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The Pathogenesis of Experimental Infections of *Cryptosporidium baileyi* in Chickens

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ABSTRACT

Groups of broiler and layer chicks were experimentally infected with 400, 20,000 and 1,000,000 oocysts of *Cryptosporidium baileyi* at either 2, 16 or 30 days of age. Clinical signs were not observed in any of the infected birds and there was no significant effect on weight gain compared to uninfected controls. Tissues examined histologically at 2, 5, 7, 14, 21 days post-infection (DPI) revealed the presence of parasites in the Bursa of Fabricius and cloaca between 7 and 14 DPI, and occasionally in the caeca and trachea. Hypertrophy and hyperplasia of the lining epithelial cells, particularly in the bursa, were the main histological changes observed. A marked heterophil response with acute inflammatory exudate and bursal cast formation was seen in some of the heavily challenged birds.

INTRODUCTION

Cryptosporidia are coccidial parasites which develop on the microvillus border of epithelial cells of their vertebrate hosts (Current and Blagburn 1990). Avian cryptosporidia have been observed in the enteric, respiratory and renal epithelium of infected birds (Goodwin 1989). Two species of *Cryptosporidium* are generally recognized as occurring in avian hosts (Blagburn 1989). Morphometric studies on oocysts (Lindsay et al. 1989) distinguish *C. meleagridis* from *C. baileyi*. Further studies may establish additional species.

Cryptosporidium meleagridis, isolated from turkeys, develops predominantly in the small intestine (Slavll 1955). *Cryptosporidium baileyi* was first reported by Current et al. (1986) from the cloaca and Bursa of Fabricius of

the domestic chicken. It is known to infect several species of galliforme and anseriforme birds (Lindsay et al. 1986a) and other tissues including the caecum, colon and respiratory tract (Current and Blagburn 1990). Clinical and pathological signs have been associated with the presence of cryptosporidia in the respiratory tract of birds (Dhillon et al. 1981; Goodwin et al. 1988; Itakura et al. 1984). Experimental infection of chickens with *C. balileyi* result in occurring disease when administered intratracheally (Blagburn et al. 1987; 1991), but not when given orally (Blagburn et al. 1987; Current et al. 1986). The objectives of the present study were to monitor the pathogenesis of experimental infections of *C. baileyi* in different age groups of both broiler and laying chickens when administered by the oral route.

MATERIALS AND METHODS

Parasite culture and challenge

Oocysts of *C. baileyi* were originally obtained from Janssen Pharmaceutica, Beerse, Belgium. Fresh oocysts for use in the study were produced by passage in Ross Hisex day old chicks. Faeces were collected in trays containing 2% potassium dichromate and 0.02% sodium azide. Oocysts were recovered by salt flotation and cleaned by centrifugation in the presence of sodium dodecylsulphate (SDS) and flocculated in the presence of 2% sulfuric acid. The oocysts were enumerated using a Neubauer haemocytometer and stored in Hank's balanced salt solution at 4°C until required for use.

Experimental birds

Male Ross Hisex and female Lohmann Brown layer chicks were obtained from a local hatchery at a day old and allowed to acclimatize for a period of 24 hrs prior to the start of the study. The birds were housed in wire floor cages (1.0 m^3, 10 birds/m^2) at an initial temperature of 30°C with 14 hrs of red light and 10 hrs of red light. All birds were fed ad libitum on a standard anticoccidial free diet prepared on site. Water was supplied via gravity feed nipple drinkers from a bottle reservoir. From 14 days of age, birds were maintained at an ambient temperature of 25°C with 14 hrs of daylight.

Experimental design

The study was conducted in 3 parts. Trial A was conducted in broiler chicks to monitor clinical effects and weight performance. Trial B was conducted in a similar manner to Trial A, but in layer chicks, whilst Trial C was designed specifically to investigate the pathological effects. Each trial was conducted according to the same protocol. Essentially, at the start of each of trials, birds

were randomized on the basis of body weight into four groups. From these, groups of 8 birds were given 400, 20,000 and 1,000,000 oocysts at either 2, 16 or 30 days of age. Remaining birds acted as uninfected controls. A summary of the trial design is given in Table 1. Individual birds were orally infected with *C. baileyi* oocysts by direct administration into the crop in 1 ml volume by clean plastic pipettes. All birds were observed daily for signs of clinical abnormalities.

