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ABSTRACT

We carried out a seroepidemiological survey to determine the infection prevalence caused by *Encephalitozoon cuniculi* in 12 industrial rabbitries located in the sanitary district of Forlì (Northern Italy). We tested, using Carbon Immuno Assay, 354 sera taken from randomly chosen rabbits (66 grandparents and 288 parents). All the units tested had a prevalence between 6.7% and 96.7% (average value = 47.5%). In the breeding units the prevalence was higher among grandparents (38,9%) than in parents (21.9%). The feed conversion index was almost the same in all rabbitries. We did not observed any statistical differences with regard to slaughter yield index but a certain level of inverse correlation with prevalence was noted. In units practising natural insemination, the prevalence was significantly higher (OD = 32.9). A statistical difference was also noted between the two genetic lines mostly used: the so-called "Y" line had a higher probability to be infected (OD = 2.56) than line "X". Also the management, judged as good in 8 units, was correlated to prevalence, since the highest values of seropositivity (>85%) were detected in 3 out of the 4 units in which the management was considered insufficient. In conclusion, we underlined the importance of adopting specific eradication plans and we have suggested the main direct measures. We also stressed the need to create groups of seronegative grand-grandparents to produce negative animals for breeding units.

INTRODUCTION

Rabbit encephalitozoonosis is a chronic parasitosis, which mainly effects rodents and lagomorphs. The etiological agent is *Encephalitozoon cuniculi* (LEVADITI 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 that help the spread of the infection.
In industrial animals, and in particular in rabbits reared for meat, the infection can cause considerable financial loss, due to mortality (up to 15%) (PATTISON 1971), to the increase of rejected animals and of reformed does, and the reduced carcass weight (GREENSTEIN 1991; VAVRA 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.
Serological surveys carried out in various countries revealed a positivity of up to 90%. In Italy the parasite has already been found histologically, in 12.6% of rabbits regularly slaughtered, and 20.4% including dead and suppressed rabbits (CRAVERO 1974; JULINI & PELLEGRINI 1981-2; JULINI, 1983; SCANZIANI 1985). In a previous study (LAVAZZA 1996) conducted in Northern Italy, we found a seroprevalence of 32.5% in the industrial rabbitries,

21.4% in the rural ones, while laboratory rabbits were entirely negative. Then we looked at how the infection developed in an industrial farm, with respect to the rabbits' age, sex, and location. 66.4% of bucks, 64.9% of does 41.8% in the age group 90-180 days and 0% in the fattening group between 75-80 days resulted seropositive. There was no statistically significant difference between the breeding sexes, breeding sheds, or between the age of the breeding bucks. However, the does' positivity varied significantly in relation to the number of litters.

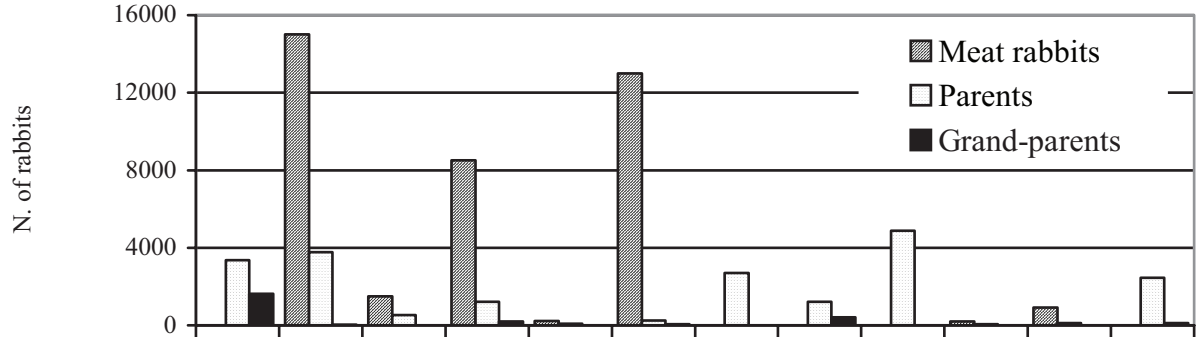
In this paper we describe the results of a survey, conducted in a district of North Italy (Azienda Sanitaria Locale of Forlì), with the aims to a) check the seroprevalence for *E. cuniculi* in all the industrial rabbitries in that area, and b) determine the correlation existing within type and attitude of rabbits, production parameters and management.

MATERIALS AND METHODS

Rabbitries and Animals

Only industrial rabbit units were considered (n. 18) and, since in the previous survey (LAVAZZA 1996) almost all meat rabbits resulted seronegative, we included in the survey all those in which both meat and breeding rabbits were present (n. 12). For each unit controlled, we described the main structural, productive and management characteristics in Table 1 and consistency in Figure 1.

