BIOTYPE AND SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS OF RABBIT *Escherichia coli*

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ABSTRACT

Non-invasive Enteropathogenic *Escherichia coli* (EPEC) represents one of the most frequent pathogens involved in fatal enteropathy of commercial rabbits in Italy. We here report the characterisation of the biotype and the resistance to antimicrobial agents of 60 strains of *E. coli* isolated from 150 rabbits affected by diarrhoeic enteropathy. Parasitological, bacterioscopical, bacteriological and histological examinations were performed on caecal samples. Biotyping was made according to CAMGUILHEM and MILON (1989), and the antibiotic resistance against 18 different antibiotics was evaluated using the Kirby-Bauer method. All the strains were isolated from *caeca* that showed catharral to haemorrhagic entero-tiflitis and, microscopically, attachment/effacement (A/E) lesions. Twelve different biotypes were identified, among which B12 and B14 biotypes were constantly present. Antibiotic resistance patterns varied considerably among the biotypes and within the same biotype among the rabbitries. Only fluoroquinolones resulted highly efficacious against *E. coli*. The results suggest to adopt quarantine measures for restocking rabbits in order to identify potential reservoirs of EPEC as well as to determine the prevalent biotype/biotypes and its/their susceptibility to antimicrobial agents.

Key words: rabbit, *Escherichia coli*, biotype, antimicrobial susceptibility.

INTRODUCTION

Escherichia coli is recognized as cause of enteric disease in many species, including rabbits. The *E. coli* strains frequently isolated during outbreaks of fatal enteropathy in the Italian commercial rabbitries are usually noninvasive strains belonging to Enteropathogenic *Escherichia coli* (EPEC) (FINAZZI *et al.* 2000, AGNOLETTI *et al.* 2003). In a previous study we described several outbreaks of colibacillosis and reported the identification in each of them of specific serotypes and biotypes, and presence of virulence factors (e.g. *eae*-gene, *af/r1*- and *af/r2*-genes) (PISONI *et al.* 2001).

A presumptive diagnosis of rabbit colibacillosis is based on the microscopic observation of bacterial attachment and effacement to the surface of the enterocytes. Definitive diagnosis is achieved through the isolation and identification of Enteropathogenic *E. coli*. EPEC strains can be differentiated from nonpathogenic ones on the basis of the serotype and biotype identification and the determination of the virulence factors Indeeed, it is important to test the susceptibility of an isolate to various antimicrobial agents to give support to the veterinary practitioners.
In this paper we describe the results of a study concerning the characterisation of the biotype and the resistance to antimicrobial agents of *E. coli* isolated from diarrhoeic post weaning commercial meat rabbits.

MATERIAL AND METHODS

We examined one hundred and fifty *post*-weaning rabbits suffering with diarrhoea and originating from 20 commercial rabbitries located in Northern Italy. Rabbits were necropsied and parasitological, bacterioscopical, bacteriological and histological examinations were carried out according to PISONI *et al.* (2001). Bacterial isolation was performed on caecal samples. All the isolated strains were identified using the API20E system (BioMerieux). A total of 60 strains of *E. coli* were isolated and biotyped as suggested by CAMGUILHEM and MILON (1989). In particular 34 *E. coli* strains were isolated from rabbits belonging to 4 large commercial rabbitries that conferred animals to our laboratory, for diagnostic purposes as part of a monitoring plan, once a month for six months. The 4 rabbitries were designated as follows: A (1800 does), B (900 does), C (1400 does) and D (1300 does). Farms A, C and D bought their parent stocks from external sources; farm B were using home restocking and only introduced semen from outside. The remaining 26 strains of *E. coli* were isolated from rabbits originating from 16 commercial rabbitries that occasionally confer animals to our laboratory for laboratory examinations.. This group has been designated as “others”.
All the *E. coli* strains were isolated from the same caecal samples that showed attachment/effacement (A/E) lesions by histopathological examination. After isolation, additional biochemical tests were performed to determine sorbose, dulcitol, sucrose, raffinose and rhamnose fermentation properties of the strains, at 4, 6, 24 and 48 hours *post* incubation. All the isolated strains of *E. coli* were further tested for their susceptibility to 18 different antibiotics using the Kirby-Bauer method (CHENGAPPA 1990).

