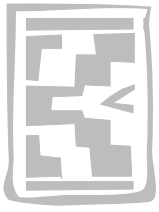

Seroprevalence of antibodies to *Encephalitozoon cuniculi* in domestic rabbits in Nigeria

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ABSTRACT

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Serum samples from 237 randomized rabbits from the five ecological zones of Nigeria, i.e. Northwest (NW), Northeast (NE), Southeast (SE), Southwest (SW) and Northcentral (NC), were evaluated for the presence of antibodies to *Encephalitozoon cuniculi* by the indirect immunofluorescent antibodies test. A titre of 10 or more was taken as positive. Thirty-nine (16.5 %) of the 237 samples were positive with 11, 10, 8, 6 and 4 seropositive rabbits occurring in the NW, NE, SE, SW and NC zones of Nigeria, respectively. Age, sex, live mass and access to grass as a feed supplement were not statistically ($P > 0.05$) associated with seropositivity, but cage type (single-versus multi-rabbit type), contact with free-range rats and previous illness were strongly ($P < 0.05$) associated with it. The practice of selling unscreened and untreated 5 to 10-week-old weaners to prospective buyers as foundation stock, use of multi-rabbit communal cages, occasional release of rabbits in runs and contact with free-range house rats should be discouraged. Regular prophylactic and curative treatments, occasional serological screening to remove carriers, and the practice of a high level of hygiene in rabbit colonies are effective control measures.

Keywords: *Encephalitozoon cuniculi,* Nigeria, rabbits, seroprevalence

INTRODUCTION

Encephalitozoonosis in animals and humans is caused by the obligate intracellular protozoan, *Encephalitozoon cuniculi*, a member of the Phylum Microspora. More than 1 000 species of microsporida exist which are classified into about 100 genera and which infect insects and vertebrate animals. (Canning & Lom 1986). To date, several species of the genus *Encephalitozoon* have been described with three strains of *E. cuniculi* being indentified (Cali, Kotler & Orenstein 1993; Didier, Orenstein, Aldras, Bertucci, Rogers & Janney 1995) most of which are opportunistic pathogens in immunocompromised contact animals and humans (Harcourt-Brown 2002).

Infection of most mammalian hosts with *E. cuniculi* occurs by ingestion or inhalation of spores from contaminated urine and faeces from infected hosts (Canning & Lom 1986). Once in the susceptible hosts, the spores invade by a germination process and undergo schizogony, an asexual cell division, followed by sporogony and maturation. Infections, characterized by muscular weakness, emaciation, urinary incontinence, vestibular signs such as head tilt, seizures, ataxia, posterior paralysis and uveitis, were first reported in rabbits by Kimman & Akkermanns (1987). Infections have also been reported in mice, guinea pigs, hamsters, dogs, cats, monkeys, and humans but systemic disease is rare in these species except in athymic or immunocompromised mice

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and neonatal dogs or foxes (Gannon 1980). *Encephalitozoon cuniculi* has been recognized as an important opportunistic pathogen in human patients suffering from the acquired immunodeficiency syndrome (AIDS) in which it causes interstitial pneumonitis, chronic diarrhoea, renal disease and wasting (Cali, Kotler & Orenstein 1993; Van Gool, Snijders, Reiss, Eeftinck Schattenkert, Van den Bergh Weerman, Bartelsman, Bruins, Canning & Dankert 1993; Didier, Visvesvara, Baker, Rogers, Bertucci, De Groote & Voss Brinck 1996). As the prevalence of encephalitozoonosis in a country may be higher than is realized (Keeble & Shaw 2006) which can have a negative effect on the results of experiments in which rabbits are used (Wilson 1979), the screening of a nation's rabbit colonies may be a worthwhile exercise.

The objectives of this survey were to determine the seroprevalence of encephalitozoonosis in domestic rabbits in different ecological zones of Nigeria in order to formulate appropriate cause-based control measures that would promote public health and produce rabbits free of the infection for experimental use in scientific studies.

MATERIALS AND METHODS

Place and time of study

The assay was conducted at the Experimental Animal Unit of the University of Ibadan, Ibadan, Nigeria from May to August 2003 on 314 serum samples obtained from rabbit colonies in the five ecological zones of Nigeria, viz. Northwest (NW), Northeast (NE), Southeast (SE), Southwest (SW) and North-central (NC) zones.

Inclusion criteria for animals

Rabbits of both sexes of all breeds and health status, in all husbandry systems, diets and preventive medicine routines that were over 4 weeks of age were included.

Study design

The survey targeted a randomized sample of 50 rabbits from each secondary school located in the capital cities of the ecological zones. (Federal authority mandated the keeping of rabbits in all federal schools for use in agricultural science and biology practicals.)

The selected schools were requested to complete questionnaires in order to collect information such as the history (origin) of rabbit colony, breed, age and sex of the rabbits in the colonies, previous and current clinical observations, feed and water consumption, routine treatments, weekly morbidity and mortality rates, housing types, urine and faecal/disposal schemes, access to wild birds or animals, and access to grass as a feed supplement.

Serum samples

Approximately 2 mℓ of blood were collected either from an incision made in the margin of the ear or the jugular vein of each of the rabbits into sterile gel tubes and allowed to clot. Centrifugation was done after the clot had retracted in order to separate serum from clot. The serum samples were stored frozen at –20 °C before testing.

Serology

A commercial heat-fixed *E. cuniculi* antigen stored at 4 °C (Sigma-Aldrich Co, England) was used. Sera were tested by the indirect immunofluorescent antibody technique (IFAT) as described by Cox & Pye (1975) for the presence of antibodies to *E. cuniculi* at a dilution of 1:10. The conjugate used was a fluorescein-isothiocyanate (FITC)-conjugated sheep IgG anti-rabbit immunoglobulin (Sigma-Aldrich Co, England). Those serum samples showing any evidence of reactivity were subsequently titrated by doubling dilutions commencing at a dilution of 1:10. The slides were examined at a magnification of 630 under a Leitz fluorescence microscope. Titres were expressed as the highest serum dilution giving a bright staining of the spore body. Titres of 10 or higher were regarded as positive, while those below 10 were regarded as negative.

Statistical analysis

The rabbits' ages and live masses as possible influencing factors on seropositivity to *E. cuniculi* were tested by the standard 2-sample t-tests (Snedecor & Cochran 1967), while other possible influencing factors such as sex, breed and health status were analyzed by the chi-squared test. In all tests, values of $P < 0.05$ were taken as significant.

RESULTS

The results of the serological screening at the five zones are summarized in Table 1, while the statistical analyses of possible influencing factors are given in Table 2. Of the 237 rabbits sampled, 39 (16.5 %) were seropositive. These 39 rabbits were

TABLE 1 Summary of the anamnestic, management, clinical and serological results of the seroprevalence survey for encephalitozoonosis in rabbits in Nigeria

