NOTE *Bacteriology*

Salmonella Typhimurium DT104 in Farmed Rabbits

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ABSTRACT. A total of 1,000 rectal samples were collected from rabbits coming from 25 rabbit farms in southern Italy. All samples were processed for isolation of *Salmonella* spp. by standard culture method based on the ISO 6579:2002 method. *Salmonella* spp. was isolated from 1/25 rabbit farms analyzed. In particular, four out of 1,000 rectal swab samples, taken from young rabbits, were serotyped as *S*. Typhimurium and phage typed as *S*. Typhimurium DT104. All the isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (ACSSuT pentaresistance type). The findings of the present study suggest the rabbit as potential carrier of *S*. Typhimurium DT104.

KEY WORDS: ACSSuT profile, Italy, rabbit, *Salmonella* Typhimurium DT104.

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World's rabbit meat production is continuously increasing. Rabbit production is concentrated in 2 major areas (Europe and Asia). As reported by EFSA [5], namely European Food Safety Authority, Italy is by far European's leading producer of rabbit meat, with Spain second, and France third. In Asia, the biggest producer is China which accounts for 99% of Asian production.

Salmonella enterica represents a leading cause of foodborne infections worldwide where *Salmonella* Enteritidis and *Salmonella* Typhimurium are considered the most common serovars isolated from human infections in Europe and the US [6, 8, 11, 17]. *Salmonella* infections occur in both developed and developing countries and are a major contributor to morbidity and economic costs. *Salmonella* can colonize and cause disease in a variety of food-producing and non-food-producing animals. Within this genus, more than 2500 serovars have been described. Although all serovars may be regarded as potential human pathogens, the majority of infections are caused by a very limited number of serovars [2, 6].

Since 2001, *S*. Typhimurium represents the prevalent serovar causing human infection in Italy. Such a predominance seems to be peculiar of Italy, as in most of the other European countries *S*. Enteritidis still remains the most common serovar isolated from human infections [8, 11, 17].

S. Typhimurium is the most frequently isolated serovar in several animal species [14]. Current scientic knowledge of *S*. Typhimurium isolation in rabbits in Italy [8] is incomplete and limited to anecdotal reports although during 1997 several salmonellosis outbreaks were reported in intensive rabbit farms in the North-Eastern regions of Italy [1]. Moreover, a study conducted in the Center-Northern regions of Italy during the period 2002–2004 by Graziani *et al*. [8] on antibicrobial resistance in *S*. Typhimurium reported 83 positive rabbits.

As no data on *Salmonella* isolation in rabbits are available for southern Italy, the present study was aimed at evaluating the prevalence of *Salmonella* in rabbits bred in southern Italy, as well as to evaluate their pattern of antimicrobial resistance.

To achieve this goal, from November 2008 to May 2009, 25 intensive rabbit farms located in southern Italy in the Campania region were investigated. Each farm was located far from the urban districts. The number of rabbits in each farm ranged from 400 to 2,500. The age of the rabbits analyzed ranged between one month and 2 years old. On each farm, 20 young (up to 4 months old) and 20 adult (i.e. older than 4 months) individuals were randomly selected and examined by rectal swabs, for a total of 1,000 rectal samples. All rabbits were apparently in a healthy body condition, although, occasionally, some animals showed signs of diarrhea. All the samples were representative of the fattening, the reproductive and the restocking units. Furthermore, environmental samples (i.e. drinking water, dust, rabbit cage and rabbit nest box swab samples) were collected.

All samples were analysed for isolation of *Salmonella* strains using the International Organization for Standardization procedure ISO 6579:2002 [10]. In particular, rectal, rabbit cage and nest box swabs, as well as 15 g of dust collected from different sites (i.e. wall, floor, and vents), were inoculated in Buffered Peptone Water (BPW; Oxoid Ltd, UK) as pre-enrichment media, and incubated at 37°C for 18 hr. After incubation, samples were inoculated into Rappaport–Vassiliadis Broth (Oxoid Ltd), as enrichment media, and incubated at 42°C for 18 hr. The cultures obtained were plating onto Xylose-Lysine-Desoxycholate Agar (Oxoid Ltd), incubated at 37°C and examined after 24 hr. Suspected colonies were inoculated onto a second selective agar, Brilliant Green Agar (Oxoid Ltd) and incubated at 37°C for 24 hr.

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Table 1. Results of antimicrobial susceptibility test of four *S*. Typhimurium DT104 isolated from 1,000 rabbit rectal swab samples

No. of strains	% of resistance to[a)]										
	A	C	S	Su	T	Ctx	Gm	K	SXT	Nx	Cip
4	100	100	100	100	100	0	0	0	0	0	0

a) Abbreviations are given in the text.