Table 1. Experimental design.

Group	Challenge dose	Age at Challenge
1	400	2 days
2	20,000	2 days
3	1,000,000	2 days
4	Uninfected control	
5	400	16 days
6	20,000	16 days
7	1,000,000	16 days
8	Uninfected control	
9	400	30 days
10	20,000	30 days
11	1,000,000	30 days
12	Uninfected control	

Faecal oocyst output

Faecal oocyst output for each group of birds was monitored by the method described by Taylor et al. (1994). Essentially faecal oocysts output was determined by collection of faeces on day 0, 5, 7, 14 and 21 post-infection and numbers of oocysts were enumerated on a Neubauer haemocytometer after concentration by a modified centrifugal salt flotation technique (MAFF 1986).

Necropsy procedure

In trial C, a total of sixty birds were examined by necropsy. One bird per group was killed by intracardiac inoculation of barbiturate at 2, 5, 7, 14, 21 DPI. Samples of tissue from the duodenum, ileum, caeca, cloaca, Bursa of Fabricius, trachea, lung, crop, proventriculus and caecal tonsil were placed in separate containers of Bouin's fluid. Additional samples were taken into 3% glutaraldehyde for scanning electron microscopy (SEM) studies. Additionally, samples were also examined for the presence of cryptosporidial oocysts by direct smearing onto a clean microscope slide and examined using a modified Ziehl-Nielsen staining technique as described by Chermette and Boufassa-Ouzrout (1988). Tissues for

light microscopy were embedded in paraffin wax, sectioned at 3 μm, stained with haematoxylin and eosin and examined histologically. For SEM, tissues fixed in 3% glutaraldehyde were processed using an osmium tetroxide thiocarbohydrazide coating technique (Malick et al. 1975), followed by dehydration in a graded series of ethanol and finally acetone. Tissues were critically point dried with liquid CO_2 and sputter coated with gold before examination on a Stereoscan S250 Mark III Scanning Electron Microscope (Cambridge Instruments).

Statistical Analyses

Data were analyzed by analysis of variance and Student's *t*-test using the statistical package MINITAB.

RESULTS

Oral inoculation of chicks with oocysts of *C. baileyi* at dosages of either 400, 20,000 and 1,000,000 resulted in patent infections in all challenge groups. No clinical signs or increased mortality were seen in any of the birds during the period of observation. In male broilers (Trial A), the levels of infection produced no apparent effect on performance. Chicks infected at 2 days of age showed no significant difference between mean body weights throughout the period of observation compared with uninfected controls. Birds inoculated with 1,000,000 oocysts at 16 days of age showed an 11% reduction in body weight by 14 DPI compared to uninfected controls although this was not statistically significant ($p=0.233$). Lower challenge groups of birds infected at 30 days of age performed better than the group of uninfected controls, although again this was not statistically significant. Growth curves for each of the infection groups of male broilers expressed as a percentage of the uninfected controls are shown in Fig. 1.

In female layers (Trial B), infection with *C. baileyi* had no significant effect on performance. Chicks infected at 2 days of age showed no significant difference in live weight gain compared with uninfected controls, although the mean body weights of the three infection groups were consistently higher than the uninfected controls at 21 DPI. All three groups of birds infected at 30 days of age had lower mean body weights at 7 DPI compared with uninfected controls. None of these differences were statistically significant. Growth curves for female layer chicks are shown in Fig. 2.

Endogenous stages of *C. baileyi* were histopathologicaly observed in groups challenged with 10^6 oocysts as early as 2 DPI. Stages of the parasite were confined to the microvillous region of epithelial cells of the cloaca and bursa. In other groups, endogenous stages were not detected on histopathology and mucosal scrapings until 5 DPI. In one section taken from a bird infected with 10^6 oocysts

at 16 days of age, parasites were seen in the trachea at 5 DPI.