S/n	Zone	School and town	Total no. of sex	Breed	Age range (in weeks)	Origin of parents stock	Feed type	Housing type	Previous clinical signs no. (age in weeks)	Serological status	Urine and faecal disposal system
1	South-West	Government College, Ibadan	50 F = 34 M = 16	NZW = 27 AC = 11 Crosses = 18	4–82	Ibadan and its environs	Commercial medicated pellets without any supplement	Wire-mesh floored cages with feed trough and inverted water bottles, 3 per cage	None	6 = positive 44 = negative	Dropping from wire-mesh floor of cages and sweeping
2	South-East	Government College, Umuiah	37 F = 25 M = 12	NZW = 37	8–63	Enugu and its environs	Commercial medicated pellets supplemented with herbs	Wire-mesh floored cages with feed trough and inverted water bottles, 4 per cage	Ocular lesions = 3 (28–41), headtilt = 2 (30–32)	8 = positive 29 = negative	Dropping from wire-mesh floor of cages and sweeping
3	North-West	Government Secondary School, Sokoto	50 F = 31 M = 19	NZW = 26 AC = 11 Crosses = 13	4–70	Bought from Zaria city (North-central zone)	Commercial medicated pellets supplemented with leguminous herbs	Large communal Wire-mesh floored cages with open feed and water troughs, 7 per cage	Headtilt = 5 (37–40), hind leg weakness = 3 (30–43)	11 = positive 39 = negative	Dropping from wire-mesh floor of cages and sweeping
4	North-East	Government Secondary School, Yola	50 F = 28 M = 22	Crosses = 8	5–101	Bought from Agricul-tural show at Zaria city (North-central zone)	Commercial medicated pellets supplemented with home table remnants	Large communal wire-mesh floored cages with open feed and water troughs, 10 per cage	Headtilt = 6 (72–92), hind leg weakness = 4 (82–94), urinary-incontinence = 8 (12–34)	10 = positive 40 = negative	Dropping from wire-mesh floor of cages and sweeping
5	North-Central	Government Secondary School, Kaduna	50 F = 33 M = 17	NZW = 30 AC = 8 Crosses = 12	5–98	Bought from Zaria city (North-central zone)	Commercial medicated pellets with leguminous herbs and supplements	Wire-mesh floored cages with feed troughs and inverted water bottles, 1 per cage	None	4 = positive 46 = negative	Dropping from wire-mesh floor of cages and sweeping; occasional release to run on wet house floor

F = Female

M = Male

NZW = New Zealand White, AC = American Chinchilla

TABLE 2 Statistical analysis of the information returned from the zonal survey of antibodies to *E. cuniculi* by rabbits in Nigeria

Rabbits variable	No. tested	Statistical test	P-value	Test results
Age	237	t-test	0.764	T= 0.05
Live mass	237	t-test	0.802	T= 0.152
Sex	237	Chi-squared	0.167	$\chi^2 = 1.58$
Housing type S or M (Singles or multi-rabbit)	50 187	Chi-squared	0.714 0.020	$\chi^2 = 1.28$ $\chi^2 = 1.43$
Access to grass (as feed supplement)	137	Chi-squared	0.342	$\chi^2 = 0.67$
Contact with other pets or wild species (on occasional release from cages)	50	Chi-squared	0.033	$\chi^2 = 0.52$
Previous illness	31	Chi-squared	0.024	$\chi^2 = 1.33$

Probability (P) values < 0.05 and their corresponding chi-squared (χ^2) values were considered significant

between 9 and 42 weeks of age and were unevenly spread across the five zones, with Northwest, Northeast, Southeast, Southwest and Northcentral zones having 11, 10, 8, 6 and 4 of them, respectively. According to their histories, 31 (79.5%) of the seropositive 39 had manifested clinical signs such as head tilt (13), hind leg weakness (7), ocular lesions (3) and urinary incontinence (8). All had responded positively to therapy with enrofloxacin (Baytril®, Bayer) or fenbendazole (Panacur®, Hoechst). Twenty-three of the rabbits that had previously manifested clinical signs were more than 36 weeks old. However, the eight that had incontinence were relatively young.

Only eight (20.5%) of the 39 seropositive rabbits had not shown any clinical signs suggestive of encephalitozoonosis at least 5 months before sampling, but they were in contact with feed and water troughs and other seropositive cage mates in their multi-rabbit cages. Thirty-five (87.7%) of the 39 seropositive rabbits were from multi-rabbit communal cages, housing 3–10 rabbits with open feed troughs, but inverted water bottles (Table 1). Only 4 (10.3%) of the seropositive rabbits were from single-rabbit cages but had occasionally been released to run on urine-contaminated floors before being re-caged (Table 1).

The 5 to 10-week-old rabbits were under-represented in the randomized zonal samples as a result of their regular sales to other schools as foundation stock.

There were no significant associations between the number of rabbits that were seropositive and the age, sex, live mass and access to grass as feed supplement, (Table 2). However, significant associations were observed between seropositivity and multi-rabbit housing, contact with other rabbits when occasionally released into runs and previous illnesses (Table 2).

