

## Evidence for cryptosporidial infection as a cause of prolapse of the phallus and cloaca in ostrich chicks (*Struthio camelus*)

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### ABSTRACT

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Cloacas of male ostrich chicks that had suffered prolapse of the phallus and cloaca were compared with cloacas of normal ostrich chicks of both sexes from the same area. Heavy infection of the cloacal and bursal tissue with *Cryptosporidium* sp. was present in all the cases of prolapse, while no cryptosporidia were observed in the normal chicks. Histopathological lesions as described in cryptosporidial infection in other species were present in the infected cloacas. These included loss of the microvillous border and epithelial hyperplasia, and degeneration, which was indicated ultrastructurally by vacuolation of the apical cytoplasm, swelling of organelles, and nuclear changes. It is suggested that these lesions, in combination with the anatomy of the male ostrich cloaca, may be responsible for prolapse of the phallus and cloaca.

### INTRODUCTION

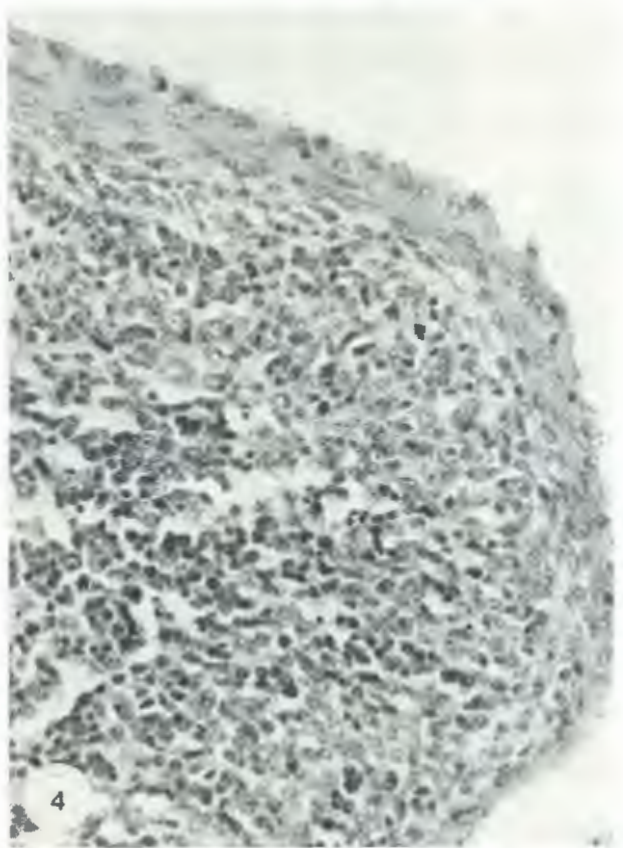
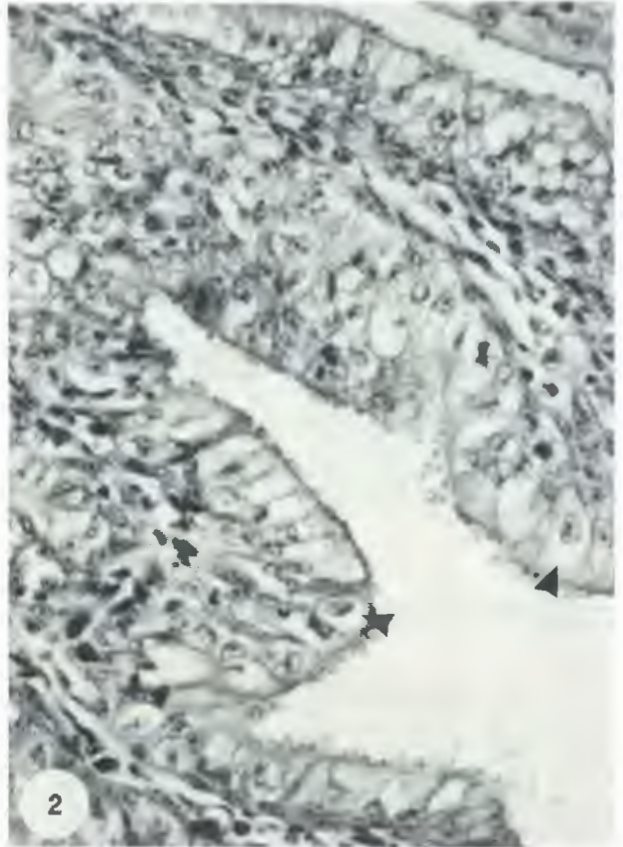
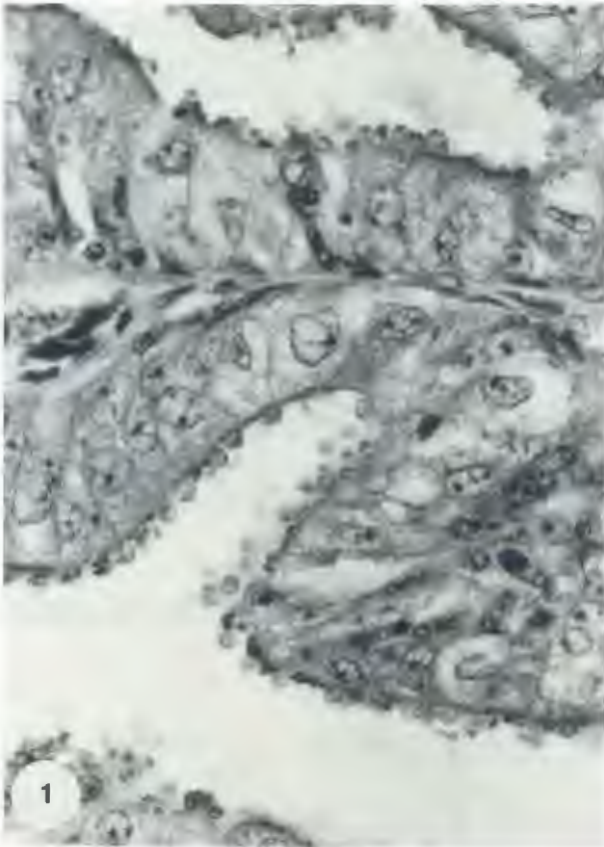
Prolapse of the phallus and cloaca, a problem affecting farmed ostrich chicks, has been associated with cryptosporidial infection (Allwright & Wessels 1993; Bezuidenhout, Penrith & Burger 1993; Penrith & Burger 1993). Infection of the cloaca and/or the bursa

of Fabricius with *Cryptosporidium* has been re-reported in a number of different bird species, including domestic chickens (Fletcher, Munnell & Page 1975; Randall 1982; Goodwin 1989; Goodwin & Brown 1989a), turkeys (Bermudez, Ley, Levy, Ficken, Guy & Gerig 1988), quail (Tham, Kniesberg & Dixon 1982), various psittacines (Doster, Mahaffey & McClearn 1979; Latimer, Steffens, Rakich, Ritchie, Niagro, Kircher & Lukert 1992), and pheasant (Sironi, Rampin & Burzoni 1991). Prolapse of the cloaca did not occur in any of the reported cases. In order to establish whether infection with *Cryptosporidium* sp. is consistent in cases of cloacal prolapse in ostrich chicks, 16 cases of prolapse in 4–6-week-old ostrich chicks from the Oudtshoorn area were examined histologically and compared with normal cloacas obtained from ostrich chicks of similar age from the same area.

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## MATERIAL AND METHODS

Cloacas, preserved in 10 % formalin, of 16 4–6-week-old ostrich chicks that had suffered prolapse of the phallus and cloaca, and of 15 normal ostrich chicks were examined histologically. Sections for light microscopy were routinely prepared and stained with haematoxylin and eosin. Selected material was prepared for electron microscopy as described below.

### Scanning electron microscopy (SEM)

Phosphate-buffered 10 % formalin-fixed bursal tissue was cut into 3 x 3 x 3-mm pieces and placed in 0.2 M sodium cacodylate (Na-C) buffer, pH 7.3. After two changes of this buffer (2 x 60 min), the tissue was put into Na-C-buffered 2.5 % glutaraldehyde (GA), pH 7.3 for 120 min. After another two changes of Na-C buffer (2 x 15 min), the tissue was dehydrated through an ascending series of ethanol (50, 70, 90, 95 and 3 x 100 %). Critical Point Drying (CPD) was through CO<sub>2</sub>, after which the samples were mounted on brass SEM viewing stubs and inspected for detritus under a stereo-microscope. The tissue was sputter-coated with gold and viewed at 5 kV in a Hitachi S-2500 Scanning Electron Microscope (Hitachi, Tokyo, Japan).

