CONTROL OF ENDEMIC *CARYOSPORA* SPECIES INFESTATION OF CAPTIVE RAPTORS

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Session 4030

Abstract: *Caryospora* spp. infestation is a major cause of morbidity and mortality of captive bred raptors. This paper describes the results of 948 faecal examinations, before and after a series of different treatment protocols. Effective methods of controlling this coccidian parasite, which has enabled the production of parasite free offspring from endemically infected breeding facilities are described.

Keywords: coccidian, *Caryospora*, raptor, bird of prey, parasite, *falconidae*

Introduction: The genus *Caryospora* comprise cyst forming protozoal parasites belonging to the 'true' coccidia (phylum Apicomplexa, class Sporozoa, subclass Coccidiasinia, order Eucoccidiortia, sub-order Eimeriidae).[1] *Caryospora* spp. infestation has previously been reported as causing significant morbidity and mortality in falconidae.[2] Although infestation has been demonstrated in wild raptors,[3,4,5] it has been reported to have a greater incidence in captive raptors.[2,3,6,7] There are 14 species of *Caryospora* identified and reported as affecting falcons, kites and owls, whilst three species are found exclusively in falcons.[1] It is considered that there may be as many as 150 different *Caryospora* species in raptors world wide.[4] Coccidial oocysts are a common finding in captive (12% in 461 samples in Germany in 1982,[6] 79% of 86 in Germany in 1994,[3] 26% in 1158 samples in Saudi Arabia in 1999 (Dr Jaime Samour, written communication November 1999) [the majority of which were wild caught rather than captive bred birds], 24% of 75 samples in the UK in 1997 [2]) but less common in wild (31% of 16 samples in the USA,[5] 0% of 247 in Germany in 1998[8]) birds of prey. The source of the birds and the level of infestation of their parents affect the incidence of faecal oocysts in any captive bird survey. However the age of the birds[6,7] is responsible for the greatest variance. The majority of *Caryospora* spp. infestations in captive birds are aquired directly[9] (i.e. definitive host to definitive host) as opposed to indirect routes.[10] Most clinical disease and the incidence of high levels of shedding is said to be found in birds less than 3 months of age.[3,6,7] The incidence of faecal shedding in wild birds is related to the rate of ingestion of relevant intermediate or paratenic hosts (frequently rodents[10]) by the birds sampled. Various therapeutic regimes have been previously advised for the control of clinical disease[3,6,7] but the author and others[3] have indicated poor responses to therapy. The aim of this work has been to survey the incidence of *Caryospora* spp. infestation in captive bred merlins (*Falco columbarius*) in the United Kingdom (UK), in whom the disease has been shown to be the greatest cause of juvenile deaths. To survey the incidence in wild merlins in the UK. To evaluate diagnostic techniques and to assess the efficacy of various therapeutic and control regimes. Whilst the clinical signs associated with *Caryospora* spp. infestation in most raptor species comprise abdominal cramps, lethargy, fluffed up appearance, weight loss, inappetance, vomition, brown or occasionally

haemorrhagic diarrhoea, in merlins clinical disease is frequently characterised by severe diarrhoea or acute death in birds between 28 to 45 days of age.[2] It is widely accepted that coccidiosis is difficult to control due to persistence of infective oocysts in the environment. Young merlins are most likely to ingest oocysts prior to 17 days of age, but do not to show clinical disease until after 27 days of age.[2] It has been shown that the prepatent period for *Caryospora* infestation in falcons is 9 to 13 days and that if the birds are treated in this period they will not only develop a partially or completely protective (depending on host and parasite species) immune response but that they will not shed oocysts.[3]

Materials

In 1996 and again in 1998 all merlin keepers in the UK registered (which was a legal requirement) with the Department of Transport and Regions were contacted requesting their assistance in providing regular faecal samples for analysis for the oocysts of *Caryospora* spp. Nine hundred and forty eight faecal samples were acquired from 168 captive merlins kept by 62 different keepers, in addition to this 72 samples were received from wild merlin nest, perch or roost sites. Samples varied with respect to quantity, water content as well as the relative presence of urate and non-avian organic material. Keepers were requested to despatch samples monthly, however many samples were not forth coming due to lack of commitment by keepers, fear of disturbing birds during the breeding season or other reasons. The keepers of all birds with positive samples were advised to use one of several different treatment regimes, and to submit further samples. The keepers administered all medications to their own birds.

