OCCURRENCE OF TYMPANIC BULLAE EMPYEMA IN COMMERCIAL RABBITS

Di Castri A.[1], Cornaggia M.[1*], Palazzolo L.[1], Rizzardi A.[1], Bottin S.[1], Viel L.[1], Foiani G.[2], Vascellari M.[2], Bano L.[1]

[1] Veterinary Diagnostic Laboratory, Istituto Zooprofilattico Sperimentale delle Venezie, Vicolo Mazzini 4, Villorba (Treviso), 31020, Italy
[2] Histopathology Laboratory, Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, Legnaro (Padova), 35020, Italy

[*]Corresponding author: mcornaggia@izsvenezie.it

ABSTRACT

Respiratory infections sustained by *Pasteurella multocida* are one of the most important cause of mortality and economic losses in rabbit industry. Middle ear can be reached from upper respiratory tract site of infection and it acts as a safe niche for *P. multocida*. In order to study the infection of this particular anatomic district, the tympanic bullae of 445 commercial New Zealand white rabbits sent to the laboratory for the routine diagnostic investigations, were inspected. Tympanic bullae empyema was the most common lesion observed (48.3%; 215/445), and *P. multocida* was the bacterial agent more frequently isolated (76.1%). Other lesions typical of pasteurellosis such as rhinitis, conjunctivitis, pneumonia, thorax empyema, splenomegaly and cutaneous abscesses, were simultaneously present. Surprisingly, in 5.6 % of animals the middle ear empyema was the only anatomo-pathological lesion observed. Rabbits affected by pulmonary pasteurellosis are commonly treated with antimicrobials that usually result widely susceptible when tested *in vitro*. Unfortunately, few pharmacokinetic studies demonstrated the capability of those drugs to reach the middle ear at effective concentrations. For this reason, the *in vitro* most effective antimicrobial might fail to treat a poor vascularized site, such as the tympanic bulla, leading to asymptomatic chronic infection difficult to detect and to control in commercial rabbitries.

Key words: respiratory disease, middle ear, tympanic bullae empyema, *Pasteurella multocida*, otitis media.

INTRODUCTION

Respiratory diseases are the most common causes of morbidity and mortality in commercial rabbits and the infection of the upper respiratory tract ("snuffles") can easily spread to the middle ear throw the Eustachian tubes (Varga, 2013).
Otitis media is the inflammation of the middle ear cavity and it is a very common but probably underestimated disease of rabbit. The clinical diagnosis of this disease and the following therapeutic treatment can be challenging (King *et al*., 2007).
The Eustachian tube is a muscular-membranous channel that links the nasopharynx to the middle ear, that is totally harbored in the tympanic bulla, a well-developed, round cavity of the temporal bone (Deeb *et al*., 2004).
Under inflammatory conditions (otitis media), tympanic bullae can be filled with abundant, purulent exudate that can reach the inner ear through the vestibular or cochlear openings, and the brain along the vestibule-cochlear nerve, causing lethal encephalitis (King *et al*., 2007; Johnson-Delaney, 2011; Bortolami and Callegari, 1999).
Pasteurella multocida is the main causative agent of rabbit respiratory disease. It is a Gram-negative cocco-bacillus, and it is divided into 5 capsular serovars (A, B, D, E, and F), which are further classified into 16 Heddleston serovars (1–16), based on the lipopolysaccharide (LPS) antigens (Carter, 1952; Heddleston *et al*., 1972). Rabbit isolates usually belong to capsulotype A, D, F and genotypes

L3 and L6 (Massacci et al., 2018). *P. multocida* often resides as commensal bacterial in rabbit's nasal cavity (Deeb *et al*., 2004) and in some conditions (i.e., high virulence strain, high bacterial load, bad microclimatic conditions and stress), it can act as primary pathogen. Signs of pasteurellosis include mucopurulent rhinitis, conjunctivitis, dacryocistitis, sinusitis, otitis, pleuropneumonia, cutaneous abscesses and septicemia. In addition, sneezing, snorting, anorexia and grooming difficulties can occur (Varga, 2013).
The involvement of the inner ear and brain are potential severe complications of the middle ear infection, with onset of vestibular signs, hearing loss, facial nerves deficits and death (Chow, 2011; King, 2007). *Encephalitozoon cuniculi* infection is the main differential diagnosis when nervous clinical signs occur (Varga, 2013).
The initial rhinitis observed in rabbits with upper respiratory tract infections, may disappear and the inflammation move into the paranasal sinuses or into the middle ear, where the infection can persist without clinical evidence (Johnson-Delaney, 2011). The purulent infection of this anatomic district may be evidenced only by means of advanced diagnostic imaging or surgical techniques that are usually employed only in pet rabbits (King *et al*., 2007; Mancinelli *et al*., 2017).
Antimicrobial treatments are the first choice in order to control rabbit pasteurellosis, but the emergence of limited resistant strains have been reported (Cucco *et al*., 2017; Wang *et al*., 2019). Furthermore, the effectiveness of the antimicrobials for the treatment of the middle ear infections could be ineffective, due to the limited blood perfusion of this anatomic district (Chow, 2011).
The aim of this study was to investigate the frequency of middle ear infections, in commercial rabbits sent to the diagnostic laboratory with a clinical history of respiratory diseases or sudden death.

MATERIALS AND METHODS

From January to December 2020, 445 commercial New Zealand white rabbits (*Oryctolagus cuniculus*) with severe respiratory clinical signs or dead without any clinical signs, were sent for post-mortem investigation to the veterinary diagnostic laboratory (Istituto Zooprofilattico Sperimentale delle Venezie, Treviso, Italy). Animals were grouped into three age classes: 74 were pre-weaning rabbits (1-30 days), 282 fattening rabbits (31-80 days) and 87 breeders (85 does and 2 bucks).
Live symptomatic animals were humanly sacrificed, according to the European and National Animal Welfare laws (EU reg. 2009/1099; EU dir. 2010/63).
The necropsy protocol was the follow: an external exam was conducted on the fur in order to evidence any external lesions (abscesses, mastitis, pododermatitis etc.). The respiratory apparatus, the outer and middle ear, trachea, lungs and thoracic cavity were accurately expected. Gross pathology examination was performed on all animals and organs following a standard protocol (Elvang, 2011).
Tympanic bullae with macroscopic lesions were collected from 10 subjects and fixed in 10% neutral buffered formalin for at least 24 hours, decalcified with a commercial decalcifying solution (Decalcifier I, Leica) and processed for routine histological examination.
In addition to the parenchymatous organs examined for diagnostic purposes, the bacteriological examination was conducted from the tympanic bullae of 110 rabbits, that contained purulent exudate. The pathological material was aseptically collected and plated on blood agar base, Baird-Parker and Eosin Methylene Blue agar plates. All the plates were incubated at 37 °C in aerobic conditions and expected after 24-48 hours.
Bacterial colonies were identified based on morphological and biochemical characteristics, and finally by means of the MALDI TOