Proceedings 4th World Rabbit Congress, 10-14 October 1988, Budapest Hungary, Vol. 3, 447-455

ENCEPHALITOZOONOSIS: ATTEMPTS TO MEASURE THE INFLUENCE OF THE SPONTANEOUS INFECTION ON THE WEIGHT GAIN OF RABBITS

István Varga and Györgyi Horváth

University of Veterinary Science, Budapest, Pf. 2, 1400, Hungary

Introduction

Encephalitozoon cuniculi Levaditi, Nicolau and Schoen, 1923 is a protozoan /Microsporidia/, an obligate intracellular parasite affecting rabbits as well as other species of rodents, carnivores, occasionally a variety of other mammalians possibly including man /see excellent review by Canning and Lom, 1986/. Blue foxes and dogs seem to be most susceptible to this infection, while highest incidence has been established worldwide in domestic rabbits with a usual course of subclinical infection. This can be acquired both by vertical and horizontal transmission. Aggregates of the microsposidia with or without inflammatory reaction are frequently detected during routine histologic examination of the brain, kidney, heart and less abundantly in the tissues of other organs. Small indented grey foci are typical on the surface of the kidney. Apart from the histopathology, demonstration of spores occasionally excreted via urine may serve the diagnosis.

Immunodiagnosis has, however, proved to be a more reliable method to detect the infection in vivo. Skin hypersensitivity test /Pakes et al., 1972/, indirect immunofluorescence test /IFT, Chalupsky et al., 1971/, complement fixation test /Wosu et al., 1977/, Indian-ink immunoreaction /IIR, Waller, 1977/, also called carbon immunoassay /CIA/, the immunoperoxidase test /Gannon, 1978/, microagglutination test /Shadduck and Geroulo, 1979/ and enzyme immunoassay /ELISA, Cox et al., 1981/ were introduced and found useful according to reports published in an increasing number over the last decade. A fairly high prevalence of encephalitozoonosis was reported also in Hungary, surveying rabbit sera collected both from small backyard colonies

and large-scale rabbit farms /Vávra et al., 1987/.

Although several aspects of the host-parasite relationship have to some extent been studied /see review by Canning and Lom, 1986/, to our best knowledge, only a single report has been published on the influence of encephalitozoonosis on the body weight of rabbits /Vávra et al., 1980/. On the ground of serodiagnosis of rabbits slaughtered at the age of 90 days, the average meat carcass of 40 positive animals proved less by 11,2 % as compared to that of 130 negative animals.

The purpose of the present study was to /1/ measure the body weight gain and feed conversion of individually caged male rabbits over a 6 weeks' fattening period /2/ compare the sensitivity and reliability of CIA with histopathological findings.

The IIR was chosen as a rapid, simple method for screening and also showing good correlation with other immunodiagnostic techniques /Waller, 1977/. Reports on partial disaccordance of IIR and IFT /Chalupský et al., 1979; Pakes et al., 1984; Kunstýř et al., 1985/, and the difficulties in reading the test in the presence of a number of unstained organisms /Kellett and Bywater, 1978/ were also kept in mind.

Materials and Methods

There were 2 experiments performed on male New Zealand White rabbits purchased from the breeding stock of the Research Centre for Animal Production and Nutrition, Gödöllő. The dates of birth of the rabbits were 20-21.2.1986 and 20-21.7.1987 in the 1st and 2nd Experiment, respectively. Rabbits used in the 1st Exp. were weaned at the age of 5 weeks and brought into our animal room at the age of 6 weeks, while those in the 2nd Exp. were weaned at the age of 6 weeks and immediately transferred into our animal room. The 72 rabbits taken at random in the 1st Exp. were caged individually in 2 blocks of new, clean, 4-floor wire mesh cages with plate walls between them and with automatic watering, and were provided pelleted feed ad libitum. The room temperature was 16-19^{o}C; a full-day faint artificial illumination and automatic ventillation was provided. The 62 rabbits in the 2nd Exp. were similarly kept in the same animal room after the cleaning and the sterilization of the cages by flame.

The rabbits were weighed and blood and faecal samples were taken weekly in both experiments. The feed consumption was re-

corded from the age of 7 weeks during a 6 weeks' period. At the age of 13 weeks, the survivors were killed for postmortem examination, and samples of kidney, brain, spinal cord, heart, liver, spleen, and appendix were taken for histological processing. The number of coccidial oocysts per gram of faeces was determined by a modified McMaster technique.

Test kit for CIA was purchased from Testman /Uppsala, Sweden/, and serum dilution 1:25 was generally used considering the manufacturer's recommendations. Occasional difficulties in the interpretation of the test emerged both at that and at higher dilutions of sera, showing different ratios of stained organisms in Brownian movement hardly distinguishable from spores of E. cuniculi by morphology with the attached carbon particles. As from 3 out of 6 batches of the original carbon suspensions Escherichia coli-like organisms had been isolated in pure culture, sera were regarded positive only in cases having less than 5/100 unstained/stained organisms.

Rabbits were retrospectively sortied in two categories at the end of the experiments in order to estimate the effect of encephalitozoonosis on the body weight gain. Animals showing both seropositivity throughout the 6 weeks or the last 4 weeks of the investigation period and histopathological changes characteristic for encephalitozoonosis were regarded as "infected" /I/ and the rest of rabbits as "control" /C/ group. Data of rabbits, that died in the course of the experiments /due to enteritis or pneumony/ or were suffering from diarrhea, that showed abscesses or head tilt due to otitis in the middle ear proven by necropsy at the end of the experiments, were excluded from the evaluation.

Biometric analysis was made by Student's t-test, analysis of variance followed by Duncan's test, calculation of the linear regression and the correlation coefficient. Metabolic body weight gain /MBWG/ was calculated on the equation as follows:

$$MBWG = \frac{\text{mean daily gain, kg}}{\text{initial body weight, kg}^{0,75}} \times 100$$

Results

Mild coccidial infection with Eimeria media, E. magna and E. perforans was established in the rabbits when delivered to

our laboratory at the age of 6 weeks. The average numbers of oocysts /±S.D./ per gram of faeces /OPG/ amounted to 7 150± ±9 442 /I-group/, 4 887±12 982 /C-group/ in the 1st Exp., and 10 531±34 124 /I-group/, 9 772±21 526 /C-group/ in the 2nd Exp. at that time. Both the OPG and the ratio of rabbits shedding oocysts fell rapidly after individual caging /Table 1/

Table 1. Number of rabbits excreting in the faeces oocysts of Eimeria sp.

Exp.	Group	No. of rabbits	Age of rabbits /week/							
			6	7	8	9	10	11	12	13
1st	I	16	11	11	7	4	11	9	5	-
1st	C	51	25	26	12	18	15	26	17	-
2nd	I	13	9	5	1	1	0	0	0	0
2nd	C	36	17	11	3	5	2	1	1	1

In none of the rabbits killed at the age of 13 weeks and of those that died or were excluded for other reasons from the experiments /5 of 72 and 13 of 62 in the 1st and 2nd Exp., resp./ could be detected any sign of hepatic coccidiosis at postmortem.

The results of serodiagnosis and histopathological findings showed good correlation and the latter will be published in a separate paper /Vetési, 1988/. The incidence of seropositive littermates /+/total/ showed a distribution in the two experiments as follows:

1st Exp.: 0/3, 0/3, 0/4, 0/1, 0/2, 0/2, 0/3, 1/2, 0/2, 1/5, 0/3, 0/4, 1/3, 2/4, 2/4, 4/5, 2/2.

2nd Exp.: 0/1, 0/1, 0/3, 0/2, 1/3, 0/3, 1/3, 1/3, 2/4, 1/1, 0/2, 1/3, 0/1, 0/1, 1/2, 0/3, 0/2, 2/4.

Highest titers complying with aforementioned criterion of 13 seropositive rabbits in the 2nd Exp. at the age of 13 weeks were recorded as follows:/1:/ 800, 1600, 800, 400, 400, 1600, 400, 800, 3200, 100, 1600, 6400, and 50.

None of the rabbits showed clinical signs characteristic of encephalitozoonosis.

Since the groups of rabbits in the two experiments showed significant differences both in the initial mean weights /F= 15,3 at 6 weeks of age/ and final weights /F= 19,2 at 13 weeks/, pooling of data of corresponding groups would mask inherent discrepancies. Thus, they are separately shown in Table 2.

Table 2. Data referring to the body weight gain /$\bar{X}$,$\pm$S.D./ of rabbits spontaneously infected with Encephalitozoon cuniculi

Exp.	Group	Number of rabbits	Mean /g/	S.D.	p[x]	Av.diff. /%/
Body weight at the age of 7 weeks						
1st	I	16	1 327	228	>0,05	-4.4
1st	C	51	1 389	203		-
2nd	I	13	1 000	130	<0,001	-18.0
2nd	C	36	1 219	194		-
Body weight at the age of 13 weeks						
1st	I	16	2 628	363	<0,05	-6.5
1st	C	51	2 811	278		-
2nd	I	13	2 165	219	<0,001	-15.7
2nd	C	36	2 569	267		-
Metabolic body weight gain between weeks 7 and 13/%/						
1st	I	16	2.525	0.273	>0.05	-5.6
1st	C	51	2.676	0.407		-
2nd	I	13	2.800	0.452	>0.05	-0.6
2nd	C	36	2.817	0.529		-
Feed consumption between weeks 7 and 13						
1st	I	16	4 950	753	>0.05	-5.0
1st	C	51	5 213	604		-
2nd	I	13	4 317	511	<0.001	-14.0
2nd	C	36	5 024	579		-
Feed conversion rate between weeks 7 and 13 /kg/kg/						
1st	I	16	3.811	0.408	>0.05	+2,6
1st	C	51	3.713	0.403		-
2nd	I	13	3.735	0.428	>0.05	-0.5
2nd	C	36	3.757	0.426		-

