A SEROEPIDEMIOLOGICAL SURVEY OF ENCEPHALITOZOON CUNICULI IN DIFFERENT ITALIAN RABBITRIES

LAVAZZA A., TINELLI F., ZANON F., MASSIRIO I.

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia "B. Ubertini", Via A. Bianchi 7, 25124 Brescia, Italy

Abstract - We made a seroepidemiological survey to find the effects of the *Encephalitozoon cuniculi* infection in rabbitries in Northern Italy. Initially, 231 sera samples were studied from 11 rabbitries of different functional uses: 3 industrial, 1 for laboratory rabbits, 1 reared in isolation and 6 rural farms. We found a seroprevalence of 32.5% in the industrial rabbitries, 21.4% in the rural ones while the laboratory rabbits were entirely negative. Subsequently, we looked at how the infection developed in one of these industrial farms, with respect to the rabbits' age, sex, and location. We studied 690 rabbits and found: 66.4% of bucks seropositive (232 sera), 64.9% of does (302), 41.8% in the age group 90-180 days (141) and 0% in the fattening group between 75-80 days (15). There was no statistically significant differences between the breeding sexes, breeding sheds, nor between the age of the breeding bucks. However the does' positivity varied significantly in relation to the number of litters. These data justified an eradication programme which has been based on the elimination of seropositive subjects and the movement of negative rabbits to a clean environment.

INTRODUCTION

Rabbit encefalitozoonosis is a chronic parasitosis which mainly effects rodents and lagomorphs. The etiological agent is *Encephalitozoon cuniculi* (LEVADITI et al. 1924), a Gram-positive microsporidium and intracellular parasite, whose entire life cycle revolves within a single host. It multiplies above all in the kidneys and the SNC and is excreted by urine, in the form of a spore. The spore is infectious and is transmitted horizontally by ingestion of contaminated food or water, or more rarely by inhalation. In intensive rearing, poor hygiene conditions, overcrowding, routine moving of the animals, the introduction of unchecked animals from outside are all factors which help the spread of the infection.

In laboratory animals, the infection is particularly important even if it is chronic or latent, indeed this factor making it particularly difficult to diagnose. However clinical manifestations can be observed during stressing experimentation. In industrial animals, and in particular in rabbits reared for meat, the infection can cause considerable financial losses, due to mortality (up to 15%) (PATTISON et al. 1971), to the increase of rejected animals and the reduced carcass weight (GREENSTEIN et al., 1991; VAVRA et al. 1980).

It is precisely this negative effect on the productivity of intensive farms that would justify control measures of *E. cuniculi*, for its gradual eradication. Such a programme would also be highly recommended for laboratory rabbit producers. In either case, before implementation, an evaluation of the infection and its seroprevalence is needed in respect to age, and category of rabbit, possible above all with a serological survey.

Surveys such as those carried out in other countries with a positivity of up to 90%, depending on the group of rabbit studied, have not yet been carried out in Italy. However the parasite has already been found histologically, in 12.6% of rabbits regularly slaughtered, and 20.4% including dead and suppressed rabbits (CRAVERO et al., 1974; JULINI and PELLEGRINI, 1981-2; JULINI, 1983; SCANZIANI et al., 1985).

We therefore carried out a serological survey in two distinct phases. First we found the rate of infection of *E. cuniculi* from groups of rabbits from different functional uses. Then, once identified a positive industrial rabbitry, we studied how the parasite spread in respect to age, sex, and locations of the animals in the sheds. We used the Carbon Immuno Assay (CIA) to determine seropositivity, which uses the aspecific absorption of carbon particles to the heavy chain of the IgG. This test is simple, easy to use, economic and it is as sensitive as indirect immunofluorescent (IFA) (WALLER, 1977), even if some Authors consider that revealing only IgG gives a positive result later than IFA (COX et al. 1977).

These data justified an eradication programme which was based on the gradual elimination of seropositive subjects and the movement of negative rabbits to a clean and disinfected environment.

MATERIALS AND METHODS

Animals and Sera

In the first phase of the survey, we examined 231 sera from 197 rabbits from the three functional uses of rabbitries.