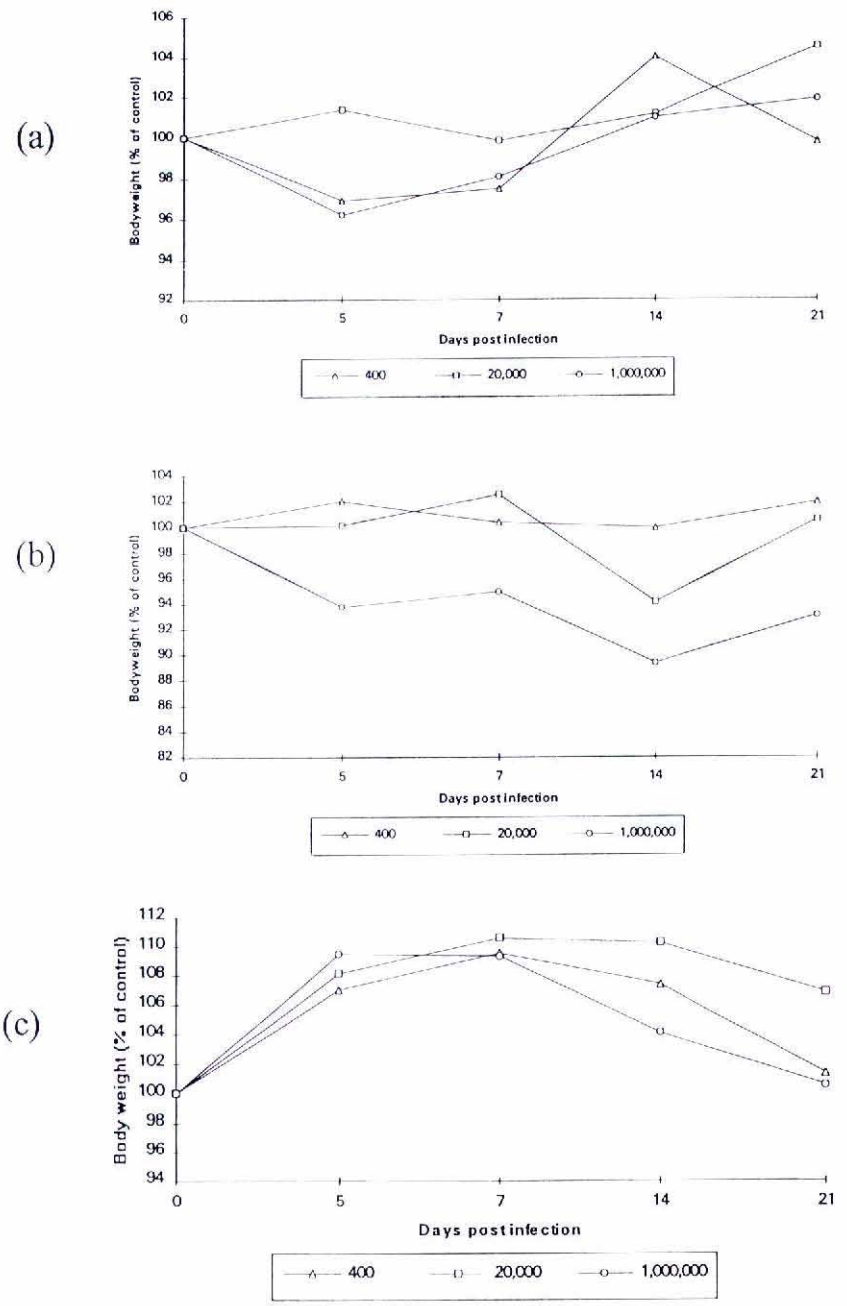
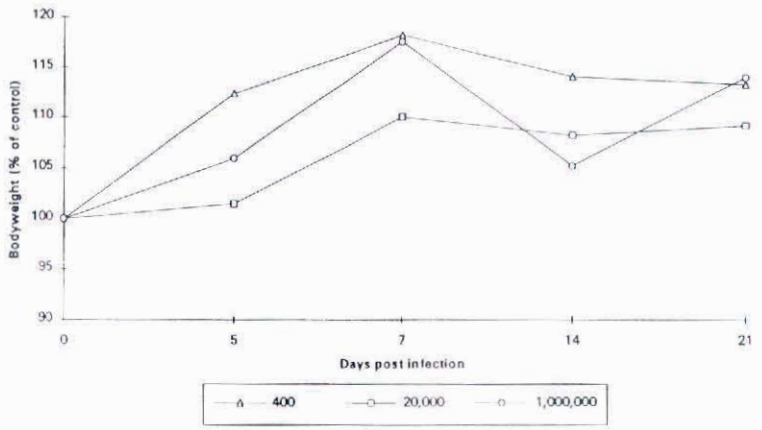
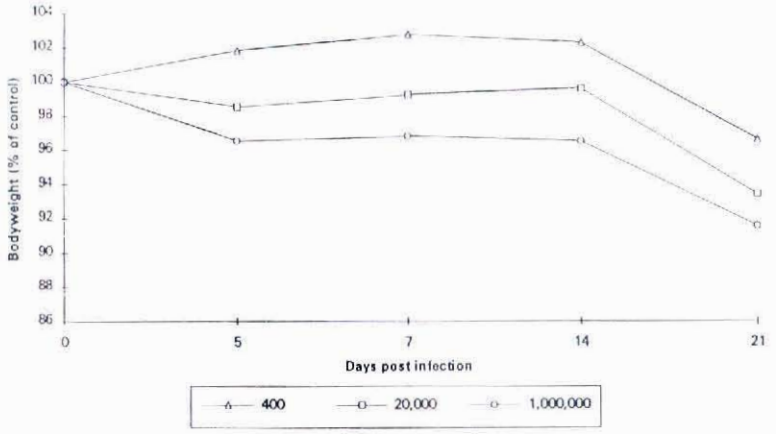


