BACTERIAL INFECTIONS IN RABBIT AS COMPANION ANIMAL: A SURVEY OF DIAGNOSTIC SAMPLES IN ITALY

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ABSTRACT

Rabbits are becoming more popular as pets and the number of diagnostic samples is now increasing. Rabbits make excellent pets because they are clean and docile. For housing, extreme temperatures should be avoided; in fact high temperatures may cause overheating and death, but also low temperatures are harmful. Good ventilation is essential and cages should be easily accessible for daily care. Cage material must be tough enough to support constant chewing but, to avoid boredom, some environmental enrichment (e.g., toys and chewing materials) should be provided. Most important is to guarantee a good hygiene of environment and animals because rabbits can develop a variety of clinical problems. Coccidiosis, caused by several species of *Eimeria*, may be subclinical or cause mild to severe diarrhoea; instead *Clostridium spiroforme* could be responsible for clostridial enterotoxemia that causes moderate to severe diarrhoea frequently resulting in death of weanlings. Respiratory diseases, generally caused by *Pasteurella multocida*, are the major cause of morbidity and mortality in rabbits. Infection may be subclinical or cause various combinations of clear to thick nasal discharge, clear to thick ocular discharge, anorexia, lethargy. The bacterial infection may also result in abscesses in subcutaneous tissues and other sites, torticollis and circling for infection of the inner ear and septicaemia. For all these pathologies the diagnosis is based on bacterial culture or parasite observation. However other pathogens are cited, such as *Staphylococcus* spp. and *Bordetella bronchiseptica.* As what regards the antibiotic susceptibility of bacteria involved in pet rabbits diseases, a few number of studies are available. The aim of this work was to evaluate the bacterial prevalence in samples from different ill pet rabbits during diagnostic procedures from 2004 to 2007. Moreover we evaluated the antimicrobial sensitivity/resistance of bacteria to some molecules used in rabbits. Thirty two samples were collected and *Pasteurella multocida* was the most isolated bacterium (7 positives/32) followed by *Pseudomonas aeruginosa* (5/32) and *Klebsiella pneumoniae* (3/32). Others microorganisms were isolated in lower percentage. The results of the antimicrobials susceptibility tests were variable, but some molecules were decidedly ineffective.

Key words: Pet rabbit, Bacteriological samples, Bacterial prevalence, Antimicrobials sensitivity.

INTRODUCTION

Rabbits are becoming more popular as pets and the most spread are the smaller breeds, such as the Dutch and Netherland Dwarf. Rabbits are generally timid and non-aggressive, although occasionally some animals could display aggressive defence behaviour including biting. For housing extremes in temperature should be avoided (Suckow and Douglas, 1997) and good ventilation is essential. The cages should be easily accessible for daily care and the cage material must be tough enough to support constant chewing and to avoid boredom some environmental enrichment (e.g., toys and chewing materials) should be provided.

Most important is to guarantee a good hygiene of environment and animals because rabbits can develop a variety of clinical problems. Coccidiosis, caused by several *Eimeria* species, may be subclinical or cause mild to severe diarrhoea; instead *Clostridium spiroforme* could be responsible for

clostridial enterotoxemia that causes moderate to severe diarrhoea frequently resulting in death in weanlings (Quinn *et al.*, 1994; Suckow and Douglas, 1997; Farina and Scatozza, 1998). As regards fungi, pet rabbits could be healthy carrier of dermatophytes, above all *Trichophyton mentagrophytes*. Upper respiratory diseases, more generally called "snuffles", are considered the most common diseases observed in pet rabbits (Langan *et al.*, 2000). Common clinical signs include nasal discharge, sneezing and conjunctivitis (Rougier *et al.*, 2006; Wagner and Fehr, 2007). The microorganism most commonly implicated with these symptoms is *Pasteurella multocida*, that is the major cause of morbidity and mortality in rabbits too. The bacterial infection may also result in abscesses in subcutaneous tissues and other sites, torticollis and circling for infection of the inner ear and septicaemia (Farina and Scatozza, 1998; Suckow and Douglas, 1997). However other pathogens are cited, such as *Staphylococcus* spp. and *Bordetella bronchiseptica* (Deeb and DiGiacomo, 1990; Deeb and DiGiacomo, 2000; Langan *et al.*, 2000). For all these pathologies the diagnosis is based on bacterial culture or parasite observation (Quinn *et al.*, 1994; Poli *et al.*, 2005).

The aim of this work was to evaluate the bacterial prevalence in samples collected from 32 different pet rabbits during diagnostic procedures from 2004 to 2007. Moreover the antimicrobial sensitivity/resistance of isolated bacteria to some molecules used in rabbits was evaluated.