Methods

All samples were mixed in saturated saline solutions and left to stand for 30 minutes to allow oocyst floatation and collection under a coverslip. The coverslip was lifted and placed onto a slide for examination using 10x magnification to assess oocyst numbers. In view of variable sample volume, desiccation and levels of non-faecal material it was considered that obtaining accurate estimates of oocyst excretion in faeces was unrealistic. All samples were scored 0 (clear), 1 to 5 (light to very heavy levels of excretion). Subsequent analysis using the Mc Master method indicated that these assessments were equivalent to the following oocyst levels. Level 1 (<1000 oocysts/g), level 2 (1000 to 5000 oocysts/g), level 3 (5000 to 10000 oocysts/g), level 4 (10000 to 100000 oocysts/g) and level 5 (>100000 oocysts/g). All positive samples were then stored at 4^{o}C in 2% potassium dichromate for subsequent measuring, sporulation and identification. No cross infection tests were performed. 690 samples were collected on a single day, 186 were collected over a three day period whilst 72 were nest scrape or roost samples. The time of day of sample collection was requested and recorded where supplied. The ages of all birds involved were recorded as it is recognised that juvenile birds are most commonly affected. In the case of 10 birds with relatively low (level 1, or 2) levels of excretion, treatment was delayed and further faecal samples were collected three or more times a day for a ten day period to investigate diurnal or post prandial variations. In an extension of the initial work, a further major breeding site (producing peregrine *Falco peregrinus*, saker *Falco cherrug*, gyr *Falco rusticolus*, merlin *Falco columbarius* and hybrids there of) was investigated. This site had previously suffered a serious incidence (70% of young birds clinically affected within the first 4 months of life)

of *Caryospora* spp. infestations of young birds. Adult birds were all treated with toltazuril (Baycox, Bayer, Bury St. Edmonds, UK)(25mg/kg po once 2 months prior to anticipated egg laying in an attempt to reduce oocyst contamination of the breeding aviaries). All off spring from the site were target treated with a single dose of toltazuril (Baycox, Bayer)(25mg/kg po) at 21 and 35 days of age. These birds were faecal screened for oocysts at 40 and 70 days of age. It was believed to be impossible and perhaps undesirable to eliminate *Caryospora* spp. from this collection and hence the aim was to produce healthy, parasite free young birds. At three other sites, merlin offspring from parents that had previously produced infected offspring despite being screened and treated were foster or hand reared and subsequently screened for oocyst excretion.

Therapeutics

Common therapeutics used against coccidiosis in falcons have not lead to satisfying results.[3] Previously recommended therapies for avian coccidiosis have involved sulfadimidine (Bimadine, Bimeda, Llangefni, UK) 50 -150mg/kg po or i/m sid 5 to 7 days, amprolium (Amprol Plus, Merial, Harlow, UK) for 6 days at 30mg/kg, or amprolium in combination with ethopabate (Amprolvet Super) at a dose of 0.5mg/kg sid for 5 days. More recently clazuril (Appertex, Petlife, Bury St. Edmunds, UK) 5 - 10mg/kg po sid has been recommended, as has toltazuril (Baycox, Bayer) at doses ranging from 7.5mg - 25mg/kg on one or two consecutive days. In a previous publication[2] the author (NF) reported the lack of efficacy of sulphonamides and clazuril (Appertex, Petlife) in treating *Caryospora* spp. infestation in merlins. It is recognised that thiamine deficiency may occur in birds suffering from coccidiosis due to vitamin utilisation by the parasite. Furthermore that hypovitaminosis may worsen leading to thiamine responsive fits when such birds are treated with amprolium (Amprol Plus, Merial) (in the absence of concurrent thiamine supplementation).