Figure 1. Growth curves for male broilers (a) infected at 2 days of age, (b) infected at 16 days of age, (c) infected at 30 days of age expressed as a percentage of the uninfected controls.

(a)



(b)



(c)

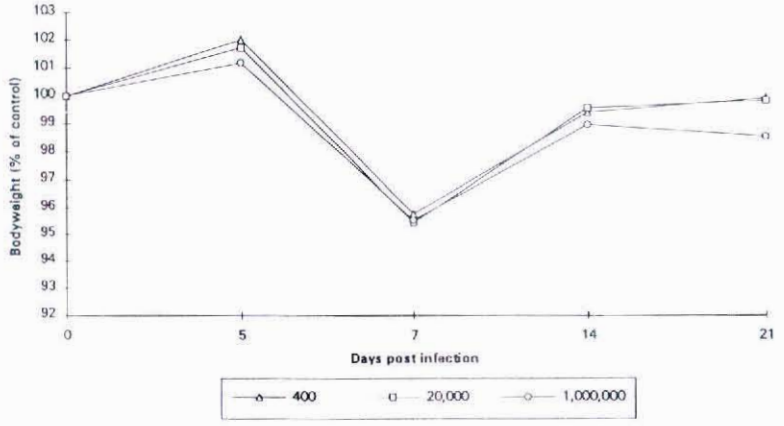


Figure 2. Growth curves for female layers (a) infected at 2 days of age, (b) infected at 16 days of age, (c) infected at 30 days of age expressed as a percentage of the uninfected controls.