Group A : Intensive meat rabbitries

Nr. 1: This farm had 40,000 animals with 1,500 breeders in 5 new alined buildings built as sheds. One of these was divided into two parts while the others were identical and smaller. The breeders were housed in the largest one (A) and three of the smaller ones (B,D,E) in about 800 wire-cages. The bucks were distributed equally in each of the sheds and lived their entire life in the same cage. They were used for natural fecundation. The does were reared with their litter mates until weaned and were moved according to the weekly cycle typical of intensive farms. Fattening and future stock rabbits from 60-70 days were reared in shed C. This was capable of housing about 3,500 rabbits in wire cages arranged in double-tier batteries. The animals had access to water ad libitum from self-drinking nipples and were fed with a special pelleted diet according to their role (meat or breeder) and to their productive period. The droppings were collected in a trough and taken away every 3 months. There was horizontal ventilation with a natural air entrance from perforated bricks at the base of the walls, and adjustable sash windows at the sides with artificial air extraction by fans on one end wall. Insecticides were both automatically and manually distributed monthly to fight flying insects and parasites. All breeders and future stock rabbits were regularly vaccinated for viral haemorrhagic disease and myxomatosis. One hundred and twenty five sera from 91 rabbits were examined in this rabbitry during the first stage, divided according to location and age (Table 1). In fact, a second sera was taken from 16 does after 4 months, and from 15 fattening rabbits after one month. A third sera was also taken from 9 does.

Nr. 2 : An average sized rabbitry (500 breeding does), rotated on a weekly cycle, consisted of 3 tunnels with the breeding does, the litters until weaning and the restocking does. There were another three tunnels for the fattening rabbits, with an on-site slaughter annex. We took a single sample of 12 sera from 11 week old rabbits. Nr. 3 : An average sized rabbitry (500 does), rotated on a three weekly cycle. It had two sheds, one larger than the other divided into two parts. One for the does and meat rabbits and the smaller one for the males and the grandparents. A single sample was taken from 17 does of different ages (from 6 months to 2 years).

| Date | n° sera | does | shed | n° | 50-90dd | shed | n° | 100-150dd | shed | n° |
|-----------|-----------|-------------|----------|---------|---------------|----------|----|-----------|------|----|
| 12/05 | 26 | 16* | A | 5 | 10 | A | 5 | - | | |
| | | | в | 5 | | В | 5 | | | |
| | [[| | Е | 6 | [| | | [| | |
| 9/6 | 25 | - | | | 19^ | C | 19 | 6 | С | 8 |
| 5/7 | 25 | • | | | - | | | 25^ | С | 25 |
| 8/9 | 30 | 10* | A | 1 | 20 | C | 20 | - | | |
| | | | в | 5 | | | | | | |
| | | | D | 2 | | | | | | |
| | | | E | 2 | | | | | | |
| 26/09 | 19 | 9** | A | 1 | | | | 10 | С | 10 |
| | | | в | 4 | | | | | | |
| | | | D | 2 | | | | | | |
| | | | E | 2 | | | | | | |
| TOTAL | 125 | 35 | A | 7 | 49 | A | 5 | 41 | С | 41 |
| | | | в | 14 | | В | 5 | ļ | | |
| | | | D | 4 | | С | 39 | | | |
| | | | E | 10 | | | | | | |
| * 10 of t | he 16 doe | s tested on | the 12/5 | were to | ested again o | n the 8/ | 9. | | | |

Group B : Laboratory rabbits

Nr. 4 : An industrial rabbitry which reared rabbits for laboratories. This structure was marked by its isolation in respect to other zootechnical farms, the internal source future stock and rigid regard for hygiene. There was a single sample taken from 33 rabbits of 13-15 weeks.

Nr. 5 : Rabbits reared in strict isolation in our institute in an isolated area equipped with an over air-pressure system and air filters. We took a single sample of 16 sera from 4-5 month old rabbits.



^ 15 of the 19 stock rabbit tested on the 9/6 were tested again on 5/7.

Group C : Rural rabbitries

A single sample of 28 sera was taken from rabbits ready for slaughter, reared in 6 small rural family establishments, all within the same Health board (USL) area.

We subsequently concentrated on Nr.1 industrial rabbitry. Over 10 months, a total of 690 rabbit sera were taken in four categories: 1) 232 naturally breeding bucks, 224 between 4-37 months, the rest of unknown age, 2) 302 breeding does, whose age was defined in numbers of litters, from 1 to 18, 3) 141 future stock rabbits between 90 and 180 days and 4) 15 fattening rabbits ready for slaughter (75-80 days). The shed when the sample was taken was noted for each subject.

Serological test

The sera were preserved at 4°C until examined with the Carbon Immune Assay (CIA), using a commercial kit produced and distributed by Testman, Uppsala, Sweden. The antigen consisted of 3×10^7 spores/ml. of heat inactivated *E. cuniculi*, then washed and suspended in PBS containing 0.1% formalin. The carbon suspension consisted of microscopic particles capable of binding aspecifically to the IgG of various types of mammals. The positive reference serum was a serum from a hyperimmune rabbit to which 0.1% NaN₃ was added and it was used at the minimum dilution of 1:20. The inactivated sera were assayed at 1:40, double the minimum significant dilution. The CIA test consisted of an initial contact of equal quantities (10µl) of the examined sera and the antigen in a U-shaped microtitre well for 5 minutes. Then 10µl of this mixture was placed in contact with an equal quantity of carbon suspension on a slide and then covered. The observation was carried out 5 minutes later, at a magnification of 600x with an ordinary light microscope. With positive sera antibodies dark-grey spores were seen against the background of carbon particles and if negative, translucent white on a brown background.

Statistical analysis

С

6-11

Total

The statistical differences of positivity were measures by an χ^2 test (GLANTZ, 1988): 1) between buck and doe breeders, 2) for the mixed population sheds (A,B,D,E), 3) age for both bucks and does.

RESULTS AND DISCUSSION

21.4

26.4

22.8

Nr. positive Group Unit Total % 'N° N° rabbits rabbits rabbits sera sera sera Α 1 125 91 44 28 35.2 32.5 2 12 33.3 4 3 17 7 41 1 В 4 33 0 0 5 0 16 0

28

197

231

 Table 2 : Serological results from the 11 rabbitries

| Table 3 : Serological results from Group C | | | | | | | | | | | | | |
|--|------|-----|------|-----|-----|-----|-------|--|--|--|--|--|--|
| Unit N* | 6 | 7 | 8 | 9 | 10 | 11 | Total | | | | | | |
| Nr. rabbits | 6 | 7 | 8 | 4 | 2 | 1 | 28 | | | | | | |
| Nr. positive | 1 | 0 | 2 | 0 | 2 | 1 | 6 | | | | | | |
| % | 16.6 | 0.0 | 25.0 | 0.0 | 100 | 100 | 214 | | | | | | |

6

61

45

The results of the CIA test on 231 of the 197 rabbits examined during the first phase of the survey are shown in Table 2. Sera positivity was respectively 26.4% and 22.8% of the total sera and animals checked. This difference was because 9 rabbits of rabbitry 1 were positive to the two or three checks that were made on them.

In the three intensive rabbitries in group A, sera positivity (35.2%) was higher than in the other two groups. The lack of positivity in group B, even if not guaranteed for the breeding does, shows that it is possible to rear rabbits free of *E. cuniculi*, even without rigid isolation such as in rabbitry Nr.5. It is sufficient to consider rabbitry Nr.4, i.e. adopting appropriate hygiene and prevention measures, including control of possible

sources of contamination, e.g. externally introduced rabbits and wild rodents (rats and mice). In the rural rabbitries, the somewhat limited data, did not show a uniform situation but rather there seemed to be considerable differences for animals of the same age, from completely negative to 100% positive (Table 3). In any case, these results suggest the probable role of hygiene conditions and managerial factors which favour the spread of the infection.

| Date | Nr. | | Do | Des | | | 50-9 | 90dd | | | 100-1 | 50dd | |
|-------|------|------|------|------|----|------|------|------|----|------|-------|------|----|
| | Sera | tot. | pos. | cap. | n° | tot. | pos. | сар. | n° | tot. | pos. | cap. | n٩ |
| 12/05 | 26 | 10* | 8 | A | 2 | 10 | 0 | Α | 0 | | | | |
| | | | | В | 4 | | | В | 0 | | | | |
| | | | | Е | 2 | | | | | | | | |
| | | 6 | 3 | Α | 2 | | | | | | | | |
| | | | | Е | 1 | | | | | | | | |
| 9/6 | 25 | | | | | 15^ | 1 | С | 1 | 6 | 2 | С | 2 |
| | | | | | | 4 | 1 | С | 1 | | | | |
| 5/7 | 25 | | | | | | | | | 15^ | 5 | С | 5 |
| | | | | | | | | | | 10 | 3 | С | 3 |
| 8/9 | 30 | 10* | 8 | В | 5 | 20 | 4 | С | 4 | | | | |
| 1 | | | | D | 1 | | | | | | | | |
| | | | | E | 2 | | | | | | | | |
| 26/09 | 19 | 9* | 7 | . A | 1 | | | | | 10 | 2 | ¢ | 2 |
| | | | | в | 4 | | | | | | | | |
| | | | | D | 1 | | | | | | | | |
| | | | | Ε | 1 | | | | | | | | |
| Total | 125 | 35 | 11** | • | | 49 | 6^^ | Α | 0 | 41 | 12^^ | С | 12 |
| | | | | | | | | в | 0 | | | | |
| | | | | | | | | С | 6 | | | | |

