67	0.0	0.0	0.0	0.0	0.0	0.0	3.13	0.115
E.canadensis	75.25	70.30	64.37	0.0	0.0	0.0	0.0	0.0	0.0	2.56	0.001
E.ellipsoidalis/alabamensis	76.52	66.82	00.00	0.0	0.0	0.0	0.0	0.0	0.0	3.00	0.012
E.brasiliensis	71.81	67.48	64.18	0.0	0.0	0.0	0.0	0.0	0.0	2.85	0.052
E.zuernii	00.00	00.00	00.00	0.0	0.0	0.0	0.0	0.0	0.0		
E.wyomingensis	70.64	67.48	66.98	0.0	0.0	0.0	0.0	0.0	0.0	5.07	0.742

Table 5: The mean percentage of sporulation of *Eimeria* oocysts at different pH

pH Levels	3.3	4.2	9.4	11.5	SED	P
Eimeria species						
E.bovis	66.17	65.11	69.47	65.30	2.88	0.410
E.auburnensis	69.17	65.84	66.99	66.50	3.28	0.762
E.canadensis	71.74	62.84	65.42	66.61	3.77	0.138
E.ellipsoidalis/alabamensis	67.58	68.80	67.14	66.80	3.50	0.994
E.brasiliensis	65.20	65.50	66.44	68.72	5.25	0.905
E.cylindrica	65.54	66.70	66.20	73.20	4.35	0.214
E.zuernii	64.00	64.40	76.30	65.30	5.35	0.103
E.wyomingensis	69.90	67.98	62.62	70.14	4.12	0.202
E.subspherica	60.35	63.10	66.96	68.76	5.97	0.509

Table 6: The mean percentage sporulation and survival of *Eimeria* oocysts in saturated saline (NaCl) for different time periods

Time Period	2 days	4 days	6 days	8 days	SED	P
Eimeria species						
E.bovis	67.64	63.46	59.98	55.58	2.86	0.005
E.auburnensis	63.78	57.34	54.98	43.68	5.14	0.010
E.canadensis	63.27	58.80	53.92	50.97	4.38	0.103
E.ellipsoidalis/alabamensis	67.14	66.04	50.24	53.72	3.32	0.001
E.brasiliensis	52.97	57.67	50.00	48.25	4.20	0.236
E.cylindrica	60.98	61.48	54.00	58.60	3.46	0.162
E.zuernii	60.58	55.20	00.00	00.00	1.84	0.000
E.wyomingensis	60.84	56.98	55.64	54.24	3.38	0.275

Table 7: The mean percentage survival of Eimeria oocysta during centrifugation at different rpm for various time periods

rpm	1000			1500			2000			SED	P
Time in minutes	10	20	30	10	20	30	10	20	30		
Eimeria species											
E.bovis	77.4	81.2	77.4	78.1	76.4	79.8	82.8	74.9	81.2	5.00	0.861
E.auburnensis	69.9	69.9	71.1	72.9	74.0	70.9	73.2	72.7	71.5	4.88	0.975
E.canadensis	81.3	78.4	77.2	80.8	77.0	79.8	83.2	81.0	82.3	3.92	0.950
E.ellipsoidalis/alabamensis	71.1	72.2	70.1	74.0	74.5	75.4	73.4	73.8	75.3	5.88	0.990
E.brasiliensis	67.8	67.8	72.3	67.8	65.3	73.1	68.1	66.9	77.6	4.55	0.882
E.cylindrica	70.9	70.4	68.4	66.3	66.8	74.2	66.1	67.7	68.6	3.78	0.327

species having completed their sporulation between 4 and 5 and 8 and 9 days at 23 to 26°C respectively, while *E.canadensis* completed its sporulation by 3 and 5 days at 23 and 26°C respectively (Jones and Parker, 1985). Coudert and Yvore (1973) found that *E.steidai* from the rabbit had completed its sporulation within 13 days at 10°C, whereas, Davies *et al.* (1963) showed that sporulation of various *Eimeria* species was completed between 2 and 6 days at 28 to 31°C. However, Marquardt *et al.* (1960) showed somewhat different figures in the case of *E.zuernii*. They showed that *E.zuernii* did complete its sporulation after one month at 10°C but showed only 1% sporulation. When the same species was kept at 8°C sporulation did occur after several months. In contrast, Nomi (1926), recorded 89-100% sporulation of *E.zuernii* oocysts after 35-40 days at 11-15°C. Wilson and Morley (1933) described in their survey that *E.zuernii* had completed sporulation within 48-72 hours at 20-25°C and that 35-100% sporulation was seen. Therefore, the results obtained in the present study on speed and percentage sporulation for different periods at different temperatures are similar to those of the above workers. There is clearly a direct relationship between increasing temperature and speed and percentage of sporulation.

