NOTE  Bacteriology

**Salmonella Typhimurium DT104 in Farmed Rabbits**

Luca BORRELLI1), Alessandro FIORETTI1), Valentina RUGGIERO1), Antonio SANTANIELLO1), Giuseppe CRINGOLI1), Antonia RICCI2), Lisa BARCO2), Lucia Francesca MENNA1) and Ludovico DIPINETO1)*

1) Department of Pathology and Animal Health, Faculty of Veterinary Medicine, University of Naples Federico II, Naples and 2) National Reference Laboratory for Salmonella, IZSVe, Legnaro (Padua), Italy

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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 serotype d 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.

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 scientific 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 antibiogram of isolates 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 plated 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.
tem for Salmonella infection 460 strains of API20-E system (bioMérieux, France). All strains were biochemically identified by using the API20-E system. All isolates were stored frozen at –80°C in 20% glycerol.

Filters were inoculated into BPW after through Millipore filters 0.22 micrometre pore size sterilized screw-capped glass bottles, and filtered 100 ml through Millipore filters 0.22 micrometre pore size. Cefotaxime testing was performed using the disk diffusion method according to the Clinical Laboratory Standards Institute M31-A2 recommendations [4, 12]. The antimicrobials tested were those included in the Enter-net 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 ciprofloxacin (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% Confidence Interval=0.13–1.10%) were serotyped as S. Typhimurium. These strains 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 Enter-net 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 ciprofloxacin (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% Confidence Interval=0.13–1.10%) were serotyped as S. Typhimurium. These strains were phage typed as S. Typhimurium DT104. All positive samples were from young rabbits of which 3 were clinically healthy and one 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 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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REFERENCES

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