Treatments: In this survey the following therapeutic regimes were tested: clazuril (Appertex, Petlife) 7.5mg/kg po sid 3 days, toltazuril (Baycox, Bayer) 8mg/kg po once, toltazuril (Baycox, Bayer) 20mg/kg po once, toltazuril (Baycox, Bayer) 25mg/kg po on 2 consecutive days, toltazuril (Baycox, Bayer) 25mg/kg po once plus movement of the bird to a previously uncontaminated area, toltazuril (Baycox, Bayer) 25mg/kg po daily x 2 followed by sulphonamide for 5 days at 50mg/kg po sid, or toltazuril (Baycox, Bayer) 25mg/kg po once weekly for 3 weeks. All positive cases, irrespective of level of excretion, were assigned to different treatment regimes subsequent faecal samples were monitored on a monthly basis. Response to therapy was assessed by repeated monthly faecal analysis following treatment. Long term monitoring is essential in determining the true efficacy of treatment for *Caryospora* spp. infestations of falcons. Temporary cessation of shedding is commonly seen with a number of agents, but is typically not maintained. Treatment of falcons with *Caryospora* spp. infestation is necessary not only to prevent clinical disease but also in an attempt to reduce the level of oocyst contamination of the aviary.

Results

Seventy two samples were examined from wild merlins, none of the samples were positive. Eight hundred and seventy six samples were examined from 168 captive merlins owned by 62 different keepers. One hundred and forty six (16.7%) of samples

were positive, with 56 (33%) of the birds testing positive on at least one occasion. Thirty four (55%) of the breeder sites were positive on at least one occasion during the study period. The ten birds whose treatment was delayed in order to monitor shedding rates excreted oocysts in between 10% and 80% of samples (average 45%). There was no apparent diurnal or post prandial variation in shedding. Rates of shedding were analysed for the first 18 months of life (Figure 1) demonstrating that shedding was significantly higher in the period 8 to 24 weeks of age, although previous authors[2,3,6,7] have reported higher levels in birds under 3 months of age, shedding has not previously been reported to extend to 5 months of age. Rates of shedding were analysed by season excluding birds (<6months of age) (Figure 2). These results show there is a significant increase in shedding rates of adult birds during the breeding season (egg laying is anticipated in early May), which inevitably leads to increased oocyst contamination of the rearing site. This seasonal increase in shedding was also demonstrated in non-breeding birds. Rates of oocyst shedding were analysed including juvenile birds (Figure 3), which show a higher incidence of shedding in the last eight months of the year. Oocyst shedding following the different treatment regimes were also assessed (Figure 4). The only treatment regime which demonstrated any significant efficacy was toltazuril (Baycox, Bayer) 25mg/kg once weekly for three weeks, which was significantly more efficacious than the same dose on two consecutive days. Although it is accepted that the control of coccidiosis is dependent on good hygiene as well as medical and/or immunological control, moving birds to a clean environment 24 hours after 2 treatments with toltazuril (Baycox, Bayer) (a reportedly effective treatment regime[3]) did not improve success rates over medication alone. In the breeding collection which had suffered the 70% incidence of clinical disease in the previous breeding season, a total of 45 young birds were treated as described above. Four and a half percent of these birds did demonstrate infestation although this did not occur until 100 and 105 days respectively, by which stage they had been under their new keepers care for a period 30 days. The remaining birds demonstrated no clinical signs or faecal oocysts of *Caryospora* spp. No oocyst shedding was found in 15 young birds, foster or hand reared from infested parents that had previously produced infested offspring.