MATERIALS AND METHODS

Bacteriological examination

Seeding of the samples
Specimens were collected by veterinary practitioners, in Lombardia region (Italy), from 32 different pet rabbits with clinical manifestations of disease. The samples, just arrived to the microbiological laboratory, were streaked on blood agar medium (Oxoid) and incubated at 37°C for 18-24 hours under aerobic atmosphere or in candle jar according to the kind of bacteria we supposed present in the sample.

Identification of isolated strains
First of all, Gram stain was performed to identify the typical bacterial microscopic morphology; furthermore, the following tests were performed: (1) evaluation of the macroscopic characteristics of colonies on blood agar plate; (2) catalase activity and (3) coagulase test with rabbit plasma. Moreover, the isolates have been transferred on *Mannitol Salt Agar* (MSA, Difco), a selective and differential medium for evaluation of staphylococcal capability to ferment mannitol; *MacConkey Agar* has been used for identification of Gram-negative bacteria fermenting or not lactose. A characteristic "swarming" has been allowed to identify *Proteus mirabilis*; *Pseudomonas aeruginosa* for production of a blue-green pigment. API galleries (API System®, BioMérieux) have been used for identification of bacterial species.

Susceptibility test (Kirby-Bauer test)
All the isolated strains have been tested for susceptibility to antimicrobial agents by the agar disc diffusion method (Kirby-Bauer test) on *Mueller Hinton Agar* (Difco). The plates have been incubated at 37°C for 18-24 h. After measuring the antimicrobial zone diameters, following the standards, the strains have been categorized as susceptible, resistant or intermediate to the drug (Quinn *et al.*, 1994; NCCLS, 2002; NCCLS, 2004; Poli *et al.*, 2005).

RESULTS AND DISCUSSION

Sex and age of pet rabbits undergone to sampling procedures are reported in Table 1. In the population of pet rabbits we have studied males are the most swabbed (68.75% vs. 31.25 of females), with an age scale ranging from 1 to 5 years (65.63%); this result is in agreement with the data reported by Rougier

et al. (2006), about the frequency of appearance of respiratory and ocular infections in pet rabbits. In fact, the mean value for age is reported approximately as 14 months.

Table 1: Age and sex of pet rabbits undergone to sampling procedures

		Number	Percentage
Sex	Male	22	68.7
	Female	10	31.2
Age	<1 year	2	6.2
	1-5 years	21	65.6
	>5 years	9	28.1

Samples came mainly from nose (swabs) with a prevalence of 37.5%, then from abscesses (in particular from mouth [dental abscesses] or abdominal cavity (28.1%) and from eyes (15.6%). These data are in agreement with the high spreading of these types of pathologies in pet rabbits as reported by literature (Langan *et al.*, 2000; Boucher and Nouaille, 2002; Rougier *et al.*, 2006; Wagner and Fehr, 2007).

Table 2: Sites of sampling

	Number	Percentage
Nose	12	37.5
Abscess	9	28.1
Eye/Conjunctiva	5	15.6
Ear	3	9.3
Vagina	2	6.2
Feces	1	3.1
Total	32	100.0

The most isolated microorganisms from the 32 samples collected are shown in Table 3. In agreement with the literature (Langan *et al.*, 2000; Boucher and Nouaille, 2002; Rougier *et al.*, 2006) *Pasteurella multocida* was the most isolated bacterium (21.9%) followed by *Pseudomonas aeruginosa* (15.6%) and *Klebsiella pneumoniae* (9.4%). The other microorganisms were isolated in a low percentage. Therefore this result underlines the important role of *P. multocida* in many infections not only in intensive breeded rabbits but also in pet rabbits (DiGiacomo *et al.*, 1991; Farina and Scatozza, 1998; Langan *et al.*, 2000; Poli *et al.*, 2005). Many samples (28%) were negative probably due to sampling mistakes or to the poor significance of the collected sample (e.g, insufficient drawn material or sampling not in the correct site) or to antibiotic treatment of animals just before sampling.

Table 3: Bacterial prevalence

Bacteria	Number	Percentage
Pasteurella multocida	7	21.9
Pseudomonas aeruginosa	5	15.6
Klebsiella pneumoniae	3	9.4
Staphylococcus aureus	2	6.2
S. aureus + Streptococcus β-haemolyticus	1	3.1
Pasteurella pneumophila	1	3.1
Escherichia coli + Streptococcus β-haemolyticus	1	3.1
Arcanobacterium pyogenes	1	3.1
Acinetobacter calcoaceticus	1	3.1
Escherichia coli haemolyticus	1	3.1
Negative	9	28.1
Total	32	100.0

Finally, Tables 4 and 5 show the results of the antibiotic susceptibility tests performed on the 7 strains of *P. multocida* and on the 5 strains of *P. aeruginosa*. In both cases the strains are particularly resistant to many molecules that it's possible to use in rabbit (Rosenthal, 2004) even if *P. multocida* is susceptible to doxycycline (6 strains/7), marbofloxacin (5/7) and enrofloxacin (4/7) as reported by literature (Hanan *et al.*, 2000; Meunier *et al.*, 2004; Rosenthal, 2004). On the contrary *P. aeruginosa* is susceptible only to marbofloxacin (3 strains/5). So these data are particularly depressing because they

show that the treatment of such infections could be very difficult above all because of the extremely enteric susceptibility of rabbits to several classes of antimicrobials (Rosenthal, 2004).