During our investigation of the effect of low temperature on survival, a significant effect of storage at low temperature for 3 months was observed with reduced sporulation of the *Eimeria* species investigated. Edgar (1954) described that storage at low temperature (0-5°C) could retard or temporarily inhibit sporulation of the oocysts of the some species. On the other hand, Long (1973) observed the effects of low temperature (4°C) on sporulation of *Eimeria* species for 14 weeks. After this period oocysts were transferred to room temperature but only 46% sporulated.

In the present investigation, an attempt was made to observe the effects of storage at subzero temperature (-20°C) for different periods on the sporulation of *Eimeria* species. A significant effect on survival was observed. Few species could survive for 1 or 24 hours, while beyond this period no survival

was seen. Coudert and Yvore (1973) considered that unsporulated oocysts of poultry *Eimeria* species could not survive after subjection to -35°C for 1 hour. Kogan (1959) also observed a similar results at -35°C. Marquardt *et al.* (1960) found that unsporulated oocysts of *E.zuernii* were unharmed by freezing at -7 to -8°C for as long as two months. After two weeks at this temperature, 92% survived and after 2 months, 74% sporulated. They further showed that *E.zuernii* also survived (37% and 5%) at -30°C for 6 and 24 hours respectively. On the other hand, Jansson (1990) considered that coccidiosis of Swedish calves may be caused by over-wintered oocysts particularly where the temperature does not fall below -7°C. The effect of subzero temperature on sporulation and survival of the species investigated by us agrees with the findings of the above workers.

Unsporulated oocysts of most of the *Eimeria* species investigated were seen to survive for 5 and 10 minutes at -70°C, but most could not survive for 1 hour. However, 56.45% *E.bovis* sporulated after one hour at -70°C. Marquardt *et al.* (1960), also recorded 92% sporulation after two weeks and 74% survival after two months in the case of *E.zuernii* when kept at -7 to -8°C. Most of the species examined survived following exposure at 38°C for 4, 8, and 24 hours, except *E.zuernii*, but none survived following exposure to 45 and 55°C. Marquardt *et al.* (1960) reported that unsporulated oocysts of *E.zuernii* did not survive when exposed to sunlight for 8 hours at 35°C, while Long (1973) considered that temperatures of 35°C or above could reduce or permanently inhibit the sporulation of oocysts of several species of *Eimeria*. Parker and Jones (1989) demonstrated that the oocysts of *E.bovis, E.ellipsoidalis* and *E.zuernii* species were destroyed by natural factors in simulated yard conditions. They found that viable oocyst numbers fell by 50% after 24 hours exposure at 48°C, 98% after 48 hours and oocysts were not detectable after 74 hours. Our results are therefore in accordance with the observations made by previous workers.

Marquardt *et al.* (1960) used a wide range of hydrogen ion concentrations on oocysts but did not find any effect, all species surviving to sporulate. Our findings are totally in agreement with above workers. During our study, a significant effect on survival and sporulation was seen when oocysts were exposed to saturated salt solution. This was most marked in the case of the most pathogenic species (Table 6). However, the deformation and collapse of the walls of the oocysts seen was reversible on washing and subsequent sporulation was recorded. Ryley and Ryley (1978) made a similar observation. They stated that after 1 to 2 days of contact with salt solution, appreciable deformation and collapse of the oocysts was observed but these effects were reversible on washing and subsequent sporulation took place. No significant effects of centrifugation at 1000, 1500 and 2000 for 10, 20 and 30 minutes were observed. All species sporulated as usual and no adverse effects were seen. Similar results were obtained by Wilson (1930) and Marquardt *et al.* (1960) both failed to find any effects of centrifugation. Sporulation was not detected in any species following exposure to anaerobic conditions.

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