Discussion

This survey has not been laboratory based, but has dealt with privately owned birds maintained and medicated were necessary by their owners. Inevitably the experimentation has been hampered by the death or loss whilst flying of study birds and by the failure to maintain sample collection by some keepers. There may have been inaccuracy in administering medication by the owners, or post administration regurgitation. The number of merlins registered in captivity in the UK during the period 1990 to 2000 has remained very constant at approximately 350, with an annual fledging of some 100 young. These data indicate an average captive life expectancy of 3.5 years. All merlin keepers were asked to collect samples and 37% of the captive merlin population have been included in this survey. As the program continued additional cases were added to the program either having been presented as clinical *Caryospora* cases, or being related to other birds being studied. The overall effect may be a skewed population with the addition of a greater percentage of oocyst positive birds than would have been found in a normal captive bird population. The results of this survey do confirm that *Caryospora* spp. infestation is a common problem in the UK captive bred

merlin population. As this infestation has been shown to be a common cause of death in merlins,[2] the author believed that there was merit in attempting to minimise or eradicate the infestations from captive merlins in the UK. Toltazuril (Baycox, Bayer) at 20mg/kg once weekly for 3 weeks is the only therapy which has been effective in achieving this final response, although some birds have had to be treated several times. Clazuril (Appertex, Petlife) although previously recommended[2] has proven ineffective. With this exception, all treatments led to a cessation of clinical signs and a temporary halt to oocysts shedding. However long term monitoring has proven these regimes to be non efficacious contrary to previous findings.[2,3,6,7] It is generally accepted that oocysts remain viable in the environment for a considerable period (at least 15 months),[9] and that to effect full control environmental cleansing is essential. However in this survey when a second group was treated with toltazuril (Baycox, Bayer) 25mg/kg and moved to previously uncontaminated accommodation their response to therapy was no better than the group which had been treated and left in a contaminated area. Furthermore when the same level of toltazuril is administered once a week for 3 weeks, a dramatically improved response rate is achieved. The results add weight to the view previously expressed[2] that the oocysts are resistant to this treatment at certain stages of the parasite development cycle. Although even with the most effective therapeutic regime there was a significant failure rate, it is noteworthy that the initial incidence of positive samples of 48%, had reduced to just 7% by early 2000. Other workers have demonstrated the development of variable levels of immunity and duration of patency with different *Caryospora* spp. in different falconidae host species.[3,6,7] In this study oocyst shedding by juvenile birds has been maintained for longer than previously reported[3,6,7] indicating a slower or less complete development of immunity. Two birds sampled in this project demonstrated overt clinical disease, with level 3 shedding, caused by the same species of *Caryospora* within a 5 month period. This is contrary to previously published expectations.[3,6,7] This finding indicates that a solid immunity is not maintained in all merlins for a period of five months. The finding of a high incidence in captive-bred birds is as expected.[2,3,6,7] A zero incidence in wild birds is consistent with the views of some authors[2,8] but contrary to others.[4,9,10] Although the clinical effect of coccidiosis is expected to be much greater in a confined area and less significant in a free living situation, it is considered that the loss or release of infested captive bred birds could pose a threat to an already threatened endogenous free living population. The intermittent nature of oocyst shedding by infested birds indicates that sample collection over a three day period has a greater chance of detecting positive cases. Although Kuhl[3] treated 16 kestrels (25mg/kg toltazuril, Baycox, Bayer) none demonstrated any abnormalities in general health, faecal consistency, feeding, moulting or behaviour. In this survey, a small minority of birds demonstrated reduced flight ability and general malaise, a slight croakiness and change of vocalisation for a period of up to 48 hours following a single oral administration of 25mg/kg. However no birds showed any significant or long term effects and this therapeutic agent is considered safe in this species at doses up to 25mg/kg po. In this survey infestation of breeding birds has been eradicated in many cases as judged by repeated faecal monitoring and oral therapy which enabled *Caryospora* free off spring to be produced. The use of parasite free foster parents or hand rearing has also enabled parasite free offspring to be produced. The process of allowing or creating *Caryospora* infection in young birds and treating them during the pre-patent period shows promise as a clinical technique to produce un-infested young birds with immunity against the parasite.

However the current project has not proven that infection did occur or that immunity was generated. Additional research is on going to further investigate control of this parasite.

Acknowledgements: The author would like to thank the Department of The Environment Transport and Regions for despatch of requests for samples and providing data on numbers of merlins in captivity. Jenny Morgan and Lansdown Veterinary Surgeons for laboratory assistance. Graham Rebecca and Ron Downing for the supply of wild merlin faecal material. The keepers of all the birds sampled for their co-operation and assistance.

