ZEBRA GRANT PROJECT

PREVALENCE OF *ENCEPHALITOZOON CUNICULI* IN A POPULATION OF WILD RABBITS IN NORFOLK, ENGLAND

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Abstract

Serum samples from 27 wild rabbits (*Oryctolagus cuniculus*) and two wild brown hares (*Lepus europaeus*) were tested for the presence of antibodies to *Encephalitozoon cuniculi* using an indirect enzyme linked immunosorbent assay. Brain and kidney samples from the same animals were also examined for the presence of lesions consistent with encephalitozoonosis. The sample population included in the survey consisted of any rabbits or hares presented to the Royal Society for the Prevention of Cruelty to Animals Wildlife Centre in East Winch, Norfolk, England over a time period of approximately nine months in 2006. All animals included in the survey were those that had to be euthanased due to injury, infection or any other trauma. None of the rabbits or hares sampled showed a positive antibody titre for *E. cuniculi* at a serum dilution of 1:20. One rabbit showed an equivocal weak positive at a serum dilution of 1:10 where a positive reaction at a dilution of 1:20 is accepted as a true positive. None of the tissues examined had lesions that were consistent with *E. cuniculi* infection. This result suggests that either *E. cuniculi* is not present in the wild rabbit population in Norfolk or is present at a prevalence rate of less than 10.5 %. A larger sample size covering different regions of the country would be necessary to assess whether the parasite is present in the wild rabbit population in England.

Introduction

Encephalitozoon cuniculi is a single-celled, obligate intracellular protozoal parasite of the genus Microsporidia. There are over 1000 species of Microsporidia, which are known to infect all major animal groups. Most species infect invertebrates, but many have been shown to infect mammals and several can infect humans. *E. cuniculi* is the species most commonly reported to infect mammals and can infect many different hosts including mice, guinea pigs, hamsters, dogs, cats, foxes, mink, monkeys, sheep, goats and humans (Didier, Didier, Snowden & Shadduck 2000, Hollister, Canning & Willcox 1990, Didier, Vossbrinck, Baker, Rogers, Bertucci & Shadduck 1995). The resulting disease appears to be more severe in monkeys and dogs (Percy and Barthold 2001). Several *Encephalitozoon* species have been recognised as being zoonoses, including *E. cuniculi*. They have been reported as causing opportunistic infections in immune compromised people, predominantly acquired immune deficiency syndrome (AIDS) patients or organ transplant recipients (Hollister and others 1990). Infection presents as diarrhoea, renal disease and keratoconjuctivitis. They have also recently been documented as causing diarrhoea in healthy individuals including adults travelling abroad and children (Didier and others 2000).

Microsporidia are characterised by producing resistant spores. The spores contain a polar filament that pushes through the host cell wall allowing the spore contents to gain entry into the target host cell, where they differentiate and multiply. The host cell eventually ruptures, releasing new spores, which can infect other host cells or be passed into the environment via urine, faeces or respiratory secretions, with infection usually occurring through ingestion or inhalation of spores (Didier, Snowden & Shadduck 1998, Pakes and Gerrity 1994). The main route of infection of *E. cuniculi* is oral although transplacental transmission and inhalation are also reported (Baneux and Pognan 2003; Cox, Pye, Edmonds & Shepherd 1980, Didier and others 1998). After ingestion, the organism is carried in the blood to target organs, primarily the central nervous system (CNS) and kidneys, however liver, lung, heart and lens may be involved (Percy and Barthold 2001). Eye lesions have been associated with intrauterine infection in rabbits (Harcourt-Brown 2004). The eventual rupture of host cells in these target organs leads to inflammatory reactions with granuloma formation. This results in focal granulomatous meningoencephalomyelitis with astrogliosis and perivascular lymphocytic inflammation in the CNS and granulomatous interstitial nephritis in the kidney (Percy and Barthold 2001).

The primary host for *E. cuniculi* is the rabbit, with antibodies first detectable in rabbit serum about three weeks post infection. Peak titres occur at six to nine weeks after infection. Serum antibodies are detectable at least two weeks before organisms can be demonstrated intracellularly and four weeks before histopathological changes can be found in the kidney. Lesions are not usually identifiable in the brain until eight weeks after antibodies are present in the serum (Cox and Gallichio 1978, Harcourt-Brown 2001). Laboratory kits have been shown to have protective maternal antibodies, which wane after weaning (Bywater and Kellett, 1978, Lyngset, 1980). Spores can be detected in urine samples four weeks after infection and large amounts of spores are excreted until two months post infection drifting to undetectable amounts by three months post infection. Spores can remain viable for at least six weeks at room temperature (Percy and Barthold 2001).

While the organism is usually attributed with causing a vague, subclinical disease, outbreaks of severe clinical disease have been reported, primarily affecting the CNS, kidneys or lens (Harcourt-Brown 2004). The subclinical presentation has been shown to depress the immune system and therefore interfere with interpretation of experimental test results (Cox 1977; Waller and others 1978). *E. cuniculi* has recently been recognised as a common cause of clinical disease in pet rabbits in the U K, causing CNS impairment of varying severity, with the most commonly noted clinical signs being vestibular disease or head tilt (Harcourt-Brown and Holloway 2003). Renal disease is also common with scarring of the kidney noted at post mortem. Although renal disease does not usually cause renal failure, occasionally weight loss, polydypsia and polyuria with haematological and biochemical evidence of renal failure are present (Harcourt-Brown 2004). Ocular lesions can also occur with the parasite localising in the lens, leading to anterior uveitis and cataract development (Stiles, Didier, Ritchie, Greenacre, Willis, and Martin, 1997).

E. cuniculi was first discovered in 1922 as the cause of motor paralysis in laboratory rabbits. Lesions and microorganisms were identified in the brains and kidneys of affected rabbits along with lesions in the spleen, liver and myocardium. Investigators also identified organisms in the urine and suggested that the route of transmission was via the urine (Wright and Craighead 1922). There was originally discrepancy among taxonomists as to the classification. In 1923, Levaditi and others identified the parasite as a microsporidian and recommended the name *Encephalitozoon cuniculi*. In the 1960s, it was determined *Encephalitozoon* should be in the Nosematidae family and was named *Nosema cuniculi*, as it was known until 1970 when electron microscopy revealed that the diplokarya characteristic of the genus *Nosema* were not present and *Encephalitozoon cuniculi* was agreed upon as the name (Didier and other 1998, Pakes and Gerrity 1994). There are three strains of *E. cuniculi* identified that can be distinguished by molecular means. Strain I is most often associated with rabbits, strain II with rodents and strain III with dogs. *E. cuniculi* strains isolated from humans in the United States have been identified as strain III while in Europe human isolates have been identified as strain I (Didier and others 1995).

Literature Review

The prevalence of *E. cuniculi* in laboratory rabbit populations in the United Kingdom (UK) and abroad has been known for many years with serological screening to cull infected animals from colonies (Cox 1976, Shadduck and Pakes, 1971). A survey of laboratory rabbit colonies in the UK in 1980 reported seroprevalences ranging from 25 to 95 percent (Gannon 1980). Recent surveys have shown serologically that the exposure to *E. cuniculi* is high in clinically healthy pet rabbits in the UK and that the parasite is causing disease in pet rabbits (Harcourt-Brown and Holloway 2003, Keeble and Shaw 2006). In one study in North Yorkshire, 125 pet rabbits were blood sampled. Populations were divided into asymptomatic rabbits (38 rabbits) and rabbits with clinical signs of possible encephalitozoonosis (87 rabbits). Six of the 26 (23 %) asymptomatic rabbits screened were found to be seropositive for *E. cuniculi*. Eight more asymptomatic rabbits were found to be seropositive in a group of 12 rabbits subsequently screened because of close contact with one of the seropositive rabbits. Sixty-nine percent (60 of 87) of the rabbits with clinical signs were seropositive, including 91 percent of the rabbits presenting with head tilt and all nine rabbits presenting with ocular lesions (Harcourt-Brown and Holloway 2003). These results indicate that *E. cuniculi* is present in pet rabbits. Although it is not possible to determine if this seropositivity indicates that the rabbit has been exposed and is clinically affected, is an asymptomatic carrier or has eliminated the parasite after mounting an immune response, the high percentage of seropositive individuals in those showing clinical signs suggests that the parasite is causing disease (Harcourt-Brown and Holloway 2003). Another more widespread survey of clinically healthy rabbits over all of the UK found a seroprevalence of 52 percent (50 of 97 rabbits) (Keeble and Shaw 2006). In this study the sample population was obtained by offering a health screen at a reduced fee for rabbits presented as new clients or pre-operative for rabbits undergoing routine surgical procedures. Keeble and Shaw (2006) point out that this is not a random sampling of the healthy population as many healthy pet rabbits are never brought to veterinary surgeries. However, it does show that a significant percent of the rabbits sampled had been exposed to *E. cuniculi*, whereas the previous study by Harcourt-Brown and Holloway (2003) reported only 23 percent of healthy rabbits as seropositive.

The first report of encephalitozoonosis in a wild rabbit was an isolated case in the USA in 1955, in which a rabbit dying of natural causes was found to have focal encephalomyelitis with organisms believed to be *E. cuniculi* present in the tissue (Jungherr 1955). There have been previous reports of *E. cuniculi* exposure in wild rabbits in the UK with three rabbits from Scotland being found to be seropositive in 1979 (Wilson 1979). Wilson (1979) also reported the organism in a wild fox as a subclinical infection, the fox having died for other reasons (Wilson 1979). In 2002, *E. cuniculi* was reported in free-ranging rats (*Rattus norvegicus*) where three of 23 tested wild rats were found to be seropositive (Müller-Doblies, Herzog, Tanner, Mathis and Deplazes, 2002). Müller-Doblies also demonstrated that laboratory rats were susceptible to oral infection with spores of *E. cuniculi*. Wild mice in Iceland have also been shown to have antibodies to the parasite (Hersteinsson, Gunarsson, Hjartardóttir and Skírnisson, 1993). This data suggests that wild animals are capable of being infected with *E. cuniculi* and that a reservoir of infection in the wild exists. Others have reported encephalitozoonosis in carnivores (Nordstoga 1972, Hersteinsson and others 1993, Hůrková and Modrý, 2006), including a prevalence of 13.3 percent reported in stray dogs in the UK (Hollister and others 1989). It has been

suggested that the source of infection in carnivores is feeding on infected rabbits or other rodents (Nordstoga 1972; Vavra and Blazek 1971).

In 1980, surveys in the UK and Australia either did not find seropositive individuals or found low seroprevalence in wild rabbit populations (Cox and others 1980; Cox and Ross 1980). Of 175 wild rabbits from England and Scotland tested in 1980 none showed significant serum antibody titres to the parasite by indirect immunofluorescence, where a positive titre was defined as a reaction at a serum dilution of 1:10. Two of the rabbits tested did have weak positive reactions at a serum dilution of 1:5 (Cox and Ross 1980). A similar result was reported in a survey in Australia in 1980, which also failed to find any significant antibody titres to *E. cuniculi* by indirect immunofluorescence. Of the 823 wild rabbits and 46 wild brown hares surveyed, 12 rabbits and four hares had weak positive reactions (titre of 5) but no lesions consistent with encephalitozoonosis were found in kidney sections from these individuals. The same study, however, demonstrated experimentally that wild rabbits were susceptible to infection via oral administration of spores (Cox and others 1980). In 1997, a new survey of the wild population in Western Australia was undertaken and exposure to *E. cuniculi* was confirmed. The serological prevalence was found to be 25 % (20 seropositive out of 81 rabbits tested) (Thomas, Finn, Twigg, Deplazes and Thompson, 1997). Thomas and others (1997) were also able to culture spores typical of *E. cuniculi* from brain tissue of some of the seropositive rabbits.

Given the recent results in Australia, a new survey of the population in the UK would be warranted, as it is possible that the pattern of disease in wild rabbits has changed since the last study was carried out in the UK. Also, the presence of the parasite in pet rabbits has recently become a significant disease issue. This is either because the incidence of the disease has increased or simply because it is being recognised and diagnosed more frequently (Harcourt-Brown and Holloway 2003, Keeble and Shaw 2006). It is not known whether infection clinically affects wild rabbits significantly or whether wild rabbits can be asymptomatic carriers acting as reservoirs of disease for pet rabbits and other mammals, including humans. Similarly, pet rabbits could act to transmit the disease to the wild rabbit population. With the potential for zoonotic disease in immunocompromised humans, it would be beneficial to be aware of the role of wild animals as reservoirs of infection or as sources of contamination of the environment with spores. Knowledge of the status of the disease in the wild population would allow for these risk factors and relationships to be investigated. Thus, a survey of wild rabbits in the UK is needed to determine the prevalence of *E. cuniculi* exposure.

Materials and Methods

Collection of samples: Between April 2006 and December 2006, sera and tissue samples were collected at the Royal Society for the Prevention of Cruelty to Animals (RSPCA) Wildlife Centre in East Winch, Norfolk, UK by the RSPCA veterinary surgeons. The sample population consisted of rabbits (*Oryctolagus cuniculus)* and hares (*Lepus europaeus)* that were admitted to the East Winch Wildlife Centre during this time period that had to be euthanased for any reason including myxomatosis infection, following trauma from a road traffic accident or any other severe trauma. Rabbits were euthanased by injection of sodium pentobarbitone solution. Tissues taken for examination included whole brain and kidneys and were stored in 10 % formal saline at room temperature until examination. Blood samples were cardiac samples obtained post mortem. Approximately 2 ml of blood were collected, instilled into serum gel tubes and centrifuged. The serum was decanted and stored at -20 ºC.

Sera: Serology for antibodies to *E. cuniculi* was performed at the Diagnostic Laboratories, Clinical Services Division at the Royal Veterinary College using an indirect enzyme linked immunosorbent assay (iELISA) to test for antibodies to *E. cuniculi* with a commercial *E. cuniculi* antigen by European Veterinary Laboratories (EVL). Positive and negative controls were run. Results were reported as negative or positive with a titre reported for any positive reactions. A positive result was reported if there was a reaction to a 1:20 dilution or higher of serum. An equivocal result or weak positive result was reported if there was a reaction to a 1:10 dilution of serum. A negative result was reported if there was no reaction to the 1:10 dilution.

Tissues in formal saline: Gross examination of organs at post mortem was conducted. The collected tissues were fixed in 10 % formal saline for at least one week followed by sectioning. Cross sections of brain tissue were made in three equally spaced regions encompassing all major regions of the forebrain, midbrain and hindbrain. These sections were fixed for two to three days prior to routine histological processing. Kidneys were bisected longitudinally prior to fixing for two to three days. Sections were stained with haematoxylin and eosin (H & E) and examined for evidence of *E. cuniculi* infection including focal granulomatous meningoencephalomyelitis with astrogliosis and perivascular lymphocytic inflammation and granulomatous interstitial nephritis. Any suspected lesions were measured and location noted. Sections containing such lesions were stained using the Brown and Brenn Gram stain for identification of any spores or organisms that may have been present.

Data Analysis: The sample size, *n*, for estimation of the prevalence of the disease in a large population of unknown size was formulated using, $n = 1.96^2 P\text{exp} (1- P\text{exp}) / L^2$, where L is the absolute precision (5 % was chosen in this case) and

the confidence interval is 95 %. *P*exp is the expected prevalence of disease and 25 % was assumed in accordance with the results of Thomas and others (1997) (Thrusfield, 2005).
The software program Win Episcope 2.0© (Blas, Ortega, Frankena, Noordhuizen and Thrusfield, 1998) was used to calculate the number of individuals which would need to be sampled to detect at least one positive individual with a probability of 0.95. This program was also used to determine the maximum probable prevalence given that all individuals tested were found to be negative with a confidence interval of 95 %.

Results

A total of 27 rabbits (*Oryctolagus cuniculus)* and two brown hares (*Lepus europaeus)* were included in the study. The most common reason reported for euthanasia was complications relating to myxomatosis virus (10 rabbits). Four rabbits had injuries resulting from a road traffic accident and four rabbits had injuries from being attacked by a carnivore. Two rabbits were orphaned, one had been caught in a trap, one young rabbit had its nest destroyed by humans and three rabbits had injuries of unknown origin. For two rabbits the reason for euthanasia was unreported. The ages and genders of the rabbits and hares (**Table 1**) included in the study are listed below. A more complete table of results is listed in **Appendix 1**.

