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# ANTIMICROBIAL RESISTANCE AND DRUG CONSUMPTION IN RABBIT FARMING

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#### ABSTRACT

In order to collect information about the use of antimicrobials (AMs) and levels of antimicrobial resistance (AMR) in 32 Italian intensive meat rabbit farms, consumption of antimicrobial agents was calculated over the period 2010-2014 and the in vitro efficacy of cefotaxime, aminosidine, colistin, enrofloxacin, chloramphenicol, tetracycline, sulfadiazine and trimethoprim was assessed against 160 *Escherichia coli* isolates collected from healthy animals. Compared to AM consumption in the first semester of 2010, the mean consumption of AMs decreased by 29% in the second half of 2014, falling to 1,898 mg/kg live weight (LW), represented mostly by tetracyclines (70.7%) and sulfonamides (11.8%). Overall, between 2010 and 2014, consumption fell for tetracyclines and polymyxins, solely represented by colistin, but increased for bacitracines, sulfonamides and fluoroquinolones. Evaluation of *E. coli* susceptibility to the eight considered antimicrobial agents yielded variable but generally high proportions of microbiological resistance (minimal inhibitory concentrations higher than EUCAST epidemiological cut off (MICs>ECOFF). Unusually high resistance levels were observed for some AMs (tetracycline, sulfadiazine and enrofloxacin) compared to recently reported levels for other food-producing animals in Europe. The study evidences a considerable decline in the use of AMs in Italian meat rabbit farms from 2010 to 2014, but consumption rates still remain very high. It also highlights an alarming spread of AMR, including resistance to AMs considered critically important for human health.

Key words: drug consumption, antimicrobial resistance, rabbit, Escherichia coli

#### **INTRODUCTION**

Over the last decade, the burden of antimicrobial resistance (AMR) has reached alarming proportions and there is widespread concern within the scientific community because many low cost antibiotics, currently used in the treatment of human infections, could become less effective in the near future (Laxminarayan et al., 2013). In particular, the appearance of multi-drug resistant organisms (MDROs) has led to a worldwide increase in human deaths due to therapeutic failures.

Many studies have demonstrated that MDROs can be transmitted to humans by various routes and the complexity of these epidemiological patterns has strengthened the belief that fighting AMR requires a holistic approach based on a one-health perspective (European Council, 2012). A key role in this scenario is played by bacterial resistance in animals, particularly food-producing animals (FPAs). FPAs can act as amplifiers of resistant microorganisms due to the concentration in a limited area of huge amounts of animals kept under antimicrobial treatment, favouring selective onset, or maintenance, or spread of bacterial resistance to AMs (Marshall and Levy, 2011). This constitutes a public health (PH) risk and numerous studies have shown that MDROs can directly contaminate animal handling staff (Pletinckx et al, 2013), foodstuffs of animal origin (Davis et al., 2015), and agricultural areas or water bodies as a result of the use of animal manure in agriculture (von Salviati et al., 2015). It has recently been reported that crops cultivated in contaminated soil can carry MDROs, and the land surrounding farms can be contaminated aerogenously (von Salviati et al., 2015). Multiple reservoirs of AMR, combined with the ability of AMR to persist at length after selective pressure has been removed (Bischoff et al., 2002), and the occurrence of multiresistance and coresistance, make the containment of AMR a real challenge. Despite difficulties, efforts must be made to extend the life of available antibiotics through prudent use of AMs and an overall reduction in drug consumption in animal breeding (European Commission, 2015).

In Europe, the ESVAC project has been set up by EMA (European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption, 2015) within this framework, to monitor the sales of AMs in the European member states, and harmonized plans for AMR monitoring in FPAs are carried out in compliance with the European legislation (European Commission, 2013). These monitoring activities do not, however, include meat rabbits, even though this FPA sector plays a sizeable economic role in some European Countries, such as Italy, France, Spain and Hungary, and it is growing in other Countries, including China. The annual reports produced by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) on sales of antimicrobial agents in France, which differentiate between consumption levels in the various animal species, indicate that medication levels in rabbits are among the absolute highest and similar only to those of veal calves (French Agency for Food, Environmental and Occupational Health & Safety, 2014). Despite reports that meat rabbits can be involved in the transmission of MDROs to humans (Agnoletti et al., 2014; Drigo et al., 2015), there is a considerable lack of structured data on AMR levels in farms rearing rabbits for meat. The aim of this study was to collect detailed information on the use of AMs and on levels of AMR in Italian industrial meat rabbit farms. AMR was recorded in commensal indicator bacteria against a panel of AMs used to treat rabbit diseases in intensive farming or of relevance for PH. In order to manage AMR risk in the rabbit sector, it is essential to make this information available.

#### MATERIALS AND METHODS

#### Study design and data collection

Consumption levels of antimicrobial drugs were assessed in 32 intensive rabbit farms in Italy between 2010 and 2014. The study was longitudinal and retrospective. The farms were selected by convenience sampling, according to size. Seventeen farms with 500-1000 does, nine with 1000-2000 does and six with over 2000 does were enrolled in the study.

In 2014, indicator bacteria were collected at each farm to test the minimal inhibitory concentration (MIC) against a panel of AMs, selected for their relevance to PH or because they are orally administered for prophylaxis purposes or to treat rabbit pathologies.

The data were analysed to provide descriptive statistics on the overall rabbit exposure to AMs, the trends of AM consumption over a five-year period, and the AMR levels in indicator bacteria.

Data on consumption of both drugs, administered parenterally or in drinking water, and medicated feed were collected from drug registers, which are endorsed and audited by the local veterinary services, from veterinary prescriptions, and from feed delivery documents, during the period 2010-2014. The total weight of live rabbits sold for slaughter at the end of the fattening periods was collected from the related commercial files and health certificates.

#### **Bacterial strain collection**

AMR was tested in commensal indicator microorganisms (*E. coli*) isolated from healthy rabbits. In 2014, five samples of fresh faeces were collected at each farm from under the cages of animals aged 35-50 days, at various points of the barn. The faeces were transported to the laboratory at  $+4^{\circ}$ C. In the laboratory, 2 g of faeces were taken from each sample and homogenized in 6 ml of Heart Infusion Broth (HIB, Laboratorios Conda). Then 0.5 ml of homogenate was inoculated in 5 ml of HIB and incubated at 37°C for 24 h. At the end of incubation, the broth culture was plated on Eosin Methylene Blue Agar (EMB, Biolife), incubated at 37°C for 24 h, and then tested for *E. coli*. For each sample a characteristic colony of *E. coli* was re-isolated on blood agar plates (Blood Agar Base, Biolife, with the addition of 5% v.v. red blood cells), and incubated at 37°C for 24 h. At the end of incubation, the pure colony was identified using MALDI-TOF MS (Microflex Biotyper LT, Bruker Daltonics) and stored in Cryobank vials at -80°C until the MIC could be tested.

#### **MIC determination**

To evaluate bacterial susceptibility to the selected AMs, the MIC was tested by broth micromethod assay, according to the CLSI Manual, VET01-A4. Assessment was made of *E. coli* susceptibility to cefotaxime (CFT), aminosidine (AN), colistin (CT), enrofloxacin (ENR), chloramphenicol (CHL), tetracycline (TE), sulfadiazine (SFD), and trimethoprim (TRI). Orally administered active ingredients commonly used to treat rabbit enteric pathologies (AN, CT, ENR, TE, SFD, TRI) were selected, in addition to antimicrobial agents

(CFT and CHL) included in the AMR monitoring plan which started in Europe in 2014 (European Commission, 2013). Each drug was tested at two-fold dilutions ranging from 0.008 to 256 mg/L.

#### Data analysis

The raw data, represented by the quantities of medicinal specialities or medicated feed used in farms in each semester, were converted into milligrams of active ingredients, taking account of their concentration.

For each farm the raw data were then converted into the total amount of AMs consumed in a semester of the year. The total amount of each antimicrobial class and each antimicrobial agent used to produce one kg of live rabbit weight (kg LW) in the same time period was calculated using the total weight of live rabbits sold to the slaughterhouse at the end of the fattening cycles.

AMR was evaluated for each tested active ingredient by calculating the MIC50 and MIC90. MICs were analysed according to the epidemiological cut off (ECOFF) (EUCAST, http://www.eucast.org), where available. The bacterial isolates displaying MICs >ECOFFs for at least three active ingredients were defined as multidrug resistant (MDR); the isolates displaying MICs  $\geq$ 64 mg/L for SFD and MICs  $\geq$ 32 mg/L for AN were considered microbiologically resistant to SFD and AN after the MIC frequency distribution.

#### **RESULTS AND DISCUSSION**

The mean consumption of AMs recorded in the 32 surveyed farms was 2681 mg/Kg LW in the first half of 2010, decreasing to 1898 mg/Kg LW at the end of 2014. In the second semester of 2014, the classes of antimicrobials prevalently used in rabbit farms were tetracyclines (70.7%), sulfonamides (11.8%), aminoglycosides (5.6%), polymyxins (4.1%), pleuromutilines (3.1%), bacitracines (1.4%), quinolones (1.3%), diaminopyrimidines (1.2%) and macrolides (0.8%). Other antimicrobials (included beta-lactams and phenicols) were consumed in negligible amounts. From 2010 to 2014, drug consumption increased for aminoglycosides (from 94.6 to 107.4 mg/kg LW), sulfonamides (from 145.9 to 224.6 mg/kg LW), quinolones (from 3.7 to 24.5 mg/kg LW), bacitracines (from 5.0 to 26.5 mg/kg LW) but decreased for tetracyclines (from 2035.7 to 1341.2 mg/kg LW), polymyxins (from 246.2 to 77.8 mg/kg LW, solely represented by colistin), pleuromutilines (from 78.3 to 58.2 mg/kg LW), macrolides (from 22.0 to 14.4 mg/kg LW) and diaminopyridines (from 24.2 to 22.6 mg/kg LW). To evaluate AMR in the 32 rabbit farms, 160 E. coli isolates were selected for the MIC test. All the E. coli isolates displayed a wild type phenotype (MICs<ECOFF) for cefotaxime (MIC50 and MIC90 = 0.063 mg/L); conversely, 21.2% of the isolates presented MICs>ECOFF for CT (MIC50= 0.5 mg/L, MIC90= 4 mg/L), 23.7% for CHL (MIC50= 8 mg/L, MIC90= 256 mg/L), 63.1% for ENR (MIC50= 0.25 mg/L, MIC90= 64 mg/L), 97.5% for TE (MIC50= 128 mg/L, MIC90= 256 mg/L), and 89.3% for TRI (MIC50 and MIC90 >256 mg/L). The MICs for AN and SFD, for which ECOFFs are not available, were MIC50 = 2 mg/L and MIC90 = 256 mg/L for AN, and MIC50 and MIC90= >256 mg/L for SFD. Out of the tested *E. coli* isolates, 95.6% were MDR and the most common resistance pattern was ENR-SFD-TRI-TE, which was detected in 57.5% of the isolates.

**Table 1:** MICs of nine antibiotics against 160 *E. coli* isolates. For each drug MIC50, MIC90, MIC range and ECOFFs are reported. In gray MICs > ECOFF.

	Antimicrobial concentration (mg/L)																			
	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥256	MIC50	MIC90	MIC range	ECOFF
Cefotaxime		4	49	96	11												0.063	0.063	0.016 - 0.32	0.25
Colistin						42	76	7	1	19	14	1					0.5	4	0.25 - 16	2
Chloramphenicol									2	40	77	3	4	4	10	20	8	256	2 - 256	16
Aminosidine							1	23	61	27	11	2	1		1	33	2	>256	0.5 ->256	n.a.
Enrofloxacin		7	38	10	4	26	24	10			7	12	4	8	8	2	0.25	64	0.016 - >256	0.125
Tetracycline								2	2					31	78	47	128	256	1 - 256	8
Sulfadiazine													2	2	2	154	>256	>256	32 ->256	n.a.
Trimethoprim						1	6	9	1							143	>256	>256	0.25 ->256	4

n.a.: not available

#### CONCLUSIONS

The data collected through this survey on AM consumption in meat rabbit farms in Italy between 2010 and 2014 point towards high consumption levels, mostly of tetracyclines and sulfonamides. However, they do also highlight a downward trend (-29%) in overall AM consumption when compared to 2010, attributable mainly to a reduction in colistin (-68%) and tetracycline (-34%) consumption. Over the same time period, a marked rise was recorded in the use of fluoroquinolones.