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Figure 1. Rate of shedding during the first 18 months of life

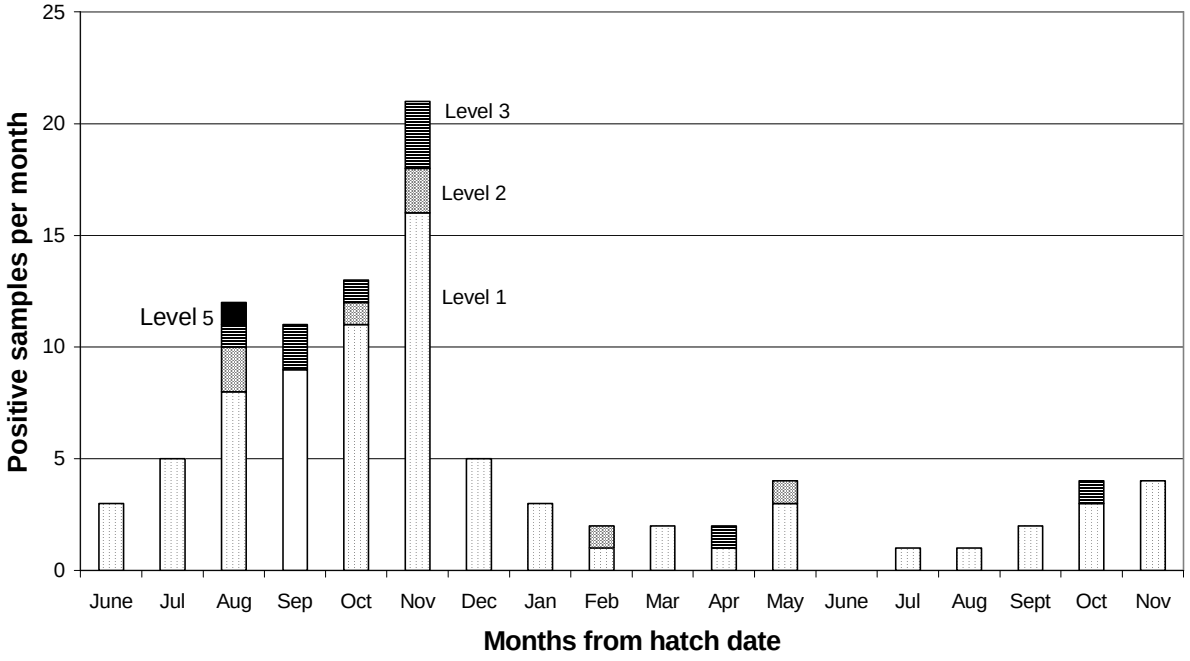

Figure 2 Monthly Oocyst Excreting incidence excluding birds <6m