DISCUSSION

The results of this survey have confirmed that *E. cuniculi* is present in a sizable proportion of apparently clinically healthy rabbits in Nigeria. In addition, they also show that age, live mass and sex of the rabbits had no influence on their seropositivity. These findings are consistent with the earlier report of Keeble & Shaw (2006) from the United Kingdom. However, other factors such as contact with wild rabbits or rats that were scored low on the chi-squared analysis might be epidemiologically important. Recently Thomas, Finn, Twigg, Deplazes & Thompson (1997) demonstrated exposure to *E. cuniculi* in wild Australian rabbits, which conflicts with the earlier negative report of Cox, Pye, Edmonds & Shepard (1980). Muller-Doblies, Herzog, Tanner, Mathis & Deplazes (2002) have also demonstrated the presence of the same parasite in the free-ranging rats, *Rattus norvegicus*. The potential danger posed to caged rabbits by wild, free-ranging rats that are sustained principally by the few food pellets dropped on the floors of the colonies require further investigation. It is also feasible that their urine and faeces contaminate feed of rabbits in open troughs as they climb on cages seeking possible entry in order to obtain the feed.

The most likely mode of infection of caged rabbits is the ingestion of spores in urine-contaminated feed in open feed troughs, since spores have been reported to survive in the environment for long periods of time (Kucerova-Pospisilova, Carr, Leitch, Scanlon & Visvesvara 1999). This possibility is supported by

the fact that more seropositive rabbits were observed in colonies containing a higher number of communal multi-rabbit cages, each housing 3–10 rabbits. Another contributing factor is possibly the policy of selling weaner rabbits of 5–10 weeks of age to other schools as foundation stock, without any prophylactic treatment. According to Lyngset (1980), young weaner rabbits suffering from an active infection of *E. cuniculi* seroconvert at 8–10 weeks of age. Such infections could spread by contact with the doe or buck, while the former may also transmit transplacentally to their offspring in the new colony. Testimony to this possibility is the fact that the NW, NE and NC stocks were all purchased in Zaria city (Table 1). In order to prevent the introduction of infection in this way it is suggested that all weaners be serologically tested before introducing them into colonies. Positive animals should be either treated aggressively or euthanazed.

The clinical signs of head tilt, ocular lesions and hind leg paresis in 23 of the seropositive rabbits that were observed about 5 months prior to the sampling in the NE, NW and SE zones are suggestive of the nervous manifestations of encephalitozoonosis that occur particularly in older rabbits. This again is corroborated by the observation of higher number of seropositive rabbits from this age group by Harcourt-Brown & Holloway (2003).

The IFAT, though rated as suitable and sensitive as the ELIZA and carbon immuno-assays for the immunodiagnosis of encephalitozoonosis (Boots 2002), could be made more useful and informative, if sampling is repeated 4 weeks later to evaluate seroconversion, or to detect the presence of both IgM + IgE with appropriate conjugates. This, if it had been done, would have assisted in predicting the age of infection, and the different status of the seropositive rabbits, which are needed for epidemiological control. The high cost of repeated screening, logistical problems and poor knowledge of the transmission of *E. cuniculi* are some of the constraints in the control of the disease.

While a national seroprevalence rate of 16.5 % may be considered relatively low, it probably points to a small rabbit industry in Nigeria.

In view of current husbandry policies, which, it seems, promote a very high prevalence of encephalitozoonosis in weaners, the following steps to control the disease are suggested: weaner rabbits should be screened before sale and infected ones removed, design and use hygienic single-rabbit cages with raised feed troughs and inverted water bottles, prophylactic treatment of animals that have been in contact with infected ones, thorough disinfection of all implements and strict hygiene practices. Rabbits for investigational studies should first be serologically tested and certified free of encephalitozoonosis.

Finally, regular disinfection of colony floors and walls with either 2 % phenol, 10 % formalin or 70 % ethanol renders the otherwise environmentally resistant spores non-infective and prevents transmission (Didier, Didier, Snowden & Shadduck 1998).

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