Table 1. Age and Gender of Rabbits *(Oryctolagus cuniculus)* and Brown Hares (*Lepus europaeus)* tested for *E. cuniculi*.

	Male	Female	Gender Unknown	Total
Rabbits				
Neonate	-	1	1	2
Juvenile	3	2	8	13
Adult	5	4	3	12
Hares				
Juvenile	1	-	1	2
Total	9	7	13	29

None of the rabbits or hares were found to be seropositive for the presence of antibodies to *E. cuniculi* by iELISA at a titre of 1:20. One rabbit showed a weak positive result at a serum dilution of 1:10. This equivocal result was from a rabbit reported to be a juvenile but of unreported gender or reason for euthanasia. One rabbit did not have serum tested due to the sample being a lipaemic sample, which would not separate. Also, none of the brain or kidney H & E stained tissue sections examined were found to have pathological lesions consistent with previous or current *E. cuniculi* infection. One forebrain tissue section had a region of very high cellularity, which was a suspected lesion although it appeared periventricular. This section was stained using Brown and Brenn Gram stain for identification of any organisms but none were found and it was concluded that the area in question was not a result of *E. cuniculi* infection.

To estimate the prevalence in our Norfolk population, with 95 % confidence and an absolute precision of 5 %, it would be necessary to sample 289 rabbits, assuming an expected prevalence of 25 % as was found in Australia (Thomas and others, 1997). Similarly if a prevalence of 5 % is assumed, 73 rabbits would be needed (Thrusfield, 2005).

To detect disease, rather than determine prevalence, only 11 animals would need to be tested in order to detect at least one case with a probability of 0.95, assuming the prevalence of disease in an infinite population is 25 %, The probable maximum level of the disease in the rabbit population given that all 27 individuals sampled were found to be negative was calculated to be 10.5 % (Blas and others, 1998).

Discussion

The recent finding of *E. cuniculi* in 52 % of pet rabbits in the UK indicates that the parasite may either be becoming more prevalent or is being recognised more readily by clinicians as causing some of the common symptoms of pet rabbits presented to veterinary surgeries (Keeble and Shaw, 2006). It would be reasonable to assume that the prevalence of the parasite in the wild population was lower than that found in laboratory or pet population due to the housing conditions of laboratory and pet rabbits, since the main method of transmission of *E. cuniculi* is thought to be through oral ingestion of spores transmitted via urine. Laboratory and caged pet rabbits would have a higher incidence of environmental contamination than is likely in wild rabbits, as wild animals can leave the burrow to urinate. While wild kits are likely to be exposed to infection via close contact with maternal urine, it has been shown in laboratory populations that kits have protective maternal antibodies to the parasite until the age of weaning, which may help prevent infection at an early stage (Bywater and Kellet, 1978, Lyngset, 1980). Even so, the prevalence of exposure in wild rabbits in Australia was found to be 25 % (Thomas and others 1997).

With the findings of high prevalence of the parasite in the pet rabbit population (Harcourt-Brown and Holloway, 2003, Keeble and Shaw, 2006), it would be plausible to suspect the presence of the parasite in wild populations. Infection could be transmitted to wild rabbits through contact with pet rabbits housed outdoors. Furthermore, with the recent report of a significant prevalence in wild rabbits in Australia (Thomas and others, 1997), it was expected to find *E. cuniculi* in the rabbits sampled in our survey of rabbits in the UK, even though a previous serological survey of wild rabbits in England and Scotland did not find evidence of significant exposure (Cox and Ross 1980). However, we did not find any cases of *E. cuniculi* in the wild rabbits or hares tested. The reasons for not finding the parasite in the wild population can be either because it is not present in the population, is present at a very low level or that the sample type and size was not appropriate or sufficient to detect exposure to the parasite.

The sample population used in this study was a convenience sample with a specified time period available for collection and analysis of samples. Therefore, sample size was dependent upon the number of rabbits admitted to the RSPCA East Winch Wildlife Centre for that specified time period. Due to the fact that this was a convenience sample it was not properly randomised such that not all units of the population had an equal opportunity of being included in the sample. The sample is not truly representative of the target population because it is biased towards diseased and possibly diseased individuals, as these are the individuals most likely to be caught and presented to the RSPCA. This was the case in the current study with 10 of the 27 sampled rabbits (37 %) having signs of myxomatoma virus infection. The remaining rabbits were involved in road traffic accidents or attacked by carnivores and thus may have been in a compromised physical state that allowed them to be attacked more easily by predators or not as able to escape predation or vehicles. It would be more probable of finding *E. cuniculi* infection in our sample population then in the healthy wild population, as diseased individuals would be more likely to have a higher parasite burden and succumb to the symptoms of infection. This method of sampling would give an overestimation of the prevalence of the disease. However, none of the rabbits or hares examined showed strong positives antibody titres and only one rabbit was found to have a weak positive result. Also, no characteristic lesions of encephalitozoonosis were found in any of the rabbit or hare tissues examined.

The two brown hares were included in the study because they came into the RSPCA during the specified time period. There is not much published information on the disease in hares but Cox and others (1980) also sampled wild hares in Australia in 1980 and although none had significant titres at that time, four hares did have weak positive reactions, which could indicate exposure to *E. cuniculi*. Our study only included two hares, which were both found to be negative for *E. cuniculi*. As it was such a small population it is difficult to draw conclusions for the presence of disease in brown hares.

