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PARASITOLOGICAL ZOONOSIS IN RABBIT MEAT: RESULTS OF SEROEPIDEMIOLOGICAL SURVEY FOR THE INVESTIGATION OF *ENCEPHALITOZOON CUNICULI, TOXOPLASMA GONDII* AND *CHLAMYDIA PSITTACI* IN ITALIAN RABBITRIES

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ABSTRACT

During June 2002 and February 2003 1,800 blood samples were collected from 5 slaughtering plants located in Campania region (Italy) which were supplied by 21 rabbitries situated in central and southern Italian regions, such as Campania, Lazio, Basilicata, Molise, Calabria. The aims of this study were to use the Carbon Immunoassay (India ink) test (CIA) and FC test to determine respectively the prevalence of specific zoonotic agents such as *E. cuniculi, T. gondii* and *Chlamydia psittaci* in fattening rabbits. For *E. cuniculi* of the total number of 1,800 sera examined, 490 were positive and they represented 27.2%. All supplying breeding farms were positive with a percentage from 10% (4/40) to 57.5% (23/40) of total sera collected from each slaughtered batch. Sera resulted less positive for *T. gondii*: of 1,800 sera only 50 (2.7%) were positive; these date were relative to 6/21 breeding farms. Serological Chlamydia screening resulted totally negative.

Key words: *Encephalitozoon cunicoli*, *Toxoplasma gondii*, *Chlamydia psittaci,* zoonosis, Carbon Immunoassay.

INTRODUCTION

Encephalitozoon cunicoli is one of the mammalian microsporidian pathogens that can affect a number of different species of animals as well as humans (XIAO *et al.* 2001). Microsporidia are ubiquitous obligate intracellular sporeforming, small pathogens that caused significant agricultural losses and interference with biomedical research. In 1922 Wright and Craighead reported the first mammalian microsporidial infection in rabbits, while microsporidial infection in human beings was first diagnosed by Magarinos Torres in 1927. In general, however, microsporidia were not routinely diagnosed. Natural ways

of infection transmission are not well known. It is believed that the disease spreads horizontally by the oro-fecal route in large breeding farms with the presence of many animals, but above all, along the oro-urinal pathway (HALÁNOVÁ *et al.* 2003). But a key role in epidemiology and pathogenesis is also due to vertical, transplacental transmission of infection (BANEUX, POGNAN 2003; HALÁNOVÁ *et al.* 2003). As concerns the diffusion of some microsporidial species such as *E. cunicoli* in human beings, they have a zoonotic character, above all in immunocompromised individuals (THOMAS *et al.* 1997; SCHOTTELIUS *et al.* 2000; HALÁNOVÁ *et al.* 2003). The human clinical pictures include diarrhoea, keratitis, sinusitis myositis, pneumonitis and cerebral lesions (ROSSI *et al.* 1998).

The prevalence of antibodies to *E. cuniculi* in domestic and laboratory rabbits has been found to range from 7.5% to 76% in clinically normal animals, with higher prevalence in rabbits suffering from other infections (THOMAS *et al.* 1997). In industrial animals, and in particular in rabbits reared for meat, the infection may cause considerable financial losses due to mortality (up to 15%), to increased number of rejected animals and to reduced carcass weight (LAVAZZA *et al.* 1996).

Another parasitological zoonosis is toxoplasmosis, caused by the protozoan *Toxoplasma gondii* (WALLER, BERGQUIST 1982). Even in this case the risk of an infection from rabbit to man is scarce, as rabbit is an intermediate host (TANTINÀ M. *et al.* 2000). In rabbits there is a high level of seroprevalence. Seropositive rabbits have cysts in their tissues and most of them remain healthy. In some circumstances illness and death among pregnant and nursing does is registered. Rabbits are frequently infected by eating feed contaminated by cat faeces containing oocysts of *T. gondii* (OKERMAN 1994). A serological screening for *Chlamydia psittaci* was done.