Figure 1: Consistency of the rabbitries controlled in the survey



	1	2	3	4	5	6	7	8	9	10	11	12
Meat rabbits	0	15000	1500	8500	230	13000	0	0	0	190	900	0
Parents	3350	3780	520	1200	74	240	2710	1200	4880	66	107	2460
Grand-parents	1630	37	0	200	0	43	0	405	0	0	0	123

Serology

We established the number of rabbits to be serologically controlled in which we could detect prevalence of 10%, with a confidence limit of 95% (Table 2). One ml of blood was taken from the ear vein of randomly chosen rabbits. All the sera were taken within a 50 days period, and kept frozen at –18°C until examined with the Carbon Immune Assay (CIA), using a commercial kit produced and distributed by Testman, Uppsala, Sweden (WALLER, 1977). The antigen consisted of $3x10^7$ 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

Table 2: Sampling criteria

Unit consistency (N. rabbits)	N. sera samples
1-22	all
23-49	22
50-99	25
>99	29

mammals. The positive reference serum was a serum from a hyperimmune rabbit to which 0.1% NaN_3 was added and used at the minimum dilution of 1:20. The inactivated sera were assayed at 1:40, double the minimum significant dilution.

Table 1: Structural, productive and management characteristics controlled in the survey

Unit	Type of production[1]	Date[2]	Insemination[3]	Feed conversion[4]	Feed supplier[5]	Slaughter yield index[6]	Genetic line[7]	Management[8]
1	SBP	1997	Ai	3.6	B	57.7%	X	G
2	WM	1996	Ai	3.8	A	59%	X	G
3	WM	1980	Ae	n.n.[9]	B	53%	X	I
4	WM	1998	Ae	3.4	A	57%	X	G
5	WM	1978	N	n.n.	V	n.n.	hybrid	I
6	WM	1992	Ai	3.7	A	55%	Y	G
7	W	1993	Ae	3.8	A	57%	Y	G
8	SBP	1993	Ae	n.n.	A	n.n.	Y	G
9	W	1996	Ae	3.8	A	57%	Y	G
10	WM	1975	N	n.n.	A	n.n.	hybrid	I
11	WM	1990	N	n.n.	A	57%	Y	I
12	W	1991	Ai	3.6	A	57%	Y	G

[1]Only the main type of rabbit produced is reported: SBP = Production and selling of selected breeding does; WM = Production of weanlings and fattening. W = Production of weanlings but not fattening. In units 2, 4, 6 and 12 some grandparents were kept for inside selection. [2]The year of construction or completely renewing of the rabbitry's sheds is reported. [3]Natural (N) when using the semen of male internally reared or artificial by using internal (Ai) or external semen (Ae). [4]The feed consumption of all categories of animals and not only meat rabbits has been included in the calculation of FC index. [5]Two different feed supplier (A and B) for most units and various types of feed (V) for one unit. [6]These data have been roughly determined using data given from the slaughterhouse. For breeding rabbits production units we considered the data originating form meat animals reared in linked units. [7]Only two different genetic line (X and Y) were used with the exclusion of unit 5 and 10 where hybrids obtained from local rabbits were reared. [8]Two different judgements (G = good and I = insufficient) were given to management on the base of hygienic conditions, type and frequency of disinfecting, separation of units, competence of workers, rates and causes of mortality. [9]Unknown or not significative for the type of production

The CIA test consisted of an initial contact of an equal quantity (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.

Table 3: Prevalence in breeding rabbits

N. unit	grandparents (%)	parents (%)
1	50.0	23.0
2	0.0	9.0
4	50.0	38.4
6	70.0	25.0
12	14.3	13.0
Av .	38.9	21.9

Statistical analysis

The prevalences were compared with the different categories of rabbits (grandparents/parents and male/female), the productive parameters (feed conversion index, slaughter yield index), the genetic line, the type of insemination and the management quality by calculating the Odds Ratio (OR) (THRUSFIELD, 1995).

RESULTS AND DISCUSSION

In the 12 rabbitries we took a total of 354 samples; of which 66 from grandparents and 288 from breeding parents. In total 168 sera (47.5%) were positive and the prevalence was the same in grandparents (31 sera corresponding to 47%) and parents (137 corresponding to 47.6%). A variable prevalence was observed in each unit but they were all positive (Figure 2). In those units where only breeding rabbits were present (unit n. 1-2-4-6-12) the seroprevalence was higher in grandparents than in parents (Table 3). This probably meant that the former category was much more involved in the diffusion of *E. cuniculi* and could act as reservoir of the infection.

