REPORT OF ENTEROPATHOGENIC *ESCHERICHIA COLI* (EPEC) ISOLATED FROM ENTERIC OUTBREAKS IN ITALIAN INTENSIVE RABBIT HERDS

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ABSTRACT

Enteropathogenic *Escherichia coli* (EPEC) can hurt the rabbit intestinal wall with the *eae* gene products characteristic of human EPEC strains; they can be classified into sero/biotypes. The present paper updates the Italian EPEC distribution and examines the isolation frequency of EPEC strains during the 1999-2004 period. The biotype classification of 6274 *Escherichia coli* field strains, the *eae* gene detection in 2106 isolates, as well as the O typing of 498 strains, allowed us to consider the B12, B14, B20, B30 eae^+ and B28 eae^+ biotypes as the most frequent panel of the EPEC strains and the B19, B31, B30 eae^- and B28 eae^- biotypes as the most frequent panel of the not EPEC strains. Compared to biotyping, O-typing did not give additional information seeing that most of O103 strains resulted EPEC, while not all EPEC belonged to O103 serogroup and several resulted not typeable. At the present colibacillosis represents one of the most important causes of enteric disease in Italian rabbit breeding units. During the last 5 years of diagnostic experience a low reduction of EPEC strains was observed from 1999 to 2002, then an increase was registered and, at present, approximately 45% of *Escherichia coli* isolates posses the *eae* gene.

Key words: *Escherichia coli* , rabbit, EPEC, biotype, O-type.

INTRODUCTION

Low levels of *Escherichia coli* are normally present in the gut of healthy rabbits (PEETERS *et al.,* 1988). In same circumstances the micro-organism can bind to intestinal enterocytes and effaces microvilli thanks to *eae* gene products (LICOIS, 1992; POHL *et al*., 1993). As in strains of human origin, attaching-effacing properties characterise the enteropathogenic *E. coli* (EPEC) strains isolated from rabbit gut (BLANCO *et al*., 1997). No enterotoxins are produced as well as no enteroinvasive ability was demonstrated in *E.coli* strains of rabbit origin. Other enteropathogenic factors have been described but

their importance in losses of commercial rabbits breading is not completely clarified . Rabbit EPEC may be classified into sero/biotypes (PEETERS *et al*., 1988). BLANCO *et al*. (1997) related the *eae* gene presence and the sero-biotype profile O103: B14; O103: B6; O26: B13; O128: B30 to enteropathogenic *E. coli* in Spanish rabbits.
In our experience the O103: B12; O103: B14; O103: B28 and the NT (not typeable) *eae* $^{+}$ B28 and B30 *E.coli* profiles are predominant in Italian colibacillosis outbreaks (AGNOLETTI *et al*., 2003).
In the present paper the bio-serotyping of Italian *E.coli* isolates is updated and the isolation frequency of the panel of EPEC strains is examined during the 1999-2004 period of laboratory activity.

MATERIAL AND METHODS

Samples

The diagnostic laboratories of Istituto Zooprofilattico Sperimentale delle Venezie carry out the public diagnostic service of infectious disease. Samples were collected from intensive rabbit farms of different Italian regions.

Biotype, O type and *eae* gene

EPEC were identified on the base of the *eae* gene as defined by Blanco *et al*. (1996). Prior to gene analysis, strains were classified as biotypes. A sample of field *E.coli* strains was O-type classified.
E.coli was isolated from the cecum content with the selective medium EMB Agar (Oxoid LTD, Basingstoke, England) and identified with the API 20E system (bioMerieux, Marcy l'Etoile, France). Approximately 70% of isolates were classified on the base of the fermentation of 5 carbohydrates using the scheme of CAMGUILHEM AND MILON (1989). The classification of *E.coli* based on the lipopolissacaride antigen (O-antigen) was carried out with reagents and methods supplied by the Laboratorio de Referencia de *E.coli*, Universidad de Santiago de Compostela - Lugo (Spain). The slow sero-agglutination test was run using a battery of monospecific antisera towards the following 18 *E.coli* somatic antigens: O-2, O-8, O-10, O-15, O-20, O-22, O-26, O-49, O-75, O-86, O-92; O-103, O-109, O-128, O-132, O-141, O-149, O-153. The *eae* gene was detected in *E.coli* genome using a PCR with eae-s and eae-a primers previously described (KARCH *et al*., 1993), amplification produces an 875-bp fragment analysed with a polyacrylamide electrophoresis.

RESULTS AND DISCUSSION

During the 5 years period (1999–2004) of diagnostic service approximately 10.000 rabbits were examined; as shown in figure 1a enteric pathology represents the main sanitary problem of Italian commercial rabbit farms. *E.coli* was isolated in different amount from the majority of examined rabbits, even from not diarrhoeic subjects.