RESULTS AND DISCUSSION

At necropsy, rabbits showed lesions consistent with dehydration and weight loss. The main gross lesion was catharral to haemorrhagic entero-tiflitis, occasionally associated to caecal impaction. No parasites were observed. Microscopically, A/E was the main lesion. The results of biochemical tests indicated that *E. coli* strains belonged to 12 different biotypes (**Table1**), being the B12, B28, B30, B14 and B31the most prevalent biotypes in Italy . Similar results were recently reported by AGNOLETTI *et al.* (2003) and CAMARDA *et al.* (2003), that also found a strong correlation between the presence of *eae*-gene and these biotypes. In our study, we isolated up to four different biotypes of *E. coli*

from different rabbits during a single outbreak of colibacillosis in the same rabbitry (**Table 1**); among these, B12 and B14 biotypes were always present. This situation should be probably to the consequence of the introduction of parents from external breeding units as indirectly proved by the fact that in the farm B, that could be considered a "closed" unit, only one biotype was isolated. Another important parameter which emerged from our data is the constant isolation of rhamnose-negative *E. coli* strains when daily mortality-rate is higher than 5‰.

Table 1. Biotypes of *E. coli* isolated in Italian rabbitries.

Biotype	A	B	C	D	Others
B 12	6	-	-	-	8
B 13	-	-	-	-	1
B 14	3	-	-	-	6
B 16	1	-	-	-	1
B 18	-	-	-	-	5
B 19	-	-	1	-	1
B 20	-	-	-	-	1
B 26	-	-	-	-	1
B 28	-	6	4	-	-
B 29	-	-	-	2	-
B 30	-	-	2	1	6
B 31	2	-	-	2	-
S	**12**	**6**	**7**	**5**	**30**

Others indicates strains of *E. coli* isolated from several rabbitries.
A, B, C, D indicate strains of *E. coli* isolated from four rabbitries monitored once a month for six months.

The results of the tests of susceptibility to antibiotics are reported in table 2 and 3. The data obtained indicates that: a) the same biotype isolated during separate outbreaks of colibacillosis in the same rabbitry showed a similar susceptibility patterns to antimicrobial agents (**Table 2**); b) different biotypes isolated in the same rabbitry during the same outbreak of colibacillosis showed different susceptibility patterns to antimicrobial agents (**Table 2**); and c) the same biotype isolated in different rabbitries showed a different pattern of resistance to antimicrobial agents (**Table 3**). Our hypothesis to explain such situation, again, is that the introduction of restocking rabbits could cause the spreading and diffusion inside the unit of new biotypes with different patterns of antibiotic susceptibility. This situation complicates the choice of the antibiotics to be used for the treatment, since the interpretation of just one antibiogram on a single E. coli isolate for each outbreaks, as usually done, could be a mistake and led to apply a wrong and not efficacious therapy. To date, there are no published data about the correlation between the biotype and the susceptibility to antimicrobial agents.

Table 2. Susceptibility to antimicrobial agents of the biotypes of *E. coli* isolated from four Italian rabbitries monitored once a month for six months.