Table 4 : Results from rabbitry Nr. 1 during the first phase

9 of these were again tested on the 26/9.

** there were 11 positive does. Of these, 7 to all three tests, 1 to two and 3 to only one.

^ 15 out of 19 restocking rabbits tested on 9/6 were again controlled on 5/7.

^ 1 rabbit was positive to both tests done

* The does during this period were repeatedly moved from a shed to another.

does and other positive mating does after the numerous movements of routine cycle rearing.

The second phase results helped to clarify the natural progress of the infection, above all in respect to age and location of the affected rabbits. 396 sera, i.e. 57.4% of the total were positive to CIA. A further batch of 13 sera (1.9%) were 50% positive, i.e. only half of the spores observed with the microscope were coloured. In effect, given that the sera were exposed to a fixed dilution, we can say that these 13 sera had a titre corresponding to the dilution (i.e. 1:40) whereas in the others the titre were greater.

 Table 5 : Distribution of the examined sera and results for each category of rabbits

| Rabbit | Total | Р | % | P/N | % | N | % |
|----------|-------|-----|------|-----|-----|-----|-------|
| Males | 232 | 147 | 63.3 | 7 | 3.0 | 78 | 33.6 |
| Does | 302 | 192 | 63.6 | 4 | 1.3 | 106 | 35.1 |
| 75-80dd | 15 | 0 | 0.0 | 0 | 0.0 | 15 | 100.0 |
| 90-180dd | 141 | 57 | 40.4 | 2 | 1.4 | 82 | 58.1 |
| Total | 690 | 396 | 57.4 | 13 | 1.9 | 281 | 40.7 |

In rabbitry Nr.1 (Table 4) there appeared to be an « active » evolution of the infection during the first phase. Fattening rabbits had a low positivity (12.2%), higher in the future stock rabbits (29.3%) and very high amongst the breeders (68.6%). While the highest rate of infection was among the does in sheds A,B,D,E, the difference between subjects <90 days old and >100 days was observed in shed C where the future stock and fattening rabbits were situated. Admitting a certain delay in the appearance of natural antibodies and bearing in mind the limitation of CIA to indicate only IgG, one possibility was infection due to contact between breeders and future stock rabbits (60-90 days) when together. This does not necessarily exclude other means of transmission, including coital by the mating bucks or subsequent contact between negative

Table 5 shows the distribution of the examined sera and their outcome for each category of rabbit. No significantly relevant difference ($\chi^2=0.07 - P=0.79$) of positivity was found between the buck breeders (66.4%) and the doe breeders (64.9%). None of the fattening rabbits of 75-80 days

P = positive sera; P/N = 50% positive sera; N = negative sera

had anti-E. cuniculi antibodies, while 41.8% of future stock subjects (90-180 days) were positive. These values correlate quite well to those we found before, above all concerning different locations, even if the age groups (50-80 days vs. 75-80 and 100-150 vs. 90-180) were slightly different. Possibly the most significant difference was that regarding the future stock, 29.3% in the first phase against 41.8% in the second. However apart from the fact that older future stock (up to 180 day) were included, we can presume that the different location of the groups in the sheds had a fundamental role. Indeed, while these latter subjects came from sheds A, B, and E where they were together mainly with breeders and their litters (positivity over 60%), the former were all from shed C together with fattening subjects and a few males. An analysis of the distribution of the examined sera and the relative positivity in respect to type of rabbit and their location is shown in Table 6. The final positivity of each shed did not differ significantly, from a minimum of 42.8% in shed C to a maximum of 66% in shed D. The values correlated to the type and numbers of rabbits sampled. In sheds B and E there was a consistent of future stock, whose positivity was lower, while shed C was affected by the small number of rabbits surveyed and that they were all bucks (positivity 75%), or fattening rabbits (positivity 0%). Comparing positivity for each category according to location we noted a distinct variation for the breeding bucks (min. 61.2% in shed B, max. in shed E of 84%) and in the future stock (min. 38.9% in shed E, max. in shed A of 54.5%). The does showed little variation (min. 60% in shed A, max. in shed B of 68.8%). It was impossible to evaluate this data for the fattening rabbits, given the small number of sera from a single (C) shed. In any case there was no statistically significant difference between the 4 sheds, χ^2 =4.56 - P=0.20.