[x] Student's t-test

The equations of the linear regression and the correlation coefficients of the weekly weight gain of rabbits between the ages of 7 and 13 weeks are also separately shown for each group as follows:

1st Exp. I: Y= -132+213X; r= 0.998
1st Exp. C: Y= -122+235X; r= 0.999
2nd Exp. I: Y= -381+197X; r= 0.999
2nd Exp. C: Y= -351+226X; r= 0.999

Discussion

Body weight of broiler rabbits is a result of many factors including genetics, nutrition, environment, age, sex, various infective agents, etc. Allocation of rabbits to groups of "positive" or "negative" concerning infection with E. cuniculi may be regarded as rough estimate on the basis of a single serologic reaction, since long, short and episodic humoral antibody response to this infection has been recognized /Kunstýř et al., 1986/. This itself may bring about certain overlap in the grouping of the rabbits, in addition to the occasional difficulties in the evaluation of the IIR. To overcome the latter, Kellett and Bywater /1978/ recorded preparations as positive in the presence of more than 5 % stained cells. We applied more rigorous criteria in the present study /see section "Materials and Methods"/ for the grouping of rabbits and for the assessment of the influence of the E. cuniculi infection on their body weight gain. Preference of 5 % border line of the unstained to the stained organisms reduced considerably the false positive reactions due partly to contamination with Escherichia coli-like bacteria.

The pooled final body weight figures in our experiments carried out on male rabbits under fairly similar conditions at an interval of 17 months are well in line with the findings of Vávra et al. /1980/ on the body weight gain decreasing effect of encephalitozoonosis in apparently healthy rabbits /11,2 % vs. 10,7 % in the present study/. In comparison with adequate controls, a reduced mean body weight in the seropositive groups of rabbits was already obvious at the age of 7 weeks. From that time on, the weight gain of the individually caged rabbits, virtually free of at least coccidial infection, could be characterised by a slightly lower coefficient of regression in the I-groups resulting in significant mean weights by the week 13. Though rabbits in C-groups tended to consume more feed, no significant difference /$p > 0.05$/ could be detected in terms of feed conversion rate or MBWG. More explicit adverse effect of the infection in the 2nd Exp., in which a high rate of rabbits had to be excluded due to intercurrent diseases, is suggestive of the significance of stress.

Experimental infection with E. cuniculi is envisaged to

yield more exact data on the weight gain of the rabbits.

Acknowledgements. The authors express their thanks to Dr. Gy. Virág for her help in purchasing the rabbits, to Dr. L.G. Molnár for his kind help by culturing the bacteria, and to Dr. E. Takács for ner advices in statistical analysis. The valuable technical assistance of Miss Zs. Kaminszky, Mrs. K. Kósa /Palenyikné/, Mrs. F. Kátai, I. Wölbling, J. Benkovics and G. Országh is also highly appreciated.

References

Canning,E.U., and Lom, J. /1986/. The Microsporidia of Vertebrates. Academic Press, London, pp. 289.

Chalupsky, J., Bedrník, P. and Vávra, J. /1971/. The indirect fluorescent antibody test for Nosema cuniculi. J. Protozool. 18. 47.

Chalupsky, J., Vávra, J. and Bedrník, P. /1979/. The use of the India-ink immunoreaction in the diagnosis of rabbit encephalitozoonosis. J. Protozool. 26. 70A-71A.

Cox, J.C., Horsburgh, R. and Pye, D. /1981/. Simple diagnostic test for antibodies to Encephalitozoon cuniculi based on enzyme immunoassay. Lab. Anim. 15. 41-43.

Gannon, J. /1978/. The immunoperoxidase test for diagnosis of Encephalitozoon cuniculi in rabbits. Lab. Anim. 12. 125-127.

Kellett, B.S. and Bywater, E.C. /1978/. A modified india-ink immunoreaction for the detection of encephalitozoonosis. Lab. Anim. 12. 59-60.

Kunstýř, I., Lew, L. and Naumann, S. /1986/. Humoral antibody response of rabbits to experimental infection with Encephalitozoon cuniculi. Vet. Parasitol. 21. 223-232.

Pakes, S.P., Shadduck, J.A. and Olsen, R.G. /1972/. A diagnostic skin test for encephalitozoonosis /nosematosis/ in rabbits. Lab. Anim.Sci. 22. 870-877.