Table 4: Results of antibiotic-sensitivity test for *P. multocida* (7)*

Antibiotic	Number resistant strains
Amikacin	6
Doxycycline	1
Enrofloxacin	3
Gentamycin	4
Kanamycin	4
Marbofloxacin	2
Cotrimoxazole	4
Chloramphenicol	6

*Number of strains tested

Table 5: Results of antibiotic-sensitivity test for *P. aeruginosa* (5)*

Antibiotic	Number resistant strains
Amikacin	3
Doxycycline	5
Enrofloxacin	3
Gentamycin	3
Kanamycin	5
Marbofloxacin	2
Cotrimoxazole	5
Chloramphenicol	5

*Number of strains tested

CONCLUSIONS

The data obtained in this research performed in a wide time period (2004-2007) showed the important role of *P. multocida* and *P. aeruginosa* in samples from ill pet rabbits and their multiresistance to some antimicrobials. So, it is much important to perform an accurate health control of these animals because the same microorganisms, isolated from them, could be potentially pathogenic for human but above all for children and elderly. Moreover it will be interesting to study the trend of antibiotic-resistance in *Pasteurella multocida* and other pathogens isolated from these pet rabbits and to compare the results with those obtained from the same bacteria isolated from intensive breeded rabbits. Because of the high diffusion of bacterial antibiotic-resistance and the low number of safe molecules we can use in rabbit, it will be a main point to find new antibiotics for therapy in the future.

REFERENCES

Boucher S., Nouaille L. 2002. Manuel pratique des maladies des lapins. *France Agricole, 272*.

Deeb B.J., DiGiacomo R.F. 1990. *Pasteurella multocida* and *Bordetella bronchiseptica* infections in rabbits. *J. Clin. Microbiol., 28, 70-75.*

Deeb B.J., DiGiacomo R.F. 2000. Respiratory diseases of rabbits. *Vet. Clin. North Am. Exot. Anim. Pract., 3, 465-480 vi-vii.*

DiGiacomo R.F., Xu Y.M., Allen V., Hinton M.H., Pearson G.R. 1991. Naturally acquired *Pasteurella multocida* infection in rabbits: clinicopathological aspects. *Can. J. Vet. Res., 55, 234-238.*

Farina R., Scatozza F. 1998. Trattato di malattie infettive degli animali. *UTET, Torino, Italy*.

Hanan M.S., Riad E.M., El-Khouly N.A. 2000. Antibacterial efficacy and pharmacokinetic studies of ciprofloxacin on *Pasteurella multocida* infected rabbits. *Dtsch. Tierarztl. Wochenschr., 107, 151-155.*

Langan G.P., Lohmiller J.J., Swing S.P., Wardrip C.L. 2000. Respiratory diseases of rodents and rabbits. *Vet. Clin. North Anim. Pract., 30, 1309-1335.*

Meunier D., Acar J.F., Martel J.L., Kroemer S., Vallé M. 2004. A seven-year survey of susceptibility to marbofloxacin of pathogenic strains isolated from pets. *Int. J. Antimicrobial Agents, 24, 592-598.*

National Committee for Clinical Laboratory Standards (NCCLS) 2002. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard M31-A2. *Villanova, PA, USA.*

National Committee for Clinical Laboratory Standards (NCCLS) 2004. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; informational supplement, M31-S1. *Villanova, PA, USA.*

Poli G, Cocilovo A, Dall'Ara P, Martino P.A., Ponti W. 2005. Microbiologia e immunologia veterinaria. *2a Ed., UTET Scienze Mediche, Milano, Italy.*

Quinn P.J., Carter M.E., Markey B., Carter G.R. 1994. Clinical veterinary microbiology. *Ed. Mosby Ltd, St. Louis, Missouri, USA.*

Rougier S., Galland D., Boucher S., Boussarie D., Vallé M. 2006. Epidemiology and susceptibility of pathogenic bacteria responsible for upper respiratory tract infections in per rabbits. *Vet. Microbiol., 115, 192-198.*

Rosenthal K.L. 2004. Therapeutic contraindications in exotic pets. *Seminars in Avian and Exotic Pet Medicine, 13(1), 44-48.*

Suckow M.A., Douglas F.A. 1997. The laboratory rabbit. *CRC Press Inc., Boca Raton, Florida, USA.*

Wagner F., Fehr M. 2007. Common ophtalmic problems in pet rabbits. *Journal of Exotic Pet Medicine, 16(3), 158-167.*