The aims of this study were to use the Carbon Immunoassay (India ink) test (CIA) and FC test to determine respectively the prevalence of specific zoonotic agents such as *E. cuniculi, T. gondii* and *Chlamydia psittaci* in fattening rabbits coming from rabbitries located in different Italian regions.

MATERIALS AND METHODS

Collection of samples

During June 2002 and February 2003 1,800 blood samples were collected from 5 slaughtering plants located in Campania region (Italy) which were supplied by 21 rabbitries situated in central and southern Italian regions, such as Campania, Lazio, Basilicata, Molise, Calabria (Table 1). All breeding farms were controlled from twice to four times, except for breeding farms 1,9,13,19 and 21 which did not supply rabbits anymore to slaughtering plants, so that further samples were not collected.

Isolation and detection

For serological investigations of *Encephalitozoon cuniculi* and *Toxoplasma gondii* the sera were preserved at –20°C until they were examined. The seropositive rabbits were determined by the Carbon Immunoassay (India ink) test (CIA), (WALLER, BERGQUIST 1982) using a commercial kit produced and distributed by Medicago AB, Uppsala

(Sweden). The serological test used to detect chlamydial complement-fixing antibodies in animals was the CF test with a commercial antigen (Dade BEHRING MARBURG Gmbh from tissutal cultures infected by Chlamydia psittaci and lyophilized after inactivation and addiction of stabilizer).

Table 1. Examined sera in rabbitries

Range related to slaughtering capacity	*Monitored supplying breeding farms*	*Batches*	*Region of origin of animals*	*Total slaughtered rabbits*	*Total sera*
A) AVELLINO 3000/day **2000–3000 animals**	***3*** (5, 8, 16)	***7***	5), 16) Campania 8) Calabria	8,350	280
B) Benevento 2000/day **1000–2000 animals**	**5** (6, 13, 14, 15, 19)	**9**	6) Molise 13), 14), 15) Basilicata 19) Campania	17,900	360
C) CASERTA 1500/day **1000 –2000 animals**	***5*** (1, 3, 4, 7, 12)	***10***	1), 3), 4), 7) Campania 12) Lazio	7,247	400
D) NAPLES 600/day **500 – 1000 animals**	***5*** (9, 10, 11, 17, 21)	***9***	9), 10), 11) Lazio 17) Campania 21) Unknown	5,100	360
E) SALERNO 300/day **500 – 1000 animals**	***3*** (2, 18, 20)	***10***	2), 18), 20) Campania	3,200	400
TOTAL	**21**	**45**		**41,797**	**1,800**

RESULTS AND DISCUSSION

Table 2 shows the results obtained during serological monitoring in each farm. In the case of a positive immunological reaction to the presence of antibodies against *E. cuniculi* in the serum, this antigen will be stained greyish-black within 5 minutes. Of the total number of 1,800 sera examined, 490 were positive and they represented 27.2%.
All supplying breeding farms were positive with a percentage from 10% (4/40) to 57.5% (23/40) of total sera collected from each slaughtered batch. Sera resulted less positive for *T. gondii*: of 1,800 sera only 50 (2.7%) were positive; these date were relative to 6/21 breeding farms. Serological Chlamydia screening resulted totally negative. This confirms the hypothesis that Chlamydiosis mainly involves the does during the first and the last week of pregnancy and the young rabbits during the first week of life (BURATTO, COLIN, 1992).

Table 2. Serological results from 21 rabbitries

SEROLOGICAL TEST											
					PATOGENI					
FARM LOCATION	BREEDING FARM	DATE OF COLLECTING	N° POSITIVE BATCHES	TOTAL ANIMALS OF THE SLAUGHTERED BATCH	*ENCEPHALITOZOON CUNICULI (E)*		*TOXOPLASMA GONDII (T)*		*CHLAMYDIA PSITTACI* ©	
					POSITIVE/40 (N°)	POSITIVE/40 (%)	POSITIVE/40 (N°)	POSITIVE/40 (%)	POSITIVE/40 (N°)	POSITIVE/40 (%)
AVELLINO	1	16/10/02	1 (E)	700	15	37.5	---	---	---	---
BENEVENTO	2	03/07/02	10 (E) 1 (E,T)	300	4	10	---		---	---
		05/11/02		300	12	30	4	10	---	---
		10/12/02		400	20	50	---		---	---
	3	17/07/02		1000	15	37.5	---		---	---
		04/11/02		913	4	10	---		---	---
		25/11/02		600	7	17.5	---		---	---
	4	01/07/02		360	23	57.5	---		---	---
		09/12/02		546	9	22.5	---		---	---
	5	01/07/02		700	15	37.5	---		---	---
		11/09/02		2000	14	35	---		---	---
		13/11/02		700	16	40	---		---	---
CAMPOBASSO	6	23/07/02	2 (E)	2200	16	40	---		---	---
		24/09/02		2200	14	35	---		---	---
CASERTA	7	09/09/02	2 (E) 1 (E,T)	500	11	27.5	---		---	---
		30/09/02		700	8	20	---		---	---
		27/01/03		928	21	52.5	16	40	---	---
COSENZA	8	17/07/02	2 (E)	1500	4	10	---		---	---
		18/09/02		1000	12	30	---		---	---
FROSINONE	9	07/02/03	1 (E)	450	9	22.5	---		---	---
LATINA	10	23/10/02	3 (E)	700	---		---		---	---
		28/11/02		800	7	17.5	---		---	---
	11	10/07/02		600	---		---		---	---
		14/02/03		410	4	10	---		---	---
	12	10/03/03		1000	14	35	---		---	---
POTENZA	13	25/06/02	4 (E) 1 (E,T)	2000	4	10	---		---	---
	14	19/11/02		2800	6	*15*	4	10	---	---
		10/12/02		1337	---		---		---	---
		05/11/02		2900	12	30	---		---	---
	15	26/11/02		2016	28	70	---		---	---
		11/02/03		2400	14	35	---		---	---
SALERNO	16	08/07/02	8 (E) 4 (E,T)	1450	23	57.5	---		---	---
		13/01/03		700	16	40	6		---	---
	17	20/02/03		320	3	7.5	---		---	---
		27/02/03		330	---		---		---	---
		20/11/02		800	5	12.5	---		---	---
	18	18/09/02		400	28	70	12	30	---	---
		04/09/02		300	6	15	6	15	---	---
		20/11/02		400	8	20	---		---	---
	19	08/01/03		400	18	45	---		---	
		08/01/03		1455	6	15	8		---	---
	20	10/07/02		150	8	20	---		---	---
		07/10/02		200	15	37.5	---		---	---
		28/01/03		300	16	40	---		---	---
UNKNOWN	21	05/12/02	1 (E)	332	15	37.5	---	---	---	---
Total positive sera/1,800 tested sera					490	27.2%	50	2.7%	0	0

CONCLUSIONS

Immunodiagnosis has proved to be a more reliable method to detect the infection in vivo (VARGA, HORVÁTH 1988). For verifying the extent of *E. cuniculi* in groups of rabbits the CIA test is quick and easy to use (LAVAZZA *et al*. 1996). The same test was found to be a rapid and realiable method for identifying rabbits that were seropositive for *T. gondii* (WALLER, BERGQUIST 1982). Fattening rabbits resulted highly positive for *E. cunicoli* and this confirms the necessity of monitoring programmes to control the spread of this zoonosis (LAVAZZA *et al*. 1996) and to control this parasite. Any attempt to reduce the infection level can succeed only if the best hygienic conditions are ensured (VIRÁG *et al*. 1986). The scarce seroprevalence registered during our research, according to other Authors, confirms that rabbit is not a carrier animal of Toxoplasmosis to human beings and that there is no zoonotic risk from this animal.

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