### Transmission electron microscopy (TEM)

The 3 x 3 x 3-mm pieces of tissue were processed as above to the final 100 % ethanol step. Tissue was then routinely prepared for resin embedding through propylene oxide into DER 332-732 Resin (EM Sciences, Fort Washington, PA). A Reichert-Jung Ultracut Ultramicrotome (C. Reichert AG., Wien, Austria) was used to cut silver to gold sections on a Microstar Diamond Knife (Micro Engineering, Huntsville) and double stained with aqueous 5 % uranyl acetate and lead citrate for 10 and 3 min, respectively. Sections were viewed at 60 kV in a Jeol JEM 1200EX TEM (JEOL Ltd, Tokyo, Japan).

## RESULTS

All the cloacas of ostrich chicks which had suffered prolapse were infected with numerous organisms identifiable as *Cryptosporidium* sp., associated with pathological changes in the epithelium. No organisms were found in any of the normal cloacas, and their epithelia were histologically normal.

Infection involved both the bursal epithelium and the glandular cloacal epithelium, and was most severe caudally. Affected areas showed moderate to massive infestation of the brush border with small (1–8 micron) spherical to ovoid organisms consistent with *Cryptosporidium* sp. (Fig. 1). Histopathological changes in the infected cloacas included epithelial hyperplasia with crowding, swelling and increased cytoplasmic vacuolation of epithelial cells. There were alterations in the appearance and alignment of the nuclei. Nuclei occurred at all levels in the cells, often crowded together and overlapping, not forming rows in the basal third of the cells. Although much of the normal cloacal epithelium, including that covering the lymphoid lobules of the bursa of Fabricius, appeared to be pseudostratified, nuclear disarray was considerably more pronounced in the presence of *Cryptosporidium*. The nuclei themselves appeared larger, lighter in colour, and more irregular in shape than in the normal tissue. They were frequently surrounded by a large, clear vacuole (Fig. 2). The nuclear membrane sometimes had marked indentations, giving the nucleus a lobed appearance. The accompanying inflammatory reaction varied from minimal, with only a few transmigrating heterophils in the epithelium and no subepithelial reaction, to fairly large numbers of heterophils in the epithelium and subepithelial tissue. In some cases a mononuclear infiltrate was also found in the subepithelial tissue. Oedema was constantly present in the subepithelial tissue.

Changes in the lymphoid tissue of the bursa were minimal. Normal bursal lymphoid lobules consisted of a darker, central medulla, a lighter outer cortical zone of lymphocytes, and a clearly demarcated epithelium which usually consisted of a single to pseudostratified layer of cuboidal epithelial cells, ranging from squamous to focally columnar (Fig. 3). In bursas infected with *Cryptosporidium*, the epithelium was usually markedly hyperplastic (Fig. 4) and occupied a wider zone, often with disappearance of the cortex. However, the lymphoid tissue itself appeared normal to only slightly reduced.