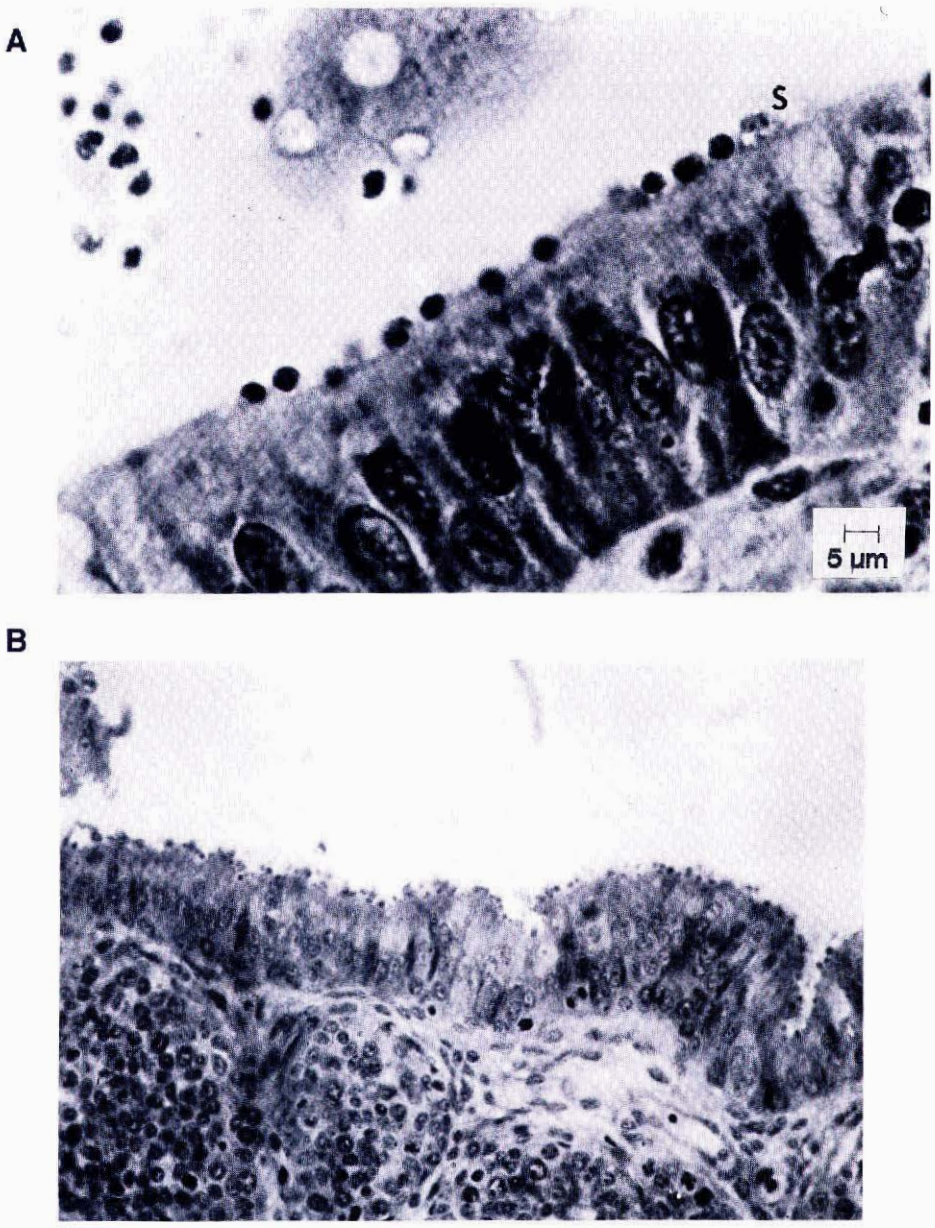


Figure 3. (A) Cloaca of a 4 day-old chicken at 2 DPI (400 oocysts). Numerous developmental stages are attached to the luminal surface of epithelial cells. Trophozoites and a maturing shizont (s) are attached to the brush border of the host cells (H&E, phase contrast). (B) Bursa of 7 day-old bird (5 DPI, 400 oocysts). The epithelial cells are already heavily parasitized, hypertrophic and showing areas of hyperplasia (H&E).

Tracheal infection was also observed in sections taken at 21 DPI from a bird infected at 2 days old with 400 oocysts. Oocysts were observed in mucosal smears of caeca from several groups, but endogenous stages were not seen in histological

sections. Hypertrophy and hyperplasia of epithelial cells, particularly those of the bursa, were the main histological changes observed in many of the sections with large numbers of parasites attached to the luminal epithelial surface. Large numbers of parasites were also observed on the luminal surface of the bursa on scanning electron microscopy. A marked heterophil response was present from about 5 DPI, in some cases involving the cortical and medullary areas of bursal follicles with many heterophils migrating into the connective tissue and through the hyperplastic epithelium. Heterophilia was not confined to the heavily parasitized bursae. Other parts of the lower intestine, where parasites were not observed, showed large numbers of heterophils within connective tissue. By 21 DPI, many of the parasites had been eliminated from the bursal epithelium which remained hypertrophic with areas of vacuolation containing necrotic cell debries. In the older age group of birds, there was an inflammatory exudate present on the surface of the epithelium and within the lumen comprising inflammatory and epithelial cells and parasites. In many of these birds, bursal casts were found on gross pathology.