The biotype classification of 6274 *E.coli* field strains and the *eae* gene detection in 2106 strains are summarised in table 2 and analysed in table 1. All 31 different biotypes were isolated from the rabbits. The B12, B14, B17, B18, B19, B20, B23, B28 B29, B30 and B31 biotypes have a frequency of isolation (expressed as N. of biotype/ N. total biotypes) that range from 1.7% to 19.2%, while most of the others biotypes are sporadic.

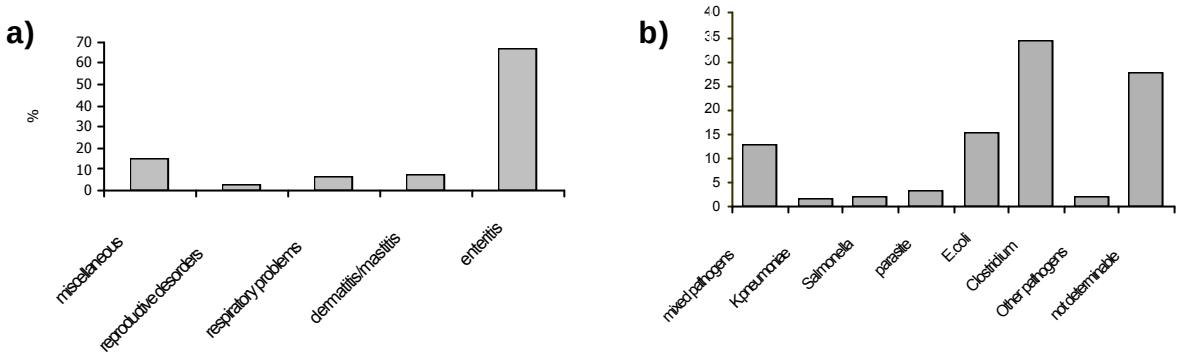

Figure 1 -a): frequency of pathologies observed in examined rabbits; -b): frequency of aetiology of enteric diseases.

At the present, results summarised in table 1 are daily applied; biotypes are grouped as "surely EPEC", "surely not EPEC" and "uncertain" on the base of percentage of eae gene possession: respectively of 95-100%, 0-15% and 15-95%. The percentage of eae gene presence is continuously updated with the examination of all "uncertain" biotypes, while samples of "surely EPEC" and "surely not EPEC" biotype are randomly sampled for eae gene evaluation. This approach allows us to quickly attribute pathologic significance to 65% of our isolates, while 35% of strains required the eae gene research. Several biotype were O-typed. As shown in table 1 serotyping was capable to identify most of O103 as EPEC strains, while not additional information were given on uncertain strains as most of which resulted not typeable (NT) with the panel of antisera used. Inside the NT strains 35% of them were eae + and classified as EPEC .

Taking into account the anatomo-pathologic findings, the evaluation of *E.coli* enteropathogenic role and results of detection of other enteropathogens bacteria, it was possible to define the colibacillosis outbreak (MILON, 1996; PEETERS *et al*., 1988). In our survey colibacillosis represents the 3rd cause of diarrhoea of commercial rabbit farms and comes after clostridiosis and the "not determinable causes" (figure 1b).

In sampled rabbits (table 2) colibacillosis is mostly related to the isolation of B12, B14, B20, B28 *eae*$^+$ and B30 eae$^+$ biotypes. On the other side the biotype B19, B29, B31 and the B30 *eae* $^-$ biotype are isolated in diarrhoeic rabbits affected with other enteric pathogens (i.e. *Clostridium* sp.) or other pathologies (dermatitis, respiratory diseases, reproductive disorders and mastitis).

Table 1. Distribution of *E. coli* biotypes among EPEC or not EPEC strains on the base of the *eae* gene presence; for each group the O-types are indicated.

Isolation significance	% *eae* $^{+}$	Biotype classification	Isolation frequence	O types (N. strains)
EPEC strains	95-100%	B12, B14, B20, B4, B8, B5, B6, B24.	24%	O103(109) ; O8-20 (2); O141(1), O15 (1); NT (5).
Not EPEC strains	0-15%	B31, B19, B17, B18, B29, B23, B27,B21, B26.	41%	O2(57); O8 (5); O103(4); O20 (4); O141(4); O22(3); O8-20 (3); O132(2); O15 (1); NT(48).
Uncertine role in disease	15-95%	B30, B28, B22, B16, B15, B25.	35%	O103(25) ; O8 (28); O153 (16); O141 (16); O8-O20 (14); O2(6); O49 (5); O132(4), O20(4); O128(2); O149(1), O15 (1); O156 (1); O86(1); NT (120);
Uncommon biotype	Not defined	B10, B1, B2, B3, B7, B11, B13	< 0.08%	O103 (3); O109(1); NT(1).

In figure 2 it is reported the isolation percentage of the most frequently isolated strains during the last 5 years of experience. It is interesting to evidence that the frequency of isolation of biotypes that possess the *eae* gene (B12, B14, B30 *eae*$^{+}$, B28 *eae* $^{+}$, and other biotype *eae*$^{+}$) was quite constantly reduced from 1999 to 2002 while increased those that do not possess the *eae* gene (B19, B29, B31, B30 *eae* $^{-}$ and B28 *eae* $^{-}$).

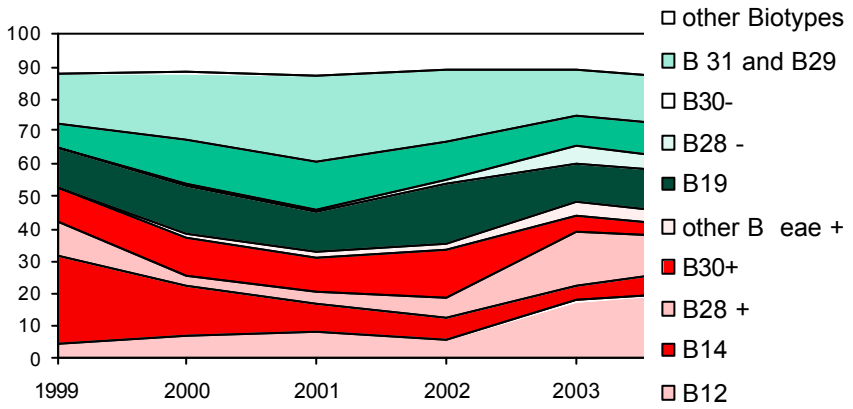

Figure 2. Percentage of isolation of *eae* $^{+}$ and *eae* $^{-}$ biotypes during the 1999-2004 period of diagnostic activity of the laboratory.

The increasing of EPEC strains prevalence observed in 2003 can be attributed to the extension of the diagnostic activity to several new intensive rabbit farms previously not controlled. This evidence supports the possibility to control the EPEC strains circulation.

Table 2. Biotype classification of *E.coli* field strains correlated with the *eae* gene presence and the O-type. Frequency of isolation is expressed as N. biotype/N. tot. biotype.

Biotype classification			Gene *eae* examination		O- Type classification (N. strains tested)
Biotype	N.	%	% *eae* +	N.	
B4	17	0.3	100	14	O103 (1);
B5	6	0.1	100	6	O103 (5); NT (1)
B6	4	0.1	100	3	O103 (1);
B8	7	0.1	100	5	
B10	5	0.1	75	4	O103 (2); NT (1);
B12	805	12.8	98	142	O103 (35); O141 (1); NT (3).
B14	488	7.8	95	102	O103 (64); O15 (1); O8-20(1);
B15	18	0.3	21	14	O103 (2); O2 (2);
B16	57	0.9	11	27	O141 (1); O2 (1); O8 (1); NT (4)
B17	217	3.5	0	27	O132 (2); O2 (3); NT (2)
B18	185	2.9	9	33	O20 (4); O8 (1); NT (3)
B19	863	13.8	4	98	O2 (9); O22 (2); O8 (1); NT (16)
B20	128	2.0	100	47	O103 (3); O8-20 (1); NT (1)
B21	14	0.2	0	5	O15 (1); O2 (1).
B22	63	1.0	63	40	O103 (9); O141 (1); NT (6)
B23	108	1.7	3	33	O103 (1); O141 (2); O2 (20); O22 (1); O8 (1); NT (14).
B24	4	0.1	100	3	
B25	5	0.1	20	5	
B26	9	0.1	0	5	
B27	62	1.0	0	15	O103 (1); O141 (1); O2 (3); NT (1)
B28	835	13.3	77	602	O103 (7); O153 (13); O128 (2); O141 (3); O156 (1); O20 (2); O49 (3); O8 (1); O8-20 (1); NT (30).
B29	158	2.5	15	60	O2(5); O8-20 (1); NT (8).
B30	1205	19.2	38	729	O103 (7); O132 (4); O153 (3); O141 (11); O149 (1); O15 (1); O2 (3); O20 (2); O49(2); O8 (26); O8-20 (13); O86 (1); NT (80).
B31	999	15.9	6	81	O103 (2); O141(1); O2 (16); O8 (2); O8-20(2); NT (4).
Other	12	<0.1	Not examined		O103 (1); O109(1);
Tot	6274			2106	498

CONCLUSIONS

Colibacillosis represents the 3rd cause of enteric pathology in Italian rabbit breading units. In our experience the *E. coli* classification based on biotype is easy to perform, rapid and economically applicable on large numbers of isolates, but the *eae* gene detection should routinely be applied for B28 and B30 biotypes. Compared to biotype, O-type did not give additional information since most of O103 strains were EPEC while

not all EPEC were O103 or O typeable. During the last 5 years of diagnostic experience a low reduction of EPEC strains was observed starting in 1999 until 2002, then an increase was registered and at present approximately 45% of *E.coli* isolates posses the *eae* gene. The B12, B14, B20, B30 *eae*$^+$ and B28 *eae*$^+$ biotypes are the most frequent panel of the EPEC strains; while the B19, B31, B30 *eae*$^-$ and B28 *eae*$^-$ biotypes are the most frequent panel of the not EPEC strains

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