Pakes, S.P., Shadduck, J.A., Feldman, D.B. and Moore, J.A. /1984/. Comparison of tests for the diagnosis of spontaneous encephalitozoonosis in rabbits. Lab. Anim. Sci. 34. 356-359.

Shadduck, J.A. and Geroulo, M.J. /1979/. A simple method for the detection of antibodies to Encephalitozoon cuniculi in rabbits. Lab. Anim. 29. 330-334.

Vávra, J., Chalupský, J., Oktábec, J. and Bedrník, P. /1980/. Encephalitozoon cuniculi /EC/ infection in a rabbit farm: transmission and influence on body weight. J. Protozool. 27. Suppl. 74A-75A.

Vávra, J., Chalupský, J., Bedrník, P., Horváth, Gy. and Varga, I. /1987/.A nyulak Encephalitozoon cuniculi-fertőzöttsége; adatok hazai előfordulásához. Magy. Áo. Lapja, 72. 499-502.

Vetési, F. /1988/. Personal communication.

Waller, T. /1977/. The india-ink immunoreaction: a method for the rapid diganosis of encephalitozoonosis. Lab. Anim.11. 93-97.

Wosu, N.J., Olsen, R., Shadduck, J.A., Koestner, A. and Pakes S.P. /1977/. Diagnosis of experimental encephalitozoonosis in rabbits by complement fixation. J. Infect. Dis., 135. 944-948.

Proceedings 4th World Rabbit Congress, 10-14 October 1988, Budapest Hungary, Vol. 3, 447-455

ENCEPHALITOZOONOSIS: ATTEMPTS TO MEASURE THE INFLUENCE OF THE SPONTANEOUS INFECTION ON THE WEIGHT GAIN OF RABBITS

István Varga and Györgyi Horváth
University of Veterinary Science, Budapest, Pf. 2, 1400, Hungary

Blood and faeces samples were taken and the body weight measured at weekly intervals from the age of week 7 to 13 in 2 experiments on a total of 134 individually caged, male New Zealand White rabbits. Feed consumption was measured which enabled the calculation of the feed conversion rate /FCR/ as well as the metabolic body weight gain /MBWG/, and te coccidial oocyst counts were continuously monitored. Indian-ink immunoreaction /IIR, called also carbon immunoassay: CIA/ was used to detect antibodies to Encephalitozoon cuniculi. Rabbits were retrospectively listed into infected /I-/ groups if both their sera showed less than 5/100 unstained/stained organisms throughout the 6 weeks or the last 4 weeks of the investigation period and showed histopathological changes characteristic of encephalitozoonosis, and the rest were regarded as control /C-/ group except those that suffered from or died of intercurrent diseases and thus were excluded from the evaluation. A remarkable difference was noted in the 2 experiments, though the final mean body weight was consistently lower in the I-groups as compared with the corresponding C-groups /by 4,4, % and 18 %, resp./. There was no significant difference in the MBWG or FCR /p>0.05/.

ENCEPHALITOZOONOSE: ETUDES SUR LES EFFETS INFLUENCANT LE GAIN DE POIDS CHEZ DES LAPINS INFECTES PAR VOIE NATURELLE

István Varga et Györgyi Horváth
Faculté de Médecine Vétérinaire, Budapest, Pf. 2, 1400, Hongrie

Dans deux epériences, nous avons mesuré, une fois par semaine, le poids de 134 lapins mâles blanc de Nouvelle Zélande entre l'âge de 7 et 13 semaines. Lors de chaque mesure, nous avons également pris des échantillons d'excréments et de sang. Nous avons aussi mesuré la consommation de nourriture, á partir de laquelle nous avons pu calculer le taux de conversion alimentaire et le gain de poids métabolique, et nous avons également mesuré régulièrement la quantité d'oocystes de coccidie dans les excréments. Pour montrer les anticorps produits contre l'Encephalitozoon cuniculi nous avons utilisé l'immunoréaction á l'encre de Chine /connu sous le nom anglais de IIR ou de CIA/. Nous avons ensuite regroupé les lapins d'une maniére rétrospective; d'un côté dans le groupe infecté /I/ ceux dont la proportion de microorganismes non peints/peints dans le sérum n'attegnait pas 5/100, et chez lesquels les changements histopathologiques caractéristiques á l'encephalitozoonose pouvaient étre montrés. D'un autre côté le groupe de contrôle /C/, mis á part les lapins qui étaient atteints d'une maladie intercurrente ou qui sont morts, et dont nous avons exclu les données lors de l'évaluation finale. Dans les deux expériences la différence était remarquable, bien que la différence de la moyenne du poids final dans le groupe I était continuellement inférieure á celle du groupe C /4,4 % et 18 %/. Le taux de conversion alimentaire et le gain de poids métabolique ne présentaient pas de différence significative /p>0,05/.

World Rabbit Science Association