Rabbitry	A				B	C			D		
Biotype	B 12	B 14	B 16	B31	B 28	B 19	B 28	B 30	B 29	B 30	B 31
Number of Strains	6	3	1	2	6	1	4	2	2	1	2
Antimicrobial agent (Disc content)											
Nalidixic Acid 30 µg	6/6	1/3	0/1	0/2	6/6	1/1	1/4	1/2	1/2	1/1	2/2
Aminosidine 60 µg	1/1	2/3	0/1	0/2	0/6	0/1	3/4	1/2	2/2	1/1	2/2
Apramycin 30 µg	6/6	0/3	1/1	2/2	6/6	1/1	1/1	1/2	2/2	1/1	2/2
Chloramphenicol 30µg	0/6	3/3	0/1	0/2	0/6	1/1	4/4	2/2	1/2	1/1	2/2
Chlortetracycline 30 µg	0/6	0/3	0/1	0/2	0/6	0/1	0/4	0/2	0/2	0/1	2/2
Ciprofloxacin 5 µg	6/6	3/3	1/1	2/2	6/6	1/1	4/4	2/2	2/2	1/1	2/2
Colistin 10 µg	6/6	3/3	1/1	2/2	6/6	1/1	4/4		2/2	1/1	1/2
Co-Trimoxazole 1.25-23.75 µg	1/6	3/3	0/1	0/2	0/6	0/1	0/4	0/2	1/2	1/1	2/2
Doxycycline 30 µg	0/6	0/3	0/1	0/2	0/6	0/1	0/4	0/2	0/2	1/1	2/2
Enrofloxacin 5 µg	6/6	3/3	0/1	0/2	6/6	1/1	4/4	2/2	2/2	1/1	2/2
Flumequine 30 µg	6/6	3/3	0/1	0/2	6/6	1/1	4/4	2/2	1/2	1/1	2/2
Gentamicin 10 µg	4/6	0/3	0/1	2/2	6/6	1/1	4/4	2/2	2/2	1/1	2/2
Neomycin 30 µg	1/6	1/3	0/1	0/2	0/6	0/1	1/4	0/2	1/2	1/1	2/2
Norfloxacin 10 µg	6/6	3/3	1/1	2/2	6/6	1/1	4/4	2/2	2/2	1/1	2/2
Polymyxin B 300 units	5/6	3/3	0/1	n.t.	0/6	n.t.	4/4	1/2	1/2	0/1	0/2
Sulfadiazina 300 units	0/6	0/3	0/1	0/2	0/6	0/1	0/4	0/2	0/2	0/1	2/2
Tetracycline 30 µg	0/6	0/3	0/1	0/2	0/6	0/1	0/4	0/2	0/2	0/1	0/2
Triple-Sulphas 300 units	1/6	3/3	0/1	0/2	0/6	0/1	0/4	0/2	0/2	0/1	2/2

CONCLUSIONS

Different studies focused to describe the different biotypes of *E. coli* affecting commercial rabbits have been previously published (OKERMAN and DÉVRIESE 1985, PEETERS *et al.* 1988, CAMGUILLHEM and MILON 1989, BLANCO *et al.* 1996). In our study we isolated several biotypes of *E. coli* from different rabbits during the same outbreak of colibacillosis: therefore we conclude that that it should be a good practice to biotype all the isolates before performing the antibiogram. During our studies we observed that antibiotic resistance emerges less frequently when a rabbitry is routinely monitored and the antibiograms on E.coli isolates frequently and repeatedly made. We observed that a biotype of *E.coli* isolated twice on the same rabbitry has usually similar behaviour *vs* antibiotics, but on the contrary the susceptibility to antibiotics easily changes when the same biotype is isolated from another rabbitry.

Table 3. Comparison of the antimicrobial susceptibility among some biotypes of *E. coli* isolated from different Italian rabbitries.