DISCUSSION

Cryptosporidium baileyi produced no clinical signs and had no significant effect on weight gain in broiler or layer chicks infected at 2, 16 and 30 days of age. Patent infections were established with as few as 400 oocysts in birds up to 30 days of age. Oocyst shedding commenced at about 7 DPI and followed the pattern previously described by Taylor et al. (1994). Oral infection with *C. baileyi* resulted in parasites confined primarily to the cloaca and Bursa of Fabricius. These findings are in agreement with those of others (Fletcher et al. 1975, Randall 1982; Tsai et al. 1983; Itakura et al. 1984; Guy et al. 1988; Levy et al. 1988; Levy et al. 1988; Goodwin and Brown 1989; Iwabuchi and Kirioka 1992). The histological findings of epithelial hyperplasia and inflammatory cell infiltration in these tissues are not usually associated with clinical signs (Fletcher et al. 1975; Randall 1982). These observations were confirmed in this, and other previous experimental studies in which chicks have been inoculated orally (Blagburn et al. 1987; Lindsay et al. 1986b). As far as can be ascertained, this is the first recorded instance of purulent exudate from the hyperplastic epithelium resulting in bursal cast formation, more typically seen with infectious bursal disease (IBD) (Randall 1991). Tracheal infections following oral inoculation have been reported by Lindsay et al. (1986b). Whilst the possibility of accidental inoculation of the respiratory tract can not be ruled out, the presence of large numbers of development stages at 21 DPI, in a bird which had been infected with only 400 oocysts, suggests that infection arose through recycling of infection. Lindsay et al.