Sample size itself may have been the biggest factor contributing to not finding the presence of *E. cuniculi* in the wild rabbits in the UK. Due to the use of convenience sampling and time constraints, it was not possible continue sampling until a predetermined critical number of individuals had been sampled. This resulted in only sampling 27 rabbits and sampling from only one region of the country. With such a small sample size, there is greater risk of the result being unusual just by chance. To detect a prevalence of 25 % with 95 % confidence, 289 rabbits would need to be tested. Even with a prevalence of 5 %, 73 rabbits need to be tested. Therefore, it is possible that the current sample size was not large enough to detect the true prevalence of *E. cuniculi* exposure in the wild population of rabbits sampled. The fact that the sample population came from only one region of the country could also have had an effect on detecting the presence of *E. cuniculi*. Although Keeble and Shaw (2006) did not report a significant difference in the prevalence of the disease in different regions of the country in pet rabbits, the current result of zero prevalence can only be applied to the region surveyed and the prevalence may differ throughout other regions of the country.

The results do suggest that the prevalence of *E. cuniculi* in the wild population sampled in Norfolk is less than that found in the wild population in Australia and much less than that in the pet rabbit population in the UK. Even with the small sample size used, it should have been possible to detect the presence of even one case of disease in the population sampled. Only 11 individuals need to be tested to detect one case of disease at a prevalence of 25 %. Therefore, testing of 27 rabbits should have been able to detect *E. cuniculi* if the prevalence was 25 %. The same calculation can be used to estimate the probable maximum level of disease in a population given that all individuals sampled were found to be negative for the disease. Analysing the results in this way indicates that if 27 individuals were tested and found to be negative for *E. cuniculi*, then the maximum probable prevalence of *E. cuniculi* in that population is 10.5 % (Thrusfield, 2005, Blas and others, 1998). Accordingly, the results suggest with a probability of 0.95 that the maximum prevalence of disease in the wild rabbit population around East Winch, Norfolk is less than 10.5 %.

Very young rabbits may not have been exposed to *E. cuniculi* or developed detectable levels of antibodies. They would then be negative on serology and have no lesions on histopathology. In any very young rabbits it is possible that antibodies passed from the does protect them from infection. Bywater and Kellet (1978) demonstrated that newborn laboratory rabbits can have high titres for *E. cuniculi* which later drop to undetectable levels, indicative of the presence of maternal antibody. Lyngset (1980) showed a similar result with the presence of maternal antibody detectable up to four weeks of age. Rabbits were then seronegative from four to eight weeks of age and some showed seropositivity

again after eight weeks, indicating exposure and active immunity developing at that time (Lyngset 1980). This may explain the one weak positive result found in the juvenile rabbit, although it is not possible to know the exact ages of the rabbits in the current study. With adults more likely to have been exposed and developed antibodies to *E. cuniculi* than juveniles, the likelihood of finding encephalitozoonosis in the population would be even less since the sample population consisted of more juveniles and neonates (57 %) than adults.

After exposure, antibodies are detectable at around three weeks with a peak antibody titre generally at six to nine weeks post infection (Cox and Gallichio 1978). The individuals in our population could have not reached detectable levels of antibodies or passed the period of peak antibody titres. Using both serology and histopathology to screen for encephalitozoonosis was done in order to pick up any rabbits, which had been infected previously but no longer had sufficient antibody titres, yet none of the rabbits had identifiable lesions. Due to the type and size of sections used it is possible to have missed lesions or organisms in the tissues examined. Although sections were made in such a way to maximise the surface area examined for lesions and encompass all regions of the kidneys and brain, other organs were not screened for lesions and/or organisms. *E. cuniculi* has also been found to cause lesions in other organs including the eyes and liver, although the CNS and renal system are considered the predominantly affected organs.

One difference in this study and the survey by Thomas and others (1997) was the type of test used for serology. Thomas and others used an indirect immunoflourescence antibody test (IFAT) using spores of *E. cuniculi* derived from in vitro culture. The test used in our survey was an iELISA with a commercial antigen (EVL). However, positive and negative controls were run and the same ELISA and commercial antigen were used in the survey of pet rabbits by Keeble and Shaw (2006), which found a 52 % seroprevalence of *E. cuniculi*. This indicates that the current protocol used was more than sufficient to identify the parasite. Also a comparison of serological assays for *E. cuniculi* by Boot and others (2000) found that there was no difference between IFAT and ELISA in detecting positive sera.

It was suggested by Cox and others (1980) that *E. cuniculi* infection put wild rabbits at such a 'biological disadvantage for survival' that natural selection pressures had eliminated the infection from wild rabbits. This could explain the current result, although it seems unlikely. A similar theory could be applied to mxyomatosis virus, which is prevalent in the wild population and has not been eradicated by natural selection. Also, most research indicates that encephalitozoonosis most often results in a mild or subclinical disease. While immune suppression is expected to affect the survival of wild rabbits, it does not seem likely to be able to assert enough selection pressure to cause self eradication from the population. Such a theory is more plausible with a rapidly lethal organism.

It is conceivable that *E. cuniculi* is present in the wild rabbit population in England although it appears to be present to a much lesser degree than has been found in pet rabbits in the UK and in wild rabbits in Australia. To confirm the results of this study it will be necessary to carry out more surveys with larger sample sizes. Subsequent surveys would also be recommended to help monitor the prevalence trends of the disease in the wild because there is still a possible risk of infection being transmitted from the domestic rabbit population to the wild population. Continued monitoring is also necessary as the pathogen is known to be a zoonosis.