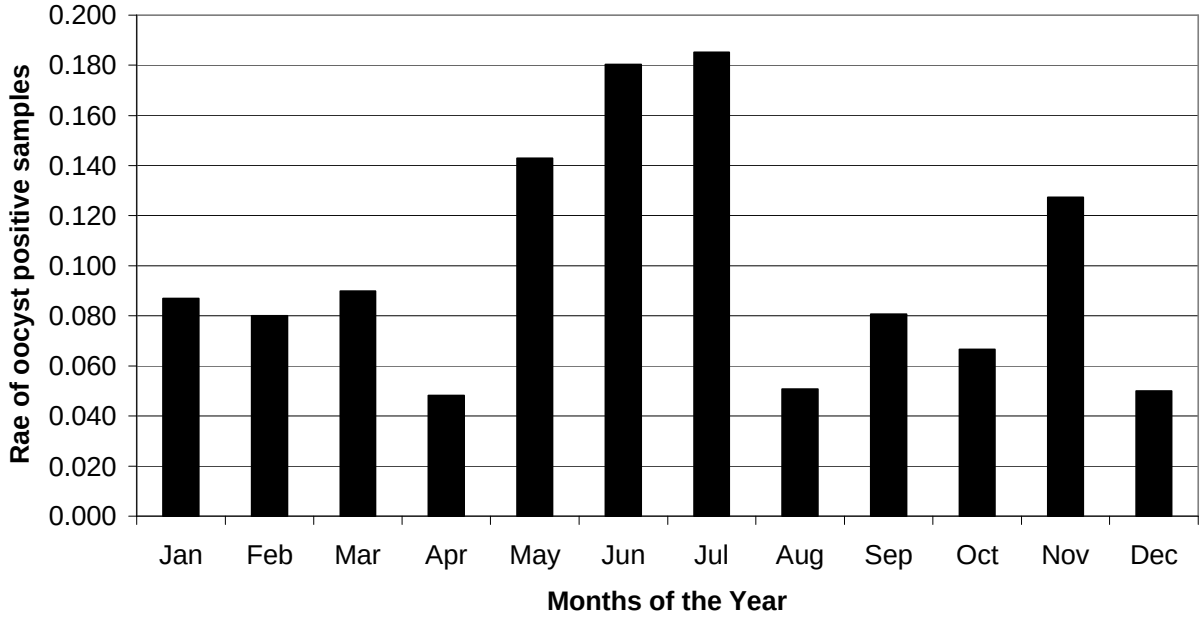

Figure 3. Rate of Oocyst positive samples by month all ages

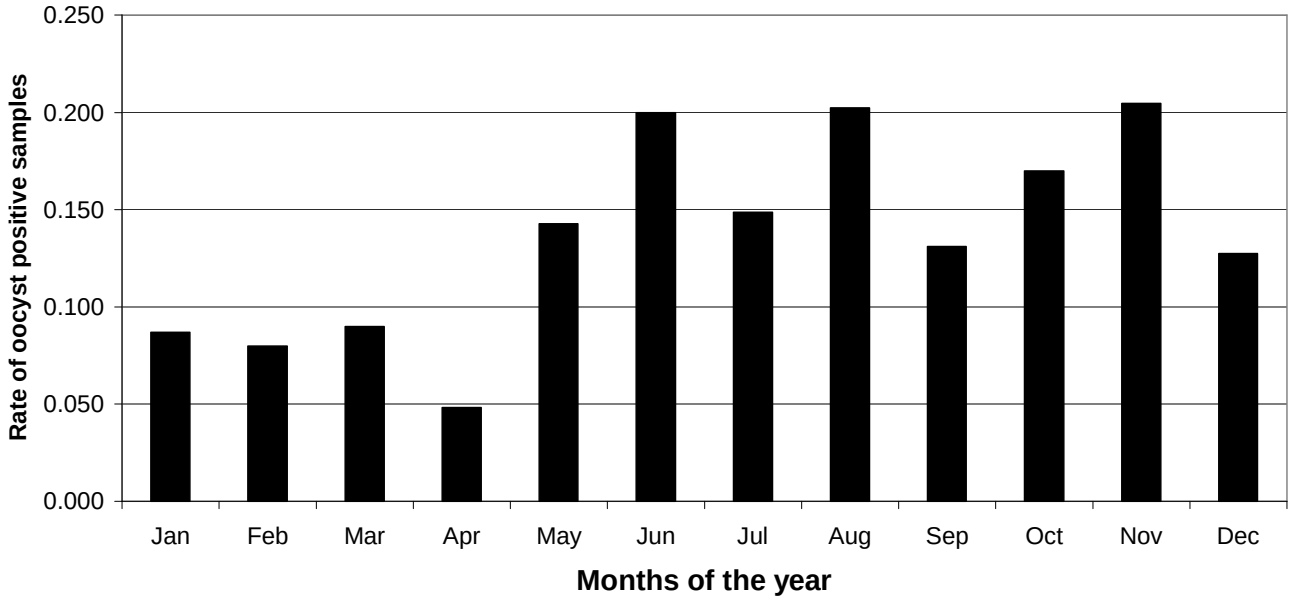

Figure 4. Therapeutic Efficacy