Table 6: Distribution of the tested sera and the relative positivity for the type of rabbits and their location.

| Rabbit | Rabbit Males | | | Does | | | | 75-80dd | | | 90-180d | d | Total | | | |
|--------|--------------|------|------|------|------|------|------|---------|-----|------|---------|------|-------|------|------|--|
| Shed | Tot. | Pos. | % | Tot. | Pos. | % | Tot. | Pos. | % | Tot. | Pos. | % | Tot. | Pos. | % | |
| A | 69 | 43 | 62.3 | 80 | 48 | 60.0 | 0 | 0 | 0.0 | 11 | 6 | 54.5 | 160 | 97 | 60.6 | |
| В | 85 | 52 | 61.2 | 61 | 42 | 68.8 | 0 | 0 | 0.0 | 94 | 39 | 41.5 | 240 | 133 | 55.4 | |
| С | 20 | 15 | 75.0 | 0 | 0 | 0.0 | 15 | 0 | 0.0 | 0 | 0 | 0.0 | 35 | 15 | 42.8 | |
| D | 33 | 23 | 69.7 | 120 | 78 | 65.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 153 | 101 | 66.0 | |
| E | 25 | 21 | 84.0 | 41 | 28 | 68.3 | 0 | 0 | 0.0 | 36 | 14 | 38.9 | 102 | 63 | 61.8 | |
| Total | 232 | 154 | 66.4 | 302 | 196 | 64.9 | 15 | 0 | 0.0 | 141 | 59 | 41.8 | 690 | 409 | 59.3 | |

Table 7 : Distribution of positive seraaccording to age and sex of rabbits

| MALES | | | | | | | | | | | | |
|---------|--------|------|-------|------|------|--|--|--|--|--|--|--|
| Age* | Nr. | Pos. | % | Neg. | % | | | | | | | |
| 4 - 6 | 50 | 29 | 58,0 | 21 | 42,0 | | | | | | | |
| 7 - 9 | 55 | 38 | 69,1 | 17 | 30,9 | | | | | | | |
| 10 - 12 | 29 | 21 | 72,4 | 8 | 27,6 | | | | | | | |
| 13 - 18 | 43 | 31 | 72,1 | 12 | 27,9 | | | | | | | |
| 19 - 24 | 29 | 14 | 48,3 | 15 | 51,7 | | | | | | | |
| > 24 | 18 | 14 | 77,7 | 4 | 22,3 | | | | | | | |
| ? | 8 | 7 | 87,5 | 1 | 12,5 | | | | | | | |
| Total | 232 | 154 | 66,4 | 78 | 33,6 | | | | | | | |
| DOES | | | | | | | | | | | | |
| Age^ | Nr. | Pos. | % | Neg. | % | | | | | | | |
| 1 - 2 | 190 | 110 | 57,9 | 80 | 42,1 | | | | | | | |
| 3 - 4 | 72 | 54 | 75,0 | 18 | 25,0 | | | | | | | |
| 5-6 | 20 | 14 | 70,0 | 6 | 30,0 | | | | | | | |
| 7 - 9 | 11 | 11 | 100,0 | 0 | 0,0 | | | | | | | |
| 10 - 12 | 7 | 6 | 85,7 | 1 | 14,3 | | | | | | | |
| > 12 | 2 | 1 | 50,0 | 1 | 50,0 | | | | | | | |
| Total | 302 | 196 | 64,9 | 106 | 35,1 | | | | | | | |
| | | 90-1 | 80gg | · · | | | | | | | | |
| Age* | Nr. | Pos. | % | Neg. | % | | | | | | | |
| 3- 4 | 55 | 22 | 40,0 | 33 | 60,0 | | | | | | | |
| 5-6 | 86 | 37 | 43,0 | 49 | 57,0 | | | | | | | |
| Total | 141 | 59 | 41,8 | 82 | 58,2 | | | | | | | |
| Ane in | months | | | | | | | | | | | |

Age expressed as number of litters

The distribution of the positive sera according to age and sex of breeders and future stock is shown in Tab. 7.

The rabbit sera have been shown in more or less equal groups to make interpretation of these data easier. Excluding the data for the rabbits of unknown age, the number of breeder bucks gradually decreased according to age, (max. 55 when between 7 and 9 months, minimum 18 when >24 months old). In contrast their positivity generally increased with age, from 58% to 77.7%, with the sole exception of breeders 10-12 months old (48.3%), which in all cases was not statistically significant $\chi^2=8.02$ -P=0.169). 62.9% of breeding does had had one or two litters and only 6.6% had had more than 6. In these cases there was also progressive positivity in respect to the number of litters (min. 57.9% for the 1-2 group, max 100% for the 7-9 group) which was statistically significant: $\chi^2 = 15.02 - P = 0.01$. It should be noted that the positivity percentage found for the breeding bucks of 4-6 months (48%) correlated well with future stock does of the same age (41.8%).

If we look at positivity divided for age for each shed, there was no clear pattern (Table 8). This was true especially for the breeding bucks, also due to the rather small number of sera for each age group considered. However the does showed an increased positivity directly correlated to age, above all in sheds A and B.

Table 8: Positivity divided for age for each shed

| Shed | | | A | | | 8 | | | С | | ſ | D | | | E | | | Total | |
|----------|---------|-----|-----|------|-----|-----|------|----|-----|------|-----|-----|------|-----|-----|------|-----|-------|------|
| Rabbit | Age* | N* | Pos | % | N* | Pos | % | N° | Pos | % | Nº | Pos | % | N* | Pos | % | N* | Pos | % |
| Maios | 4-6 | 13 | 9 | 69.2 | 27 | 14 | 51.8 | 5 | 4 | 80.0 | 3 | 1 | 33.3 | 2 | 1 | 50.0 | 50 | 29 | 58.0 |
| | 7-9 | 19 | 12 | 63.2 | 17 | 12 | 70.6 | 14 | 10 | 71.4 | 5 | 4 | 80.0 | | | | 55 | 38 | 69.1 |
| | 10 - 12 | 15 | 11 | 73.3 | 9 | 7 | 77.8 | | | | 3 | 1 | 33.3 | 2 | 2 | 100 | 29 | 21 | 72.4 |
| | 13 - 18 | 11 | 5 | 45.5 | 14 | 11 | 78.6 | 1 | 1 | 100 | 9 | 7 | 77.8 | 8 | 7 | 87.5 | 43 | 31 | 72.1 |
| | 19 - 24 | 5 | 2 | 40.0 | 11 | 3 | 27.3 | 1 | | | 9 | 7 | 77.8 | 4 | 2 | 50.0 | 29 | 14 | 48.3 |
| | > 24 | 6 | 4 | 66.7 | 4 | 2 | 50.0 | | | | 1 | 1 | 100 | 7 | 7 | 100 | 18 | 14 | TT.7 |
| | 2 | | | | 3 | 3 | 100 | | | | 3 | 2 | 66.7 | 2 | 2 | 100 | 8 | 7 | 87.5 |
| | Total | 69 | 43 | 62.3 | 85 | 52 | 61.2 | 20 | 15 | 75.0 | 33 | 23 | 69.7 | 25 | 21 | 84.0 | 232 | 154 | 66.4 |
| Does | 1-2 | 54 | 26 | 48.1 | 23 | 10 | 43.5 | | | | 97 | 64 | 66.0 | 16 | 10 | 62.5 | 190 | 110 | 57.9 |
| | 3-4 | 22 | 18 | 81.8 | 12 | 9 | 75.0 | | | | 16 | 10 | 62.5 | 22 | 17 | 77.3 | 72 | 54 | 75.0 |
| | 5-6 | 3 | 3 | 100 | 8 | 6 | 75.0 | | | | 7 | 4 | 57.1 | 2 | 1 | 50.0 | 20 | 14 | 70.0 |
| | 7-9 | 1 | | | 11 | 11 | 100 | | | | | | | | | | 11 | 11 | 100 |
| | 10 - 12 | 1 | 1 | 100 | 5 | 5 | 100 | | | | | | | 1 | 0 | 0.0 | 7 | 6 | 85.7 |
| | > 12 | | | | 2 | 1 | 50.0 | | | | | | | | | | 2 | 1 | 50.0 |
| | Total | 80 | 48 | 60.0 | 61 | 42 | 68.8 | | | | 120 | 78 | 65.0 | 41 | 28 | 68.3 | 302 | 196 | 64.9 |
| 75-80dd | | | | | | | | 15 | 0 | 0.0 | | | | | | | 15 | 0 | 0.0 |
| 90-180dd | 3-4 | 11 | 6 | 54.5 | 44 | 16 | 36.4 | | | | | | | | | | 55 | 22 | 40.0 |
| | 5-6 | | | | 50 | 23 | 46.0 | | | | | | | 36 | 14 | 38.9 | 86 | 37 | 43.0 |
| | Total | 11 | 6 | 54.5 | 94 | 39 | 41.5 | | | | | | | 36 | 14 | 38.9 | 141 | 59 | 41.8 |
| Total | | 160 | 97 | 60.6 | 240 | 133 | 55.4 | 36 | 15 | 42.8 | 153 | 101 | 66.0 | 102 | 63 | 61.8 | 690 | 409 | 59.3 |

CONCLUSIONS

This survey had basically two aims. Firstly to verify the extent of E. cuniculi in groups of different function rabbits and secondly to survey the epidemiologic progress in one industrial meat rabbitry, in order to reduce the rate of infection and improve productivity. The CIA test proved to be quick, easy to use and initially allowed us to find positivity with a titre of at least 1:40 in 22.8% of the 197 rabbits checked from 11 different rabbitries, subdivided into 3 industrial meat producers, 2 for laboratory rabbits and 6 rural ones.

As expected, we found the highest positivity in the intensive rabbitries (32.5%) where usually poor hygiene, overpopulation, cyclic moving of the animals and the introduction of external animals aid infection. In particular, the overall picture of rabbitry Nr. 1 showed an "active" evolution of the E. cuniculi infection, which led us to continue the survey for a better definition of the progress of the infection between the various age classes and sheds. An analysis of the data showed a high rate of infection amongst breeding bucks (66.4%), does (64.9%) but lower amongst future stock rabbits (42.8%), while the fattening rabbits were negative. The age group in which the rabbits were most likely to become infected with subsequent development of specific immunity was the 80-90 day one. The probability to contract the infection increased however depending on environmental causes, especially from spore contamination. The simultaneous presence of large numbers of breeders, their movement, both within their sheds and from one shed to another, and finally also from natural breeding, could all explain the easy spread of the infection in subjects over the slaughter age, destined for future stock. Indeed under these conditions, these rabbits would come into contact with the spores more easily. The spores are highly resistant and are expelled by infected rabbits for periods up to even two months. It is at least probable that there was a similar pattern in four of the five sheds (A,B,D and E). Indeed there was increased positivity of breeders correlated with ageing. It still remains to better define the infection progress in shed C, even if in theory, there were less favourable conditions for the spread of the parasite.

In this rabbitry, the *E. cuniculi* infection should be considered a considerable pathological reality, even if apparently without dangerous effects on the animals health or their productivity. Evidently, the usual preventive sanitary measures are insufficient to interrupt the life cycle of the parasite, and therefore, to reduce the seroprevalence, a specific plan of direct prophilaxis should be adopted. Considering the high rate of positivity found, such a plan should be at least over five years. The more immediate alternative of eliminating all seropositive rabbits would greatly reduce the number reared and would therefore cause chaos in production. A possible eradication plan could be based on the following points: 1) a serological control and quarantine of all externally bought breeders; 2) switch from natural to artificial fecundation; 3) the gradual re-sanitation of sheds. They would be first emptied, and after an adequate all out sanitation period and a radical cleaning and disinfection they would be re-populated with young seronegative does (circa 90 days); 4) the selection of breeders from other sheds, eliminating the older seropositive rabbits; 5) a greater division of different categories of rabbits; 6) the confinement of future stock rabbits to shed C, where buck breeders should also be taken away; 7) serological monitoring of samples from different aged rabbits and productive categories throughout the 5 sheds. An assurance of the total negativity of fattening and future stock rabbits chosen for the repopulation of the sheds must be considered essential.

An economic evaluation in terms of cost/benefit should be made of the returns if this plan is implemented, to confirm the initial assumption of greater productivity from the healthy animals.

Acknowledgements - We would like to thank Prof. G.F. MANDELLI and Prof. D. GALLAZZI for having critically evaluated the work, Dr. S. BARBERIS and Dr. G. GRILLI for sampling the sera, Mr. G. BOZZONI for technical assistence and Dr. R. COATES of the 'Centro Linguistico dell'Università di Brescia' for the English translation.

REFERENCES

- COX J.C, GALLACHIO, H.A., PYE D., WALDEN N.B., 1977. Application of immunofluorescence to the establishment of an Encephalitozoon cuniculi-free rabbits colony. *Lab. Anim. Sci.*, 27 (2), 204-209.
- CRAVERO G.C., VALENZA F., PELLEGRINO C., VIGLIANI E., 1974. Sulla neuropatologia spontanea del coniglio. Nuova Veterinaria, 50, 154-165.
- GLANTZ S.A., 1988. Statistica per discipline biomediche. Mc Graw-Hill Libri Italia, Milano, Italy.
- GRENSTEIN G. DROZDOWICZ C.K., GARCIA F.G., LEWIS L.L., 1991. The incidence of *Encephalitozoon cuniculi* in a commercial barrier-maintained rabbit breeding colony. *Lab. Anim.*, **25**, 287-290.
- JULINI M., 1983. Ulteriori indagini sulla incidenza della encefalitozoonosi nei conigli. Ann. Fac. Med. Vet. Torino, 29, 98-105.
- JULINI M., PELLEGRINO N. 1981-82. Incidenza della encefalitozoonosi nei conigli macellati. Ann. Fac. Med. Vet. Torino, 28, 1-11.

- LEVADITI C., NICOLAU S., SCHOEN R., 1924. L'étiologie de l'encéphalite épizootique du lapin, dans ses rapports avec l'étude expérimentale de l'encéphalite léthargique Encephalitozoon cuniculi (nov. spec.). Ann. Inst. Pasteur (Paris), 38 (8), 651-712.
- PATTISON M., CLEGG F.G., DUNCAN A.L., 1971. An outbreak ofencefalo-myelitis in broiler rabbits caused by NOSEMA CUNICULI. Vet. Rec., 88, 404-405.
- SCANZIANI E., FINAZZI M., GALLAZZI D., 1985. Identificazione istologica di Encephalitozoon cuniculi e Toxoplasma gondii. Riv. Zootec. Vet., 13 (1), 21-27.
- VAVRA J., CHALUPSKY J. OKTABEC J., BEDRINK P., 1980. Infection in a rabbit farm: trasmission and influence on body weight. J. Protozool., 27 (3 suppl), 74A-75A.
- WALLER T., 1977. The india-ink immunoreaction: a method for the rapid diagnosis of encephalitozoonosis. Lab. Anim., 11, 93-97.

Indagine sieroepidemiologica per Encephalitozoon cuniculi in allevamenti cuniculi italiani a

diverso indirizzo produttivo - E' stata condotta una indagine sieroepidemiologica al fine di evidenziare gli effetti dell'infezione da *Encefalitozoon cuniculi* in allevamenti di conigli del Nord Italia. Dapprima sono stati controllati 231 sieri provenienti da 11 allevamenti di diverso indirizzo produttivo: 3 industriali, 6 rurali, 1 di conigli da laboratorio ed 1 gruppo di animali allevati in condizioni di isolamento. E' stata evidenziata una sieroprevalenza del 32,5% negli allevamenti industriali, del 21,4% in quelli rurali mentre i conigli di laboratorio sono sempre risultati negativi. Nella seconda fase è stata analizzata l'evoluzione dell'infezione in un allevamento industriale in funzione dell'età, sesso e dislocazione nei diversi capannoni. Sono quindi stati prelevati 690 sieri ed è stata evidenziata una positività del 66,4% tra i maschi (232 sieri), del 64,9% fra le femmine in riproduzione (302), del 41,8% nei conigli di età compresa fra 90 e 180gg (141) e del 0% tra i conigli di 75-80gg di età (15). Statisticamente non significative erano le differenze tra i sessi, tra i diversi capannoni e tra i maschi di diversa età. Viceversa la positività nelle femmine variava significativamente in rapporto al n° di parti. I risultati ottenuti giustificano l'adozione di un piano di controllo, basato principalmente sull'eliminazione dei sieropositivi, e lo spostamento dei negativi in ambienti puliti e disinfettati.