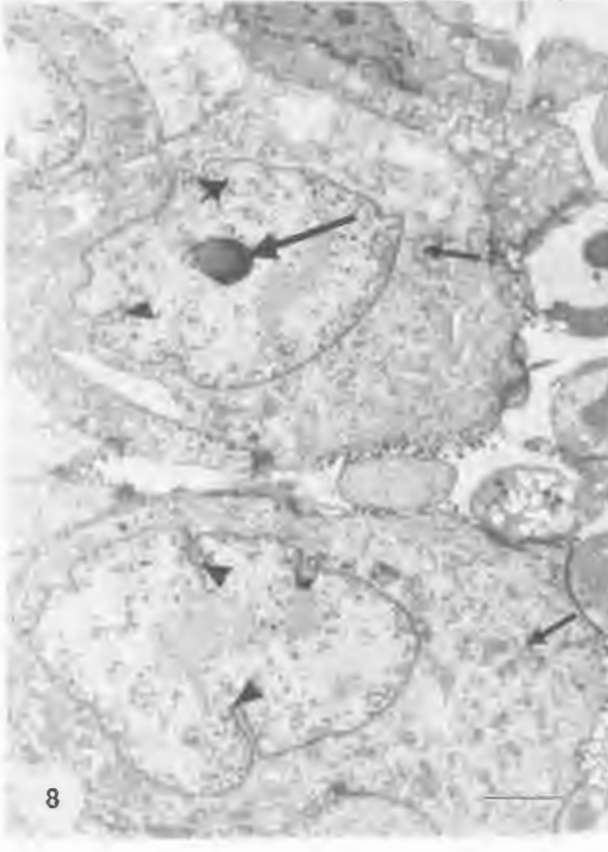
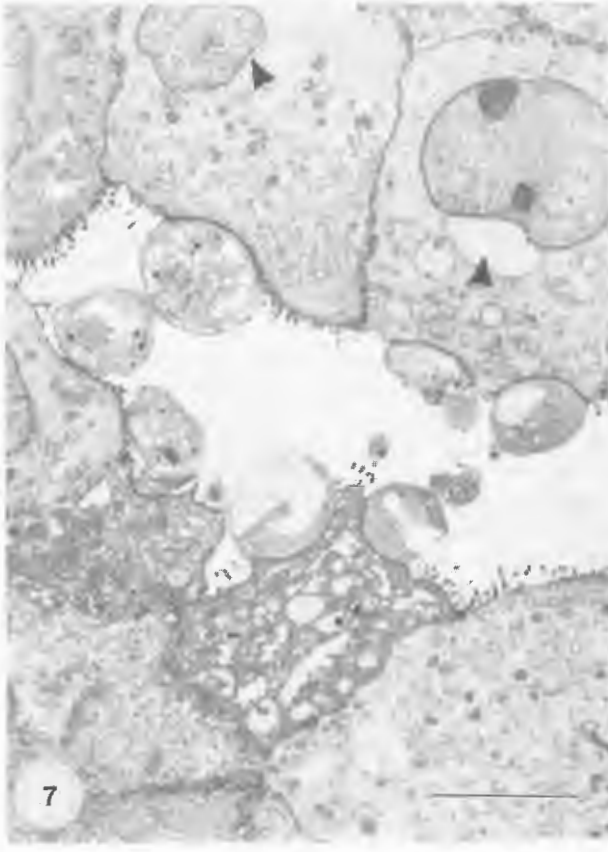
Ultrastructural changes included reduction and loss of the microvillous border (Fig. 5, 6, 7), swelling of mitochondria, accumulation of numerous vacuoles in the apical cytoplasm of epithelial cells, and sometimes dilation of rough endoplasmic reticulum, with formation of cisternae (Fig. 6, 7). Margination of nucleolar material was also observed, as well as the formation of deep indentations in nuclear membranes (Fig. 8).

FIG. 1 Cloacal epithelium of ostrich chick heavily infected with *Cryptosporidium* sp. (280 x)

FIG. 2 Cloacal epithelium of ostrich chick infected with *Cryptosporidium* sp. showing nuclear disarray and nuclei surrounded by a clear vacuole (arrowheads) (90 x)

FIG. 3 Normal ostrich chick bursa showing low cuboidal epithelium and well defined cortex and medulla (90 x)

FIG. 4 Ostrich bursa with cryptosporidial infection showing epithelial hyperplasia and loss of definition of cortex and medulla (90 x)



## DISCUSSION

Microscopic lesions in the epithelium of bursas and cloacas of the ostriches examined agree well with previous reports of cryptosporidial infection. Histopathological changes that have been described in cryptosporidial infections, include shortening and sometimes fusion and atrophy of the intestinal villi (Proctor & Kemp 1974; Belton & Powell 1987), and changes in the epithelial cells and the microvillous border, with varying degrees of inflammatory infiltration. Proliferative changes involving epithelial hypertrophy and hyperplasia are constantly reported (Panciera, Thomassen & Garner 1971; Kovatch & White 1972; Fletcher *et al.* 1975; Randall 1986; Goodwin 1989; Goodwin & Brown 1989a). Other reported epithelial changes include sloughing of cells (Panciera *et al.* 1971; Kovatch & White 1972; Randall 1986; Ritter, Ley, Levy, Guy, & Barnes 1986), the presence of shortened columnar or immature cuboidal epithelial cells (Panciera *et al.* 1971; Inman & Takeuchi 1979; Tzipori 1983) and flattening of epithelial cells in dilated crypts (Panciera *et al.* 1971). Tissue reaction varied from no or minimal inflammatory response (Randall 1986; Ritter *et al.* 1986) to variable leukocyte infiltration, involving transmigrating heterophils (Randall 1982; Goodwin & Brown 1989a) or lymphocytes (Inman & Takeuchi 1979), mixed infiltrates of polymorphonuclear and mononuclear cells (Goodwin & Brown 1989b; Elangbam, Qualls, Ewing & Lochmiller 1993), or lymphoplasmacytic infiltrates (Goodwin & Krabill 1989), apparently depending on the duration of the infection (Goodwin & Brown 1989a), and possibly the species and site of infection as well. Oedema of the subepithelial tissue was a fairly consistent feature of the lesions (Tzipori 1983; Goodwin & Brown 1989b). Bermudez *et al.* (1988) stated that the cryptosporidial infection induced in turkey poults in their study was unaccompanied by any pathological changes.