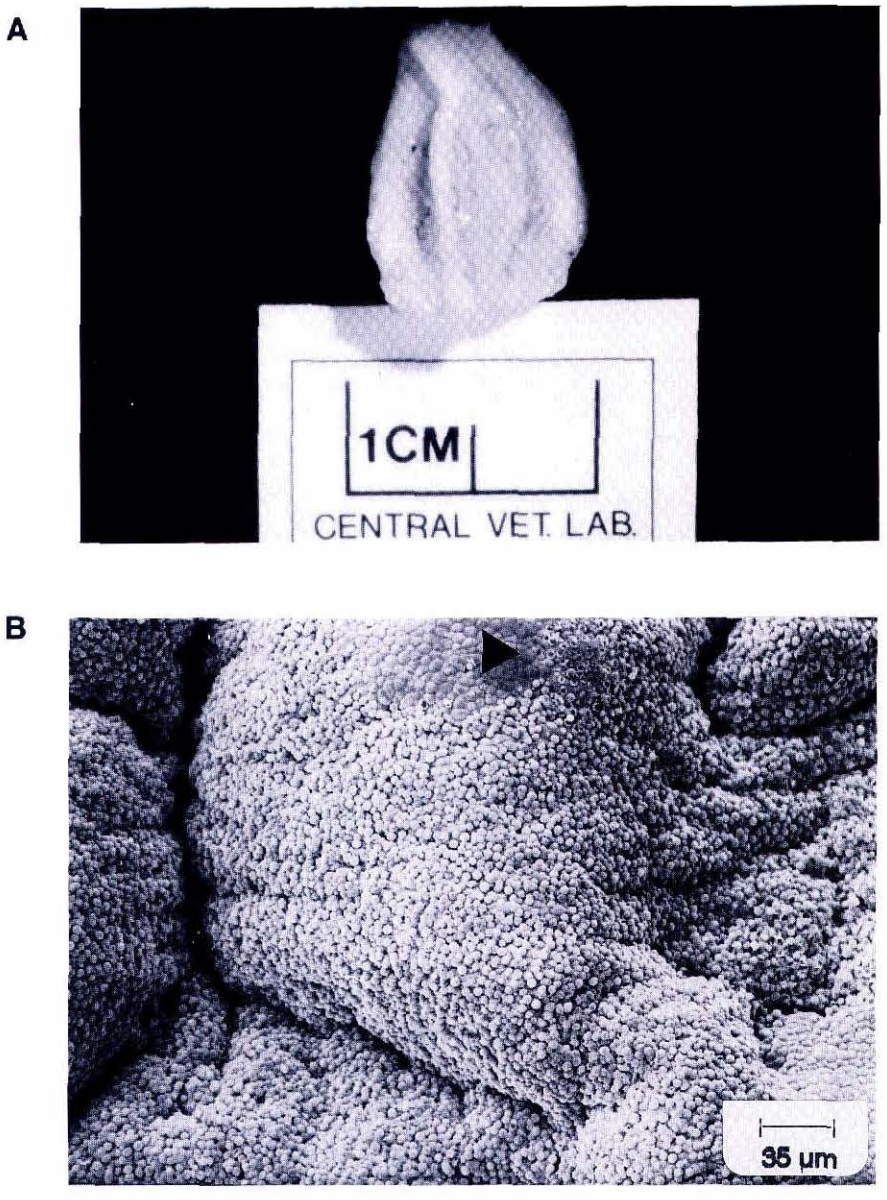


Figure 4. (A) Bursal cast fond on gross pathology in 30 day-old bird (14 DPI, 20,000 oocysts). (B) Scanning Electron Micrograph (SEM) of the bursa of a 23 day-old chicken (7 DPI, 400 oocysts). Large numbers of parasites can be seen over the surface of the bursa. An area of parasite detachment (arrowhead) can be seen with remnants of the parasitophorous membranes and attachment zones clearly visible.

(1988) demonstrated that as age at infection by the intratracheal route increased, susceptibility to respiratory disease decreased. Similar observations were made by Goodwin and Brown (1989) in 26 day-old broilers. In this study, the tissue

distribution and numbers of parasites present following oral infection, was much reduced in the older groups of birds. Current and Snyder (1988) demonstrated that 14 day-old broilers were capable of clearing endogenous stages of *C. baileyi* from the cloaca and cloacal bursa by 14 DPI following challenge with 10^6 oocysts. The period of patency does, however, appear to be influenced by the challenge dose, with lower challenge doses resulting in a longer tissue parasitemia (Taylor et al. 1994). Saltanova et al. (1991) have suggested that birds with bursal cryptosporidiosis suffer an impaired immunological response with the depression of humoral and cell mediated immunity closely correlated to the dose of oocysts given. Clearly, the nature of the hosts immunological responses to *C. baileyi* requires further investigation.

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REFERENCES

Blagburn, B. L. 1989. Avian Cryptosporidiosis. pp. 27-42. *In:* Proceedings of 1st International Workshop on Cryptosporidiosis, Angus, K. W. & Blewett, D. A. (Eds), Animal Disease Research Association, Edinburgh.

Blagburn, B. L., Lindsay, D. S., Giambrone, J. J, Sundermann, C. A. & Hoerr, F. J. 1987. Experimental cryptosporidiosis in broiler chickens. *Poul. Sci. 66:* 442-449.

Blagburn, B. L., Lindsay, D. S., Hoerr, F. J., Davies, J. F. & Giambrone, J. J. 1991. Pathobiology of cryptosporidiosis (*C.baileyi*) in broiler chickens. *J. Protozool . 38:* 25S-28S.

Chermette, R. & Boufasssa-Ouzrout, S. 1988. Cryptosporidiosis: A cosmopolitan disease in animals and man, Technical Series No. 5, pp. 59-60. Office International des Epizooties, Paris.

Current, W. L. & Blagburn, B. L. 1990. *Cryptosporidium*: Infections in Man and Domestic Animals. pp. 155-185. *In:* Coccidiosis of man and domestic animals, Long, P. L. (Ed), CRC Press Inc, Boca Raton.

Current, W. L. & Snyder, D. B. 1988. Development and serological evaluation of acquired immunity to *Cryptosporidium baileyi* by broiler chickens. *Poul. Sci. 67:* 720-729.

Current, W. L., Upton, S. J. & Haynes, T. B. 1986. The life cycle of *Cryptosporidium baileyi* n. sp. (Apicomplexa, Cryptosporidiidae) infecting chickens. *J. Protozool. 33:* 289-296.

Dhillon, A. S., Thacker, H. L., Dietzel, A. V. & Winterfield, R. W. 1981. Respiratory cryptosporidiosis in broiler chickens. *Avian Dis. 25:* 744-751.