This is the first survey of wild rabbits in the UK in 25 years. The results suggest that *E. cuniculi* is either not present in the wild population in East Winch, Norfolk, at this time or is present at a prevalence of less than 10.5 %. Future surveys with a larger sample size and encompassing different regions of the county are necessary before claiming that the disease is not present in the wild rabbit population of the UK.

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Appendix 1

Summary of Results

	Age*	Wt (kg)	Sex	Reason for Euthanasia	Location found
1	J	0.172		Attacked by carnivore	Shernbourne, Norfolk
2**	Adult	0.969		RTA	West Dereham
3	J	0.171		RTA	East Rudham
4	J		M	Myxo	King's Lynn
5	Adult	1.116	M	Attacked by carnivore	Hunstanton
6	N	0.057		Attacked by Carnivore	King's Lynn
7	Adult	0.805	F	RTA	Marham
8	N	0.137	F	Home destroyed by digging – human	Whittlesey
9	J	0.326	M	Trapped	East winch
10	J	0.188	F	Injury unknown	Coldham
11	J	0.261	M	Injury unknown	King's Lynn
12	J	0.11			Norwich

13	**J**	**0.107**			**Norwich**
14	J	0.131		Orphan	Norwich
15	J	0.111		Orphan	Norwich
16	Adult	1.462	F	Myxo	Upwell
17	J	0.178		RTA	Anmer
18	J	0.194	F	Attack by Carnivore	Dunham
19	Adult	1.25	F	Myxo	RAF Harham
20	J			Myxo + injury	Wormegay
21	Adult			Myxo	Bury St. Edmunds
22	Adult	1.22	M	Myxo	Outwell
23	Adult	0.867/0.790		Injury unknown	Blackborough end
24	Adult	1.257	M	Myxo	King's Lynn
25	Adult	0.952	F	Myxo	
26	Adult	1.366	M	Myxo	Terrington St. John
27	Adult	1.326	M	Myxo	Heacham
Hare1	J	1.429	M	RTA	Litcham
Hare2	J	0.293		Found recumbent	Sporle

Entry in Bold gave an equivocal weak positive antibody titre to *E. cuniculi*

*Age classes – Neonate, Juvenile, SubAdult, Adult, Aged

* No blood tested – lipaemic/would not separate

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ASSESSMENT OF THE WELFARE STATUS OF A SAMPLE OF CAPTIVE ELEPHANTS IN KERALA

Shilpi Prasad

Abstract

This study assessed the welfare status of 34 captive elephants in Kerala, South India. Information was collated on various factors such as age, type of bedding, diet, and number of hours worked per day by each elephant. Scores were given to each of the elephants for sores caused by chains, pressure sores and the general condition of the elephants' feet. It was found that there was no correlation between the age of the elephants and the severity of the foot problems, and no correlation between the age of the elephants and the incidence of heel cracks. Nail cracks were a significant problem and were seen in 23.5% of elephants. Heel cracks were another major problem and were seen in 64.7% of cases. However my conclusion overall is that the captive elephants in this study were kept in good condition.

Introduction

In many traditional elephant-keeping establishments in South-east Asia, captive elephants (*Elephas maximus*) do not enjoy what Webster (1984) terms "the five freedoms" (freedom from malnutrition, from thermal and physical discomfort, from injury and disease, from fear and stress, as well as freedom to express most normal patterns of behavior). Hence the maintenance of captive working elephants is often accompanied with ethical dilemmas associated with traditional management practices, welfare and conservation of the species.

The psychological and physical health of captive elephants can be influenced by many factors including environment, diet and management (Csuti and others 2001). Addressing inadequacies in the environment which predispose captive elephants to trauma can lead to significant improvements in welfare, as can the provision of appropriate social and foraging opportunities (Veasey 2006). Nutrition is essential in the management of captive elephants (Hatt and others 2006) and if there are any deficiencies or toxicities in the diet, this can affect the health of the animal (Crissey 2005). Management is also important as elephants need contact with conspecifics to ensure they have adequate social behaviour (Fowler and others 2003).

Csuti and others (2001) have identified that foot problems are the principal cause of ailments in elephants and this is why foot care is the most common procedure carried out in elephants (Fowler and others 2003). As noted by Csuti and others (2001) lack of exercise causes severe problems in elephants' feet. Additional lesions may be caused by other factors.

The purpose of this study was to:
- Record the type of superficial injuries in elephants and to investigate the factors associated with these injuries.
- Collect data regarding the age of the elephant.
- Collect data regarding the design of the holding facilities and availability of food and water.
- Record the number of hours worked per day by the elephants.
- Document the type of restraining methods if any used by the elephant owners.
- Document the owners' attitude towards modern veterinary practices by gaining information regarding medical treatment of the elephants and the owners' willingness to seek veterinary advice.
- Get practical experience on wound-handling methodology.

Materials and Methods

The necessary data was collated using a questionnaire-based form for each elephant (see attached Annexe A). The ages of the elephants were identified from records or by consultation with the mahouts. Information was gained about the type of substrate used for bedding in the holding facilities. Availability of food and water was ascertained by discussion with the mahouts. The number of hours worked per day by the elephants was noted, as was the methods of controlling the animals. The mahouts were verbally questioned about their opinions regarding medical treatment of the elephants and when they decided that veterinary intervention was necessary. The degree of sores caused by the chains was assessed and the pressure sores from the elephants rubbing themselves against the ground was established. Information was collated on the condition of the feet with particular notice being made to the nails and soles and also to the degree of lameness, if any, that may have resulted. All these measurements were made using an arbitrary scale of 0 to 5, similar to the body condition score used in livestock, with 0 representing no sores or healthy nails and soles with no lameness, going up to a score of 5 indicating chronic lesions that may have contained pus, or severely damaged nails and soles resulting in considerable lameness. The scoring systems that were used are represented in the tables below:

Sores from chains (recorded for both fore and hind feet)

Score	Condition
0	No sores are visible
1	Minimal superficial abrasions that are only just visible
2	Superficial abrasions are clearly evident
3	The sores have exposed the dermis and there is bleeding
4	The sores are covered by granulation tissue indicating a chronic problem
5	There is a chronic problem and there is also pus present as a draining tract

Pressure sores (recorded for both fore and hind legs)

Score	Condition
0	No pressure sores visible
1	Mild lesions (with smooth or partial corrugation of the skin) where the elephant has made contact with the ground
2	Superficial abrasions of the skin
3	Clear abrasions caused by rubbing against the ground
4	Lesions with open wounds
5	Lesions with thick granulation tissue and chronic abcessation as a result of prolonged pressure

General condition of feet

Score	Condition
0	Healthy soles (foot pads). The nails have no cracks and are slightly rounded to allow even distribution of pressure. The skin above the nail is smoothly attached to the nail with no gap at all. The elephant has no lameness.
1	Healthy soles where the nails have minimal or no cracks or openings. The nail is short and fairly rounded to allow even distribution of pressure. The skin above the nail is smoothly attached to the nail with no gap at all or has a very small gap. The elephant has virtually no lameness.
2	The soles have minimal but definite cracks or openings. Nails have small cracks or openings only just permitting the entry of dirt. The nail is slightly too long but still fairly rounded. The skin above the nail is attached to the nail with a small gap. The elephant has no obvious lameness but due to the slight increase in length of the nail, there may be a slight uneven distribution of pressure.
3	The sole is cracked and there is visible dirt lodged in the sole. The nail is longer than it should be but has not yet developed cracks. The skin above the nails is still attatched to the nail but is dry and dehydrated. The elephant has some minor lameness.
4	The sole is cracked and dirt is lodged in the soles in pockets. The nail is long and has developed cracks. The skin above the nails is poorly attatched to the nail and the skin is dry and dehydrated and has cracks where dirt has also accumulated. There is obvious evidence of lameness and it is clear that weight is not being distributed evenly.
5	The sole is cracked and dirt is lodged in the soles in pockets. By appearance this seems to have been going on for some time. The nail is long and has developed cracks and holes in which dirt is lodged. The skin is now penetrating the foot. The elephant is struggling to walk and there is a clear animal welfare problem.

A median score was calculated for the chain sores (an average of the 2 values from the fore and hind feet) and the same was done for the pressure sores. This gave 3 values out of 5 for each of the factors. Additionally for each elephant, any treatment that was being administered for each of the 3 conditions was noted. This allowed correlations to be made between the ages of the elephants and the respective treatments they received, or between the severity of the conditions and the respective treatments they received. Data was analysed using the SPSS package.

Results

The ages of the elephants ranged from 11 to greater than 70. The data was collated and statistically analysed using a Fisher's exact test. The tables show the correlation between age of the elephants and the general condition of their feet (Table set 1) as well as correlations between age of the elephants and cracked heels (Table set 2).

Table 1a

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
agesubcatergory * generalsubcatergory	34	100.0%	0	.0%	34	100.0%

Table 1b

agesubcatergory * generalsubcatergory Crosstabulation

Count

		generalsubcatergory				Total
		1.00	2.00	3.00	4.00	
agesubcatergory	1.00	4	6	0	0	10
	2.00	10	10	3	1	24
Total		14	16	3	1	34

Table 1c

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	2.176[a]	3	.537	.683		
Likelihood Ratio	3.273	3	.351	.489		
Fisher's Exact Test	1.808			.683		
Linear-by-Linear Association	.460[b]	1	.498	.627	.347	.167
N of Valid Cases	34					

a. 6 cells (75.0%) have expected count less than 5. The minimum expected count is .29.

b. The standardized statistic is .678.

Ta

	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
agesubcatergory * heels	34	100.0%	0	.0%	34	100.0%

Table 2b

agesubcatergory * heels Crosstabulation

Count

		heels		Total
		1.00	2.00	
agesubcatergory	1.00	5	5	10
	2.00	7	17	24
Total		12	22	34

Table 2c

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	1.342[b]	1	.247	.432	.221	
Continuity Correction[a]	.584	1	.445			
Likelihood Ratio	1.311	1	.252	.432	.221	
Fisher's Exact Test				.271	.221	
Linear-by-Linear Association	1.302[c]	1	.254	.432	.221	.159
N of Valid Cases	34					

a. Computed only for a 2x2 table

b. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.53.

c. The standardized statistic is 1.141.

Heel cracks were a major problem with 64.7% of the elephants suffering from this, while 55.9% of them had a score of 3 or greater for the general condition of their feet. 20.6% of the elephants had holes in the soles of their feet and the same percentage had cuticle feathering of some degree. Cracks in the nails were evident in 23.5% of the cases and of these 11.8% had 2 or more cracks in their nails. One elephant had a broken nail of its fifth nail on its right forefoot and a second elephant had loss of part of its fifth nail on its left forefoot. Black tracts were present on the soles of the feet of 4 elephants.

Discussion

Due to the small sample size, the ages of the elephants had to be put into only 2 subset catergories. The data had to be coded to enable use of the SPSS package. Since the ages of the elephants were not always accurate, this had to be coded for recognition. For example an elephant with an age of less than 50 years had to be coded as 50. The chain sores, pressure sores and the general condition of the feet were coded too. These were each separated into 2 catergories.