Ultrastructural changes described include alterations in the microvillous border, changes in the appearance of the nuclei and nucleoli, and degenerative changes in the cytoplasmic organelles, with increased vacuolation of the cytoplasm (Tadeja-Simborio & Itakura 1993).

*Cryptosporidium*-associated bursitis in birds may or may not involve the lymphoid tissue. Goodwin & Brown (1989a) described disturbed mucosal epithelial growth and inflammatory lesions in naturally occurring bursal infection of chickens, without significant lesions in the lymphoid tissue. In contrast, Sironi *et al.* (1991) reported epithelial hyperplasia accompanied by atrophy of lymphoid follicles in cryptosporidial bursitis in pheasant. Respiratory cryptosporidiosis was diagnosed in 4-week-old quails (Tham *et al.* 1982). Their bursae were heavily infested with cryptosporidia, and showed epithelial hyperplasia, with folding of epithelium into spaces in the lymphoid follicles, created by depletion of medullary lymphocytes (Tham *et al.* 1982).

Previously reported cloacal and bursal cryptosporidial infections in birds were asymptomatic (Fletcher *et al.* 1975; Doster *et al.* 1979; Tham *et al.* 1982; Bermudez *et al.* 1988; Goodwin & Brown 1989a; Sironi *et al.* 1991; Latimer *et al.* 1992), although in some cases the birds were ill as a result of intercurrent infections with other pathogenic organisms (Latimer *et al.* 1992) or respiratory cryptosporidial infection (Tham *et al.* 1982). Oedema and haemorrhage of the bursa have been described (Fletcher *et al.* 1975), and epithelial hypertrophy and inflammation are invariably present. These did not give rise to clinical signs. In ostriches, prolapse of the phallus and cloaca is virtually restricted to male chicks (Bezuidenhout *et al.* 1993). In the present study, all the prolapsed cloacas came from male chicks, while the normal cloacas were derived from eight female and seven male chicks. The anatomy of the male cloaca, with the large phallus that is extruded during urination, apparently predisposes to the development of clinical signs; female chicks have only a rudimentary phallus (Bezuidenhout *et al.* 1993). It is therefore probable that any cause of an increase in volume of the cloacal tissue in the male ostrich chick could result in prolapse of the phallus and cloaca. However, potential causes of swelling other than *Cryptosporidium* have not yet been identified. The fact that *Cryptosporidium* has been found to infect the bursa of a wide variety of avian species, including domestic chickens (Fletcher *et al.* 1975; Goodwin & Brown 1989a), red-lored

FIG. 5 SEM view of bursal epithelial surface showing *Cryptosporidium* sp. (arrows) and marked loss of microvilli (arrowheads)  
Bar = 2  $\mu$ m

FIG. 6 TEM view of bursal epithelial surface showing *Cryptosporidium* sp. (arrows) and reduction of the microvillous border  
Bar = 1  $\mu$ m

FIG. 7 TEM view of bursal epithelium colonized by *Cryptosporidium* sp. showing reduced microvillous border, increased vacuolation of apical cytoplasm of epithelial cells, and paranuclear vacuoles (arrowheads)  
Bar = 2  $\mu$ m

FIG. 8 Cloacal epithelial cells infected with *Cryptosporidium* sp. showing increased rough endoplasmic reticulum-forming cisternae (small arrows), margination of nucleolar material (large arrow), and indentation of the nuclear membrane (arrowheads)  
Bar = 2  $\mu$ m

parrots (Doster *et al.* 1979), common quail (Tham *et al.* 1982), ring-neck pheasants (Sironi *et al.* 1991), and citron-crested, lesser sulphur-crested, and umbrella cockatoos (Latimer *et al.* 1992), indicate it as a common cause of bursal and cloacal epithelial hyperplasia. It would be of great interest to know whether asymptomatic cloacal cryptosporidiosis occurs in female ostrich chicks, but this has not yet been observed as far as we know.