Fletcher, O. J., Munnell, J. F. & Page, R. K. 1975. Cryptosporidiosis of the Bursa of Fabricius of Chickens. *Avian Dis. 19:* 630-639.

Goodwin, M.A. 1989. Cryptosporidiosis in Birds: A review. *Avian path. 18:* 365-384.

Goodwin, M. A. & Brown, J. 1989. Effect of *Cryptosporidium baileyi* on broilers infected at 26 days of Age. *Avian Dis. 34:* 458-462.

Goodwin, M. A., Latimer, K. S. Brown, J., Steffens, W. L., Martin, P. W., Resurreccion, R. S., Smeltzer, M. A. & Dickson, T. G. 1988. Respiratory Cryptosporidiosis in Chickens. *Poul. Sci. 67:* 1684-1693.

Guy, J. S., Levy, M. G., Ley, D. H., Barnes, H. j. & Gerig, T. M. 1988. Interaction of reovirus and *Cryptosporidium baileyi* in experimentally infected chickens. *Avian Dis. 32:* 381-390.

Itakura, C., Goryo, M. & Umemura, T. 1984. Cryptosporidial infection in chickens. *Avian Path. 13:* 487-499.

Iwabuchi, I. & Kirioka, H. 1992. Retrospective examinations on *Cryptosporidium* in the Bursa of Fabricius in growing layers. *J. Jap. Vet. Med. Ass. 45:* 317-319.

Levy, M. G., Ley, D. H., Barnes, H. J., Gerig, T. M. and Corbett, W. T. 1988. Experimental cryptosporidiosis and infections bursal disease virus infection of specific-pathogen-free chickens. *Avian Dis. 32:* 803-811.

Lindsay, D. S., Blagburn, B. L. & Sundermann, C. A. 1986a. Host specificity of *Cryptosporidium* sp. isolated from chickens. *J. Para. 72:* 565-568.

Lindsay, D. S., Blagburn, B. L., Sundermann, C. A., Hoerr, F. J. & Ernest, J.A. 1986b. Experimental *Cryptosporidium* infections in chickens: Oocyst structure and tissue specificity. *Am. J. v. Res. 47:* 876-879.

Lindsay, D. S., Blagburn, B. L., Sundermann, C. A. & Giambrone, J. J. 1988. Effect of broiler chicken age on susceptibility to experimentally induced *Cryptosporidium baileyi* infections. *Am. J. v. Res. 49:* 1412-1414.

Lindsay, D. S., Blagburn, B. L. & Sundermann, C. A. 1989. Morphometric comparison of the oocysts of *Cryptosporidium meleagridis* and *crytpotsporidium baileyi* from birds. *Proc. Hel. Soc. Wash. 56:* 91-92.

Ministry of Agriculture Fisheries and Food (MAFF) 1986. pp. 85-87. Manual of Veterinary Parasitological Techniques, Reference Book 418, HMSO, London. Malick, C. E., Wilson, R. B. Stetson, D. 1975. Modified thiocarbohydrazide produce for scanning electron microscopy: routine use for normal, pathological, or experimental tissue. *Stain Tech. 50:* 265-269.

Randall, C. J. 1982. Cryptosporidiosis of the Bursa of Fabricius and Trachea in

Broilers. *Avian Path. 11:* 95-102.

Randall, C. J. 1991. *In:* A colour atlas of Diseases and disorders of the Domestic Fowl and turkey. Wolfe Publishing Ltd, London.

Saltanova, N. P., Golovkina, L. P. & Pavlasek, I .F. 1991. Immune status in experimental cryptosporidiosis in chickens. *Veterinariya 12:* 38-40.

Slavin, D. 1955. *Cryptosporidium meleagridis* sp. n. *J. comp. Path.* 65: 262-266.

Taylor M. A., Catchpole J., Norton C. C. and Green J. A. 1994. Variations in oocyst output associated with *Cryptosporidium baileyi* infections in chickens. *Vet. Para. 53:* 7-14.

Tsai, S. S., Ho, L. F., Chang, C. F. & Chu, R. M. 1983. Cryptosporidiosis in domestic birds. *Chin. J. Microbiol. Immunol. 16:* 307-313.