Overall the welfare status of the elephants was of a high standard. Each elephant has its own mahout that cares for it and in all cases the mahout has worked with the same elephant for several years. The elephants are trained by their mahouts and respond to approximately 45 local commands. All the elephants observed in this study had the opportunity to socialise with conspecifics. Fowler and others (2003) have noted that this is important for the development of correct social behaviour. The elephants were also able to express their normal behaviour such as digging, which strengthens the muscles of the legs and feet and as well as tendons and joints (Csuti and others 2001).

During ***musth*** the elephants are kept chained up continuously for a few months at a time. This causes lesions around the ankles and even though the chains are relocated onto different feet, the lesions do not heal before the chains are replaced around the original foot. Hence the injury is perpetuated and this leads to a chronic problem. It is difficult to solve this problem because ropes are not strong enough to restrain a potentially very aggressive elephant in musth. Additionally since many of the elephants were in musth, it was not possible to go close enough to them to take photographs or to assess any lesions.

Pressure sores occur on the elbows and knees of the elephant from sitting down and rising. This can lead to superficial lesions or chronic lesions with granulation tissue.

The elephants get adequate rest for most of the year. They are only exercised for 3 hours daily during which time they are taken to the water to be washed and are expected to carry their food. During festivals they work 8 hours per day.

Although most captive elephants are fed hay as the basic diet (Fowler and others 1986), the elephants in this study received palm leaves, which is sometimes complemented with treats such as bananas. This appeared to be satisfactory and palm leaves are a cost efficient and readily available source of food. Older elephants or ones that have difficulty chewing due to poor condition of their teeth receive boiled rice. Most of the elephants get water ad lib, but some elephants have water provided to them.

Most of the elephants are kept on a bedding of sand, which due to its abrasive nature prevents overgrowth of the sole. At one temple the elephants were kept on earth. Black tracts were identified in some elephants' feet but these should be trimmed away.

As noted by Fowler (1980) fungal and bacterial pathogens can affect the nails and this was seen in one elephant. The fungus had spread between two of its nails.

Cracks in the nails were common and horizontal, vertical and diagonal cracks were evident. Nutritional and genetic factors may have contributed to this (Fowler 1980). However, Fowler (1978) has commented that the main reason for cracks in the soles is usually due to wet conditions and poor sanitation. This disagrees what is said by Csuti and others (2001), which is that nail cracks are the result of abnormal pressure on the nail which is caused by repetitive movement. The middle nail on the hind feet can be predisposed to cracks if an elephant gets up and down frequently on

hard surfaces, especially if the nails are allowed to grow too long (Custi and others 2001). This was seen in two cases. Overall, most cracks in nails are seen in older elephants (Csuti and others 2001). However, analysing the results for the general condition of the feet, the findings do not support this statement. The Fisher's exact value is 0.683 and since this is greater than a p value of 0.05, this implies that the result is not significant i.e. there is no correlation between the age of the elephant and the general condition of the feet.

Heel cracks were also evident in many of the elephants and this is again caused by keeping the elephants in wet conditions and with inadequate sanitation (Fowler 1978). This correlates with my observations because when elephants were hosed with water they were sometimes situated in one place hence their feet would have remained wet for some time. However the analysis of the results does not agree with this. The Fisher's exact value is 0.271 and since this is greater than a p value of 0.05, this implies that there is no correlation between the age of the elephant and the chance of cracked heels.

There are several improvements that can be made to ensure better welfare of the elephants. If the elephants were washed then moved to a dry location this may reduce the incidence of cracked nails. As has been noted by Fowler (1993) there should be regular manicure of the cuticles. Cuticle feathering can be solved in a cost effective way by applying vegetable oil or mineral oil to them on a daily basis which causes them to soften. (Fowler 1978). Black tracts should be trimmed away to healthy sole horn (Fowler 1980). This will ensure that there are no pockets in which dirt can lodge hence creating a nidus for infection. Elephants require an abrasive bedding to prevent overgrowth of the nails and soles of their feet, but it is this abrasive bedding that contributes to the pressure sores. Therefore perhaps the elephants could be treated with oils on their elbows and knees to soften the skin, in a similar manner to that suggested by Fowler for managing the cuticles.

This study could have been improved in terms of accuracy if the elephants had records detailing their age and their medical history. Since there were no such records in the majority of cases, the information was entirely dependant upon that given by the mahouts. Although digital photographs were taken that provided a record of the lesions and feet condition for each of the elephants, the interpretation of these photographs was subjective. However, since the photographs were judged relative to one another, this was satisfactory.

In terms of further study it would be better to have a larger sample size so that more accurate correlations can be made between the ages of the elephants and the severity of the lesions. It would also be helpful to assess elephants from more locations so that the variables for bedding and type of food would be varied. This would allow for further and more detailed analysis.

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Annexe A

Details about elephant

Name/no. of elephant: _____Sex: M F

Age (yrs): _____ Number of hours worked per day: _____

Substrate used for bedding in the holding facility: _____

Type of food: _____

Amount of water provided per day: _____

Sores from chains (from 0 to 5): front feet ___ hind feet ___ Average ___ Description of sores:

_____

Details of any treatment being used:

_____

Pressure sores (from 0 to 5): front feet ___ hind feet ___ Average ___
Description of sores: _____
Details of any treatment being used:
_____
General condition of feet (from 0 to 5): _____
Description of sores: _____
Details of any treatment being used:
_____
Remarkable comments from medical history of previous months: -
_____
Type of restraining method used by mahout:
_____
Willingness to seek veterinary advice:
Low Medium High
Reason for above: _____