Cloacal prolapse was the cause of death in all the ostrich chicks from which affected tissue was submitted. In some of the cases in which cryptosporidial infection was reported in association with prolapse, *Cryptosporidium* was also present in the small intestine (Allwright & Wessels 1993; Penrith & Burger 1993), and in one case in the pancreatic ducts (Allwright & Wessels 1993). Intestinal samples were received from only a few of the ostrich chicks examined in this study, but apart from the first one (Penrith & Burger 1993) the infection appears to have been confined to the cloaca.

The source of infection has not yet been identified. In general, *Cryptosporidium* is transmitted by the faecal-oral route. Oocysts shed by either sick animals or asymptomatic carriers are responsible for the contamination of the environment, including the water supply (Current 1986; Chermette & Boufassa-Ouzrout 1988; Goodwin & Krabill 1989). Shedding of *Cryptosporidium* oocysts by healthy adult ostriches has been reported (Gajadhar 1993). The ostrich chicks in this study were raised from artificially incubated eggs and had no contact with adult ostriches, excluding them as a source of infection. The species of *Cryptosporidium* involved is undetermined, the taxonomy of the group being in a state of flux (Tzipori 1983; Levine 1984; Current 1986; Goodwin 1989; Goodwin & Krabill 1989). Experimental transmission of *Cryptosporidium* from mammals to birds has met with little or no success, while successful transmission of the organisms occurred from chickens to turkeys and ducks, but not to quail (Lindsay, Blagburn & Sundermann 1986). Attempts to infect chickens with oocysts of *Cryptosporidium* shed by adult ostriches were unsuccessful (Gajadhar 1993), and the author suggested that the species involved was probably distinct from the two species known to infect chickens, *Cryptosporidium baileyi* and *C. meleagridis*. Levine (1984) postulated a single species in birds, but the above failures at cross infection do not favour his theory, and most authors accept the probable existence of at least three species in birds (Goodwin 1989). Apart from the species of birds mentioned above, in which bursal cryptosporidiosis has been reported, faecal shedding of oocysts has been reported in peafowl, ducks, geese, budgerigar, macaw, and a tundra swan (Ley, Levy, Hunter, Corbett & Barnes 1988). Infection of the environment or water by another avian species seems to be the most likely source of the organism.

Severe clinical manifestations of cryptosporidial infections are generally considered to be associated with immune incompetence (Chermette & Boufassa-Ouzrout 1988; Goodwin 1989; Zu, Fang, Fayer & Guerrant 1992; Koudela & Hermanek 1993; Webster, Pow, Giles, Catchpole & Woodward 1993). Various studies have shown that both cell-mediated and humoral-immune responses can modulate cryptosporidial infection (Zu *et al.* 1992; Hill, Dawson & Blewett 1993; Koudela & Hermanek 1993). Immune modulation was found to alter both the number of parasites colonizing intestinal epithelium and the site of colonization, which extended further proximally in immunodeficient subjects (Hill *et al.* 1993; Koudela & Hermanek 1993). While the reason for the clinical manifestation of cloacal prolapse in ostrich chicks with cryptosporidial infection is considered to be anatomical, the degree of pathology of the cloacal epithelium is undoubtedly determined by the number of parasites present. All the samples examined were extremely heavily infested. It is possible that immunocompetent ostrich chicks might be able to keep infestation down to reasonable levels, preventing the degree of swelling required to cause prolapse. Chemotherapy is generally unsuccessful in the control of cryptosporidiosis (Chermette & Boufassa-Ouzrout 1988; Naciri, Mancassola, Yvoré & Peeters 1993; Yang & Healey 1993), only some of the newer generation glycoside antibiotics having shown some measure of success experimentally (Fayer & Ellis 1993a, b). From a practical point of view, it seems that the control of cryptosporidiosis in ostrich chicks would involve reducing stress as far as possible in order to encourage immune competence, rigorous disinfection of the environment, and limiting contact with other birds.

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