From a PH perspective, it should be noted, on the one hand, the high proportion of strains resistant to drugs considered critically important for human health, including fluoroquinolones and colistin, and on the other, the absence of extended spectrum beta-lactamase- and carbapenemase-producing *E. coli*.

The levels of microbiological resistance recorded for certain AMs (TE, SFD, ENR) were considerably higher than the levels reported for other FPAs in Europe (EFSA and ECDC, 2015). The results of the study prompt the need to boost schemes to reduce drug consumption (currently adopted by rabbit breeders in Italy on a voluntary basis) in order to bring about a further significant reduction in AM consumption, including more incisive intervention by PH authorities.

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### 1 - MESSAGE

To provide the Public Health services with data on antimicrobial consumptions and antimicrobial resistance in the meat rabbit sector, we selected 32 Italian intensive rabbit farms and monitored their antimicrobial consumptions during 2010-2014 and the resistance pattern of commensal *E. coli*. Results pointed out a decreasing trend of antimicrobial consumption during the study period but also the need to bring about a further significant reduction. The levels of microbiological resistance recorded was considerably high but the absence of extended-spectrum beta-lactamase and carbapenemase- producing *E. coli* should be noted.

# **2 - INTRODUCTION**

Rabbits are raised for meat in industrial holdings in many countries, yet they are considered a minor species. Consequently there is no information on the antimicrobial consumption in reports published yearly by the European Medicines Agency in this food producing sector.

## **3 - MATERIALS AND METHODS**

- The antimicrobial consumptions of 32 Italian intensive rabbit holdings were monitored during 2010-2014.
- We calculated for each semester:
  - a the amounts of active substances (AS) required to produce one kg of rabbit body weight at slaughter age (mg AS/kg LW)
  - b the number of animal daily doses (ADD) required to produce one kg of rabbit body weight (N. ADDkg)
- An analysis of consumption trends during 2010-2014 has been made.
- 160 E. coli strains were collected in these farms in order to evaluate the susceptibility to eight antimicrobial agents.

4 - RESULTS



Fig. 1 - Time trend of N. ADDKg in 32 farms. The linear regression over time is displayed with a red line (the trend is statistically highly significant: p<0.05 )



**Fig. 2** - Distribution of antimicrobial classes used in late 2014 (ADDKg).



 $\ensuremath{\textit{Fig. 3}}$  - The boxplot describes the variability of N. ADDKg in each farm during the study period





<sup>a</sup> EUCAST E. coli ECOFFs are not available for aminosidine. On the base of MICs distribution were considere non-wild type the bact displaying MIC>8mg/L. <sup>b</sup> EUCAST E. coli ECOFFs are not available for sulfadiazine and the sulfametoxazole ECOFF was used.

Fig. 4 - The heatmap presents how the therapeutic approach of all holdings over time changed. The black color indicates a high usage of antimicrobial agents, a soft grey color indicates a low usage instead.

 Table 1 - MICs of eight antimicrobial agents against 160 E. coli isolates. For each drug we reported:

 MIC50, MIC90, MIC range, ECOFF (EUCAST epidemiological cut off) and the percentage of non-wild type strains. In light blue color MICs > ECOFF.

# **5 - CONCLUSIONS**

The joint effort for a prudent use of antimicrobials in veterinary medicine seems to be working in the Italian meat rabbit sector, however the overall consumption of antimicrobials, including those of critical importance for human therapy, such as colistin and enrofloxacin, is still among the highest in animal species raised for food production. Also the levels of microbiological resistance recorded for certain AMs were considerably high. Nevertheless the absence of extended-spectrum beta-lactamase-producing *E. coli* should be pointed out. The results of the study prompt the need to boost schemes to reduce drug consumption in order to bring about a further significant reduction in antimicrobial consumption.