Biotype	B 12		B 14		B 16		B 19		B 28		B 30			B 31	
Rabbitry	E	A	E	A	E	A	E	C	B	C	E	C	D	A	D
Number of Strains	8	6	6	3	1	1	1	1	6	4	6	2	1	2	2
Antimicrobial agent (Disc content)															
Nalidixic Acid 30 µg	0/8	6/6	0/6	1/3	1/1	0/1	0/1	1/1	6/6	¼	4/6	½	1/1	0/2	2/2
Aminosidine 60 µg	3/8	1/6	3/6	2/3	0/1	0/1	0/1	0/1	0/6	¾	0/6	½	1/1	0/2	2/2
Apramycin 30 µg	0/8	6/6	0/6	0/3	1/1	1/1	1/1	1/1	6/6	1/1	3/6	½	1/1	2/2	2/2
Chloramphenicol30µg	8/8	0/6	6/6	3/3	1/1	0/1	1/1	1/1	0/6	4/4	3/6	2/2	1/1	0/2	2/2
Chlortetracycline 30 µg	0/8	0/6	0/6	0/3	0/1	0/1	0/1	0/1	0/6	0/4	0/6	0/2	0/1	0/2	2/2
Ciprofloxacin 5 µg	6/8	6/6	3/6	3/3	1/1	1/1	0/1	1/1	6/6	4/4	5/6	2/2	1/1	2/2	2/2
Colistin10 µg	6/8	6/6	6/6	3/3	1/1	1/1	1/1	1/1	6/6	4/4	6/6	2/2	1/1	2/2	½
Co-Trimoxazole 1.25-23.75 µg	8/8	1/6	3/6	3/3	0/1	0/1	0/1	0/1	0/6	0/4	0/6	0/2	1/1	0/2	2/2
Doxycycline 30 µg	0/8	0/6	0/6	0/3	0/1	0/1	0/1	0/1	0/6	0/4	0/6	0/2	1/1	0/2	2/2
Enrofloxacin 5 µg	2/8	6/6	2/6	3/3	1/1	0/1	0/1	1/1	6/6	4/4	3/6	2/2	1/1	0/2	2/2
Flumequine 30 µg	0/8	6/6	0/6	3/3	1/1	0/1	0/1	1/1	6/6	4/4	5/6	2/2	1/1	0/2	2/2
Gentamicin 10 µg	1/8	4/6	0/6	0/3	1/1	0/1	0/1	1/1	6/6	4/4	3/6	2/2	1/1	2/2	2/2
Neomycin 30 µg	3/8	1/6	3/6	1/3	0/1	0/1	0/1	0/1	0/6	¼	0/6	0/2	1/1	0/2	2/2
Norfloxacin 10 µg	8/8	6/6	6/6	3/3	1/1	1/1	1/1	1/1	6/6	4/4	6/6	2/2	1/1	2/2	2/2
Polymyxin B 300 units	4/8	5/6	3/6	3/3	1/1	0/1	0/1	n.t.	0/6	4/4	2/6	½	0/1	n.t.	0/2
Sulfadiazina 300 units	0/8	0/6	0/6	0/3	1/1	0/1	0/1	0/1	0/6	0/4	0/6	0/2	0/1	0/2	2/2
Tetracycline 30 µg	0/8	0/6	0/6	0/3	0/1	0/1	0/1	0/1	0/6	0/4	0/6	0/2	0/1	0/2	0/2
Triple-Sulphas 300 units	0/8	1/6	0/6	3/3	1/1	0/1	0/1	0/1	0/6	0/4	0/6	0/2	0/1	0/2	2/2

E indicates strains of *E. coli* isolated from several rabbitries.
A, B, C, D indicate strains of *E. coli* isolated from four rabbitries monitored once a month for six months.
n.t.: not tested.

Regarding the pattern of resistance to different antibiotics we notedat several biotypes are resistant to antimicrobial agents (e.g. cloramphenicol, polimixine B) never used in Italian rabbit breeding in the last ten years. In addition the *E. coli* isolates often showed resistance to sulfonamides and tetracyclines. Only fluoroquinolones were highly efficacious. Although commercial rabbit breeding is characterized by a high knowledge of the physiology and the behaviour of the rabbit, performance and health status are always influenced by the presence of EPEC. At the time of introduction of parents in a rabbitry, it should be very important to adopt quarantine measures. During this period, it could be established if the animals carry EPEC, what kind of biotype/biotypes and its/their susceptibility to antimicrobial agents.
Further studies to determine the serotype and the presence of *eae*-, *af/r1*- and *af/r2*-genes of the *E. coli* isolates will be carried out.

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