MONITORING ON RABBIT MEAT PRODUCTION CHAIN

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Abstract - On the basis of EEC and Italian laws 3 slaughterhouses and 37 rabbit farms have been monitored in Veneto Region. Monitoring consisted in performing serological and bacteriological tests on samples collected from slaughtered animals, coming from different farms. These zoonosis have been monitored: Toxoplasmosis, Chlamydiosis, Salmonellosis, Listeriosis, Staphylococci and E. coli infection and Dermatomycosis.
Slaughterhouse monitoring gave positive results for each disease, with prevalence rates varying from 5,4% (Salmonella) to 97,2% (coagulase-positive Staphylococci).Environmental monitoring of 8 farms that had previously resulted positive, showed a very high frequency of Staphylococcosis and Dermatomycosis.

INTRODUCTION

Italy is the world biggest rabbit meat producer (1). The 80% of Italian production comes from commercial farms (1). In Italy the highest rate of rabbit production is in Veneto Region, where there are 964 intensive farms (about 354.000 does) and 13 rabbit slaughterhouses (about 13.000.000 slaughtered animals per year) (2).

D.P.R. 30/12/1992 n°559, which is the Italian application of Directive 91/45/EEC, regulates rabbit meat production and distribution and introduces a « production chain » approach, where veterinary surveillance and inspection activity are extremely important.

D.P.R. 559 states, in particular, that (omissis):

1) rabbits for meat production must come from farms not submitted to veterinary restrictive measures and periodically examined for their hygienic and sanitary condition.

2) rabbits are excluded from human consumption if at post mortem examination signs of communicable diseases are found. Also the Directive 92/117/EEC Zoonosis Order , still not introduced in Italian legislation, states (article 5, paragraph 1) the necessity of notifying zoonosis trends and sources in:

- farm animals
- food of animal origin
- raw materials and/or food from countries outside EEC or traded within EEC
- man

Zoonosis monitoring is particularly important in such a productive and legal frame, also to collect epidemiological data about production chains. The traditional clinical approach to animal pathology must be integrated with an hygienic approach, to assess the zoonosis epidemiological risk.

The aim of this research (*) is the development of a zoonosis monitoring method, to outline possible risks both in rabbit farms and slaughterhouses. The research is developed in three steps:

- setting up of a production chain information system
- identification of zoonosis to be monitored
- definition of sampling and monitoring methods

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MATERIALS AND METHODS

Information system

This system was based on census and data collection previously set up in cooperation with Veneto Epidemiological Unit (C.R.E.V.). Data are collected using 5 forms previously described (2) and concerning:
1) farms census (Veterinary Services)
2) slaughterhouses census (Veterinary Services)
3) farms characteristics description(Veterinary Services)
4) technical-sanitary management (Farmer)
5) slaughterhouse monitoring (Veterinary Services)

Zoonosis to be monitored

These have been defined referring to Italian legislation, to Directive 92/117/EEC, to literature (3) (4) (5) (6), to prevailing pathology in our area, and to monitoring feasibility related to practical conditions. The following diseases have been monitored:
- Toxoplasmosis (Toxoplasma gondii)
- Chlamydiosis (Chlamydia spp.)
- Salmonellosis (Salmonella spp.)
- Listeriosis (Listeria monocytogenes)
- Staphylococcosis (coagulase-positive Staphylococci)
- E. coli infections- Dermatomicosis (Trichophyton and Microsporum)

Sampling and monitoring methods

Monitoring was based on two different sampling sites: slaughterhouse and farm.

Slaughterhouse - 3 over 13 slaughterhouses of Veneto Region have been chosen, as they represented different situations of production and hygiene management:
- slaughtering only 1 day per week
- slaughtering 3 days per week
- slaughtering 5 days per week

The following samples have been taken twice in the three slaughterhouses:
a) during slaughtering: 20 blood samples for each batch, to perform serological test (Toxoplasmosis and Chlamydiosis)
b) after slaughtering and before chilling: 5 samples of abdominal skin for each batch (corresponding to 25 cm2 and 25 grams each) to perform microbiological exams (Salmonella spp., Listeria monocytogenes, E. coli, coagulase-positive staphylococci).

3.2 Farms - In the three slaughterhouses batches from 37 different farms have been monitored:
- 6 suppliers of the 1 day per week activity slaughterhouse
- 9 suppliers of the 3 days per week activity slaughterhouse
- 22 suppliers of the 5 days per week activity slaughterhouse

The 8 farms from which positive animals at the slaughterhouse monitoring originated, have been subsequently visited. During farm inspection environmental monitoring has been performed, to verify if there was any correlation between farm hygiene and sanitary situation and positive results obtained at the processing plant.
In each farm these samples have been taken:
- 30 swabs from cages floor
- 30 swabs from nest-boxes walls and floor
- 15 swabs from walls
- 15 swabs from fans

To assess the degree of air microbial contamination, two Petri plates, containing the below listed media, have been exposed for 30 minutes in each farm section:
- Mycobiotic agar (Dermatophytes)
- Baird-Parker (coagulase-positive Staphylococci)
- MacConkey or EMB agar (E. coli)

- XLD agar (Salmonella)

Samples have been carefully taken and labelled to avoid cross-contamination, kept at fridge temperature and immediately sent to Istituto Zooprofilattico. Samples have been processed according to standard methods for food analysis and to monitoring plans techniques in other species. Serological tests have been performed as described below:

- antibodies anti-Toxoplasma: passive hemagglutination with a commercial kit (Sclavo)
- antibodies anti-Chlamydia: CFT with an antigen produced by our Institute.

RESULTS AND DISCUSSION

Table 1 shows results obtained during bacteriological and serological monitoring in each slaughterhouse. The number of suppliers which resulted positive during monitoring and minimum and maximum values for coagulase-positive Staphylococci and E. coli (C.F.U/cm2) are also shown. Results obtained in the 8 monitored farms are summarized, in relation to the kind of sample and of laboratory procedure, in Table 2.

Table 1: Veneto Region slaughterhouses monitoring result

Bacteriological test	Slaughterhouse 1 Suppliers (9)	Slaughterhouse 2 Suppliers (6)	Slaughterhouse 3 Suppliers (22)
Coag + staf. (C.F.U.)/cm2 skin	9	6	21
n° positive batches (farms)			
min-max	4 - 6068	6 - 1263	10 - 10529
E. coli (C.F.U.)/cm2 skin	6	6	21
n° positive batches (farms)			
min-max	1 - 37000	4 - 2207	1 - 3086
L. monocytogenes	0	0	4
n° positive batches (farms)			
Salmonella	0	1	1
n° positive batches (farms)			
Serological test			
Toxoplasma	0	0	3
n° positive batches (farms)			
Chlamydia	0	1	2
n° positive batches (farms)			

From these results some considerations arise, in relation to:

- information system
- monitored zoonosis
- sampling and monitoring methods

The forms used for data collection appear to be useful and sufficient to supply information about production hygienic and sanitary status.

The veterinarians of the Health Service, who had to fill in 4 or 5 forms, well cooperated with us, whereas such a cooperation was less effective with farmers, having to fill in the form referring to animals technical and sanitary husbandry. Nevertheless very useful these farm forms are, because they give information about controls to be subsequently performed. So we studied an easier form, that farmers could better appreciate.

All the monitored zoonosis appeared to be present, with different prevalence rates. It's also possible to comment each single disease.

3 over 37 (8,1%) serologically screened farms were positive for Toxoplasmosis. Other animals from these farms have then been sampled at the slaughterhouse, submitting each case of spleen enlargement to histological examination. None of the 96 samples showed typical Toxoplasmosis lesions. The absence of clinical signs and lesions in animals, together with the scarce possibility of human infection (7), lead us to consider the continuous monitoring of this disease as not cost-effective.

Table 2: Veneto region farms monitoring results

Farms	Cages	Nests	Fans	Walls	Exposed plates fattening	Exposed plates does
	Coagulase positive Staphylococci					
1	+	+	-	+	+	+
2	+	-	+	-	+	+
3	+	+	+	-	+	+
4	+	+	-	-	+	+
5	+	+	-	+	+	+
6	-	-	-	-	+	+
7	+	+	-	+	+	+
8	+	+	+	-	+	+
	Escherichia coli					
1	-	-	-	-	-	-
2	-	-	-	-	+	-
3	-	-	-	-	+	+
4	-	-	-	-	-	+
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	-	+	-	+	+

Farms	Salmonella Cages, nests, fans, walls	Listeria m.	Dermatophytes: exposed plates Fattening	Does
1	-	-	+	+
2	-	-	+	+
3	+ (nests only)	-	+	+
4	-	-	+	+
5	-	-	+	+
6	-	-	+	+
7	-	-	-	-
8	-	-	+	+

During serological Chlamydia screening, in farms evidenced as positive (5,4%), 8 more blood samples have been taken, from animals that had aborted. In none of the collected samples anti-Chlamydia antibodies were found. A different monitoring and diagnosis approach can be proposed for this disease:
a) serological screening by means of CFT
b) ELISA test (antigen capture) on vaginal swabs from does that showed reproductive disorders)
ELISA test positivity must be confirmed with a bacteriological test.

Farms from which batches positive for Salmonella came (2/37=5,4%) resulted negative when sampled. In one case only nests swabs were positive, but for a different Salmonella serotype. This fact lead us to consider slaughterhouse positivity as due to cross-contamination. Having similar results been previously obtained in Verona province (8), further observations should be carried out.
Similarly, Listeria isolations (4/37=10,8%) have not been confirmed in farms, so cross-contamination can be supposed.
Coagulase-positive Staphylococci have been very frequently isolated (36/37=97,2%), more often than in previous surveys (8). This result can be correlated with the high diffusion in farms of Dermatomycosis (see table n02), of which Staphylococci infection can be a sequel.
Dermatomycosis as well is still a widespread and persistent zoonosis, as frequently reported (9) (10) (11) (12) (13) (14) (15) (16). For this disease a continuous monitoring both in farms and in slaughterhouses may be proposed, because of the infection frequency, also among personnel.
E. coli isolation rate was also very high (33/37=89,1%). This can be related to the careless evisceration of animals with diarrhoea. These bacteria so become important as markers of processing hygiene level. However the presence of E. coli O157 H7 strains, dangerous from a public health point of view, is still to be demonstrated.

Eventually, on the basis of a recently published survey (17) and of Directive 92/117/EEC, it seems that Campylobacteriosis should be added to other zoonosis to be monitored in rabbits.
Farms and slaughterhouses census allows also in rabbit production to set up statistically and geographically representative monitoring plans. Sampling and monitoring methods we used appear to be handly and effective both in slaughterhouses and in farms. Veterinarians assigned to meat inspection found slaughterhouse monitoring particularly useful to achieve a precise idea of sanitary situation.
Reliable results, particularly for staphylococci infection, have been obtained by farms environmental swabbing, even if this monitoring didn't directly involve animals. For air monitoring, plate exposure system has been chosen for practical and economic reasons, although the surface air system (SAS) can be more reliable (18). Nevertheless have interesting results been obtained, particularly for Dermatomycosis.

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Riassunto - In applicazione di normative nazionali e comunitarie,si sono effettuati dei monitoraggi in 3 macelli e 37 allevamenti cunicoli del Veneto.Il monitoraggio prevedeva l'esecuzione di esami sierologici e microbiologici da cp. prelevati da animali macellati, provenienti dai diversi allevamenti.Sono state monitorate le seguenti malattie: Toxoplasmosi, Chlamydiosi, Salmonellosi,Listeriosi, Stafilococcosi, Colibacillosi,e Dermatomicosi.I monitoraggi del macello hanno evidenziato positivit' per tutte le malattie, con prevalenza variabile dal 5,4% (Salmonella) al 97,2% (Stafilococchi coag.+).I monitoraggi ambientali, eseguiti in 8 allevamenti risultati positivi in precedenza, hanno evidenziato una elevatissima frequenza delle infezioni da Stafilococco e da Dermatomiceti.