The prevalence in male and female was respectively 46% and 47%, which is not significant. With respect to feed conversion index, no statistical difference was demonstrated. A certain level of inverse but not significant correlation, was shown regarding slaughter yield index (Figure 3). Interestingly the higher value (59%) was obtained in unit 2, which has the lower prevalence (6.7%). This agrees with the assumption of greater productivity from the healthy animals suggested by VAVRA et al. (1980).

Figure 2: Distribution of prevalence in the 12 rabbitries

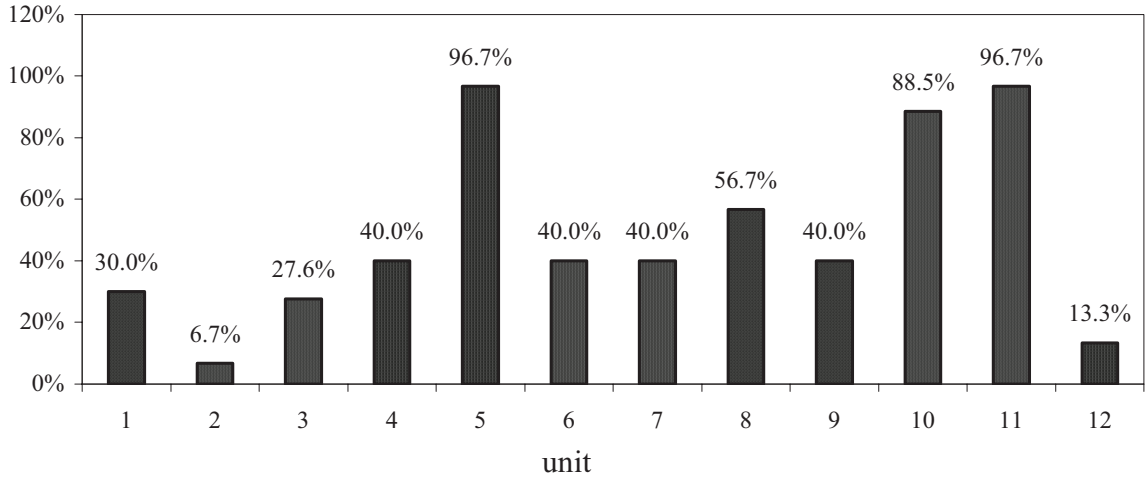
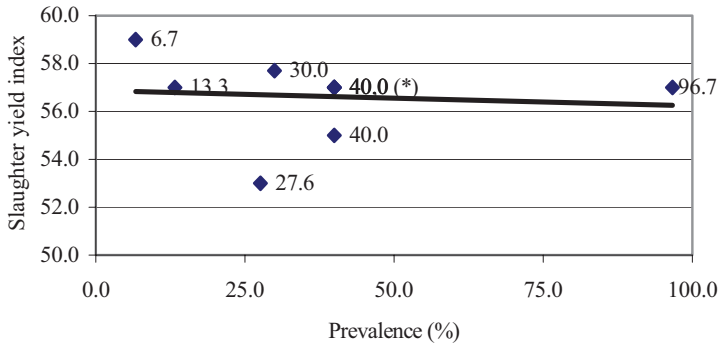


Figure 3: Correlation between prevalence and slaughter yield index.



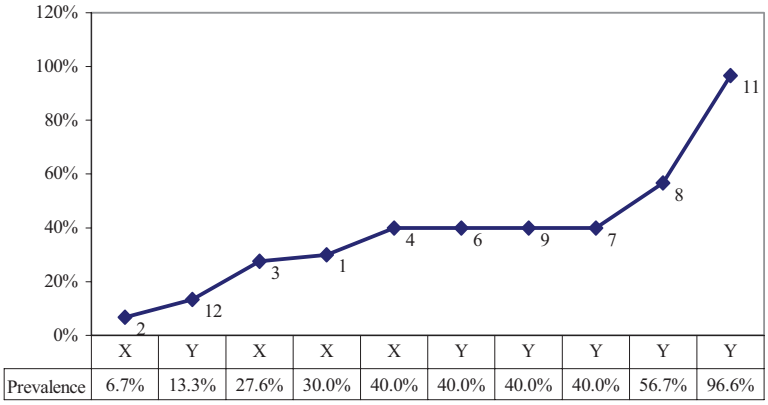
Unit	Slaughter yield index	Prevalence (%)
1	57.7	30.0
2	59	6.7
3	53	27.6
4	57	40.0
6	55	40.0
7	57	40.0
9	57	40.0
11	57	96.7
12	57	13.3

(*) this value is referred to 3 distinct units (n. 4-7-9)

In units (n. 5-10-11) where natural insemination was applied we detected the highest seroprevalence, respectively 96.7%, 88.5% and 96.6%. The statistical analysis indicated that such rabbitries had a 33 times higher probability to be infected than those using artificial insemination (OR = 32.9). Therefore, natural insemination must be considered a consistent way of diffusing the infection. Different mechanisms could be

involved: a healthy male could be infected during copulation by does that are excreting spores with urine; an infected male could infect healthy does with semen contaminated with spores during the excretion along urinary transit. Again, a male, infected or not, could act as passive vector of spores to several healthy does, due to the contamination of the penis as consequence of coupling with an infected doe.