With respect to drinking water, samples were collected in 100 m*l* sterilized screw-capped glass bottles, and filtered through Millipore filters 0.22 micrometre pore size (Millipore, U.S.A.). Filters were inoculated into BPW after which the isolation followed the procedure described above. All isolates were biochemically identified by using the API20-E system (bioMérieux, France). All strains were stored frozen at –80°C in 20% glycerol.

Salmonella isolates were serotyped according to the Kauffman-White scheme, then, isolates identified by serotyping as *S.* Typhimurium, were phage typed according to the PHLS Colindale system. The analyses were carried out in collaboration with the National Reference Laboratory for Salmonella (IZSVe, Legnaro, Italy).

The antimicrobial susceptibility testing was performed using the disk diffusion method according to the Clinical Laboratory Standards Institute M100-S16 and National Committee for Clinical Laboratory Standards M31-A2 recommendations [4, 12]. The antimicrobials tested were those included in the Enternet reference panel [16]. Specifically, ampicillin (A, 10 μg), chloramphenicol (C, 30 μg), streptomycin (S, 10 μg), sulfamethoxazole (Su, 300 μg), tetracycline (T, 30 μg), cefotaxime (Ctx, 30 μg), gentamycin (Gm, 10 μg), kanamycin (K, 30 μg), sulfamethoxazole-trimethoprim (STX, 23.75/1.25 μg), nalidixic acid (Nx, 30 μg), and ciprooxacin (Cip, 5 μg) were used.

Salmonella spp. was isolated from 1/25 rabbit farms analyzed. In particular, four out of 1,000 rectal swab samples (0.40%; 95% Condence Interval=0.13–1.10%) were serotyped as *S*. Typhimurium. These strains were phage typed as *S*. Typhimurium DT 104. All positive samples were from young rabbits of which 3 were clinically healthy and one showed diarrhea. In contrast, the environmental samples were constantly negative except for 2 nest boxes housing the positive rabbits.

With respect to antimicrobial susceptibility testing, *S*. Typhimurium DT 104 strains showed multiple drug resistance (resistant to 3 drugs). In particular, all the isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (ACSSuT pentaresistance type). In contrast, all the isolates were susceptible to cefotaxime, gentamycin, kanamycin, sulfamethoxazole-trimethoprim, nalidixic acid, and ciprooxacin. Results of antimicrobial susceptibility test are summarized in Table 1.

In Italy, *S.* Typhimurium were widely isolated from several food-producing animals. In fact, from 1999 to 2001, Busani *et al*. [3] reported during the Italian surveillance system for Salmonella infection 460 strains of *S.* Typhimurium isolated from different animals and derived meat products: swine (*n*=231), rabbit (*n*=92), cattle (*n*=62), poultry (*n*=40), and pigeon (*n*=34). Furthermore, a study conducted by Graziani *et al*. [8], from 2002 to 2004, reported 1908 isolates of *S.* Typhimurium from human (*n*=755), bovine (*n*=122), swine (*n*=632), chicken (*n*=94), turkey (*n*=117), rabbit (*n*=83), and pigeon (*n*=105); among them 242 strains were phagetyping as *S.* Typhimurium DT104. In southern Italy, although all isolates were phagetyped as *S*. Typhimurium DT104, *S*. Typhimurium is scarce in rabbits.

S. Typhimurium can cause severe enteritis with high mortality percentages in fattening rabbits; in doe rabbits *S*. Typhimurium produces enteritis and metritis usually associated with abortions and heavy losses inside the nests [15]. In suckling rabbits *S*. Typhimurium can cause diarrhea, haemorrhagic enteritis, splenomegalia, and fibrinous peritonitis. In the present study, however, all *Salmonella*-positive rabbits were in healthy conditions although one rabbit showed diarrhea. The environmental samples were negative except for the nest boxes housing the positive rabbits. This finding showed environmental contamination by positive animals through their feces. In fact, it has been demonstrated that *Salmonella* possess the capability to survive in external environments during transmission from one host to the next for several days [18]. Although most reports on *Salmonella* infection in rabbits were observed before the diffusion of industrial productions [13], failure with health standards may cause the spread of *Salmonella* also in industrial farms [9].

With respect to the antimicrobial susceptibility testing our findings are similar to other studies [7, 8], showing the multiple resistance as a common feature in *S*. Typhimurium. Interestingly, as reported by Graziani *et al*. [8] the ACSSuT profile is typically associated with phage type DT104 and the ASSuT profile, could be considered an important cause of human infections in Italy. Thus, our results suggest the role of rabbit as a potential reservoir of drug-resistant *Salmonella* and underline the need of integrated surveillance systems that consider *Salmonella* prevalence in Italian rabbits to reduce the consumer health risks.

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