Figure 4: Correlation between prevalence and genetic lines.



With regard to the genetic lines, we did not considered the 2 units (n. 5-10) in which local hybrids were present. In the remaining units some statistically significant differences were noted: in those 4 units (n. 1-2-3-4) where the "X" line was present, the prevalence was lower or slightly higher than the average value (39.1%), calculated over the 10 units in which either line "X" or "Y" was present. On the contrary all 6 units (n. 6-7-8-9-11-12) where the line "Y" was used had an equal or higher prevalence than the average value. This meant that the genetic line "Y" had a 2.5 times higher probability to be infected than line "X" (OR=2.56). This could be alternatively due to a lower resistance to infection of rabbits of line "X" or, more probably, to a higher rate of infected animals in the group of grand-grandparents and grandparents of line "Y".

The management was judged as good ("G") in 8 units (n. 1-2-4-6-7-8-9-12) and insufficient ("I") in the other 4 (n. 3-5-10-11). As shown in Table 4, the three units with the highest prevalence had insufficient management but contemporarily they were using natural insemination. This meant that such units had a 7 times higher probability to be infected than rabbitries with a good level of management, but also that management is probably less important as conditioning factor than the type of insemination.

Table 4: Correlation between prevalence and management

Unit	**2**	**12**	**3**	**1**	**4**	**6**	**9**	**7**	**8**	**10**	**11**	**5**
Manangement	G	G	I	G	G	G	G	G	G	I	I	I
Prevalence (%)	6.7	13.3	27.6	30.0	40.0	40.0	40.0	40.0	56.7	88.5	96.6	96.7

CONCLUSIONS

In the sanitary district in which we conducted the survey (ASL of Forlì), Encephalitozoonosis should be considered endemic and the high prevalence detected in all categories of rabbits, including grandparents and parents, indicates that such infection is highly diffusive. This situation, considering the data of our previous survey (LAVAZZA 1996) and the data (personal data not reported) originating from various areas of North Italy is probably common to most industrial rabbitries.

Moreover, our results clearly confirmed the negative effect caused by *E. cuniculi* infection on productive parameters (feed conversion index and slaughter yield index), and the importance mainly of the application of artificial insemination instead and then of a good management to limit and prevent the diffusion of the parasite.

The higher prevalence observed in one genetic line and the levels of positivity detected among grandparents, which were higher than in parents, suggest that in the diffusion of the infection, the breeding rabbits are the "reservoir" which maintain and favour the dissemination of spores.
In conclusion, the *E. cuniculi* infection should be considered a extensive pathological reality, and only apparently it does not cause dangerous effects on the animals health or their productivity. Since the usual preventive sanitary measures are insufficient to interrupt the life cycle of the parasite, a specific plan of direct prophylaxis should be adopted to reduce the seroprevalence or, better, to eradicate the infection.
The first step should be to create groups of seronegative grand-grandparents in order to produce negative animals for breeding units. The negativity of such rabbits should also be certified and be included as sanitary qualification in commercial protocols.
Then, a specific plan of direct prophylaxis should be adopted, even considering various levels of strictness in order to permit all farmers to apply it. A possible eradication plan could be based on the following points: 1) Serological control and quarantine of all externally bought breeding rabbits. 2) Switch from natural to artificial insemination. 3) 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. 4) Selection of breeding parents and elimination of the seropositive rabbits. 5) Serological monitoring of samples from different aged rabbits and productive categories to assess the total negativity of fattening and future stock rabbits.
Finally, 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.

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REFERENCES

CRAVERO G.C., VALENZA F., PELLEGRINO C., VIGLIANI E., 1974. Sulla neuropatologia spontanea del coniglio. *Nuova Veterinaria,* **50:** 154-165.

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.

LAVAZZA A, TINELLI F, ZANON F, MASSIRIO I. 1996. A seroepidemiological survey of Encephalitozoon cuniculi in different italian rabbitries. *Proceedings of the 6th World Rabbit Congress. Toulouse, France, 9-12 July 1996,* **vol 3.** p. 81-87 .

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 of encefalo-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.

THRUSFIELD M. 1995 Veterinary Epidemiology 2nd *Blackwell Science Ltd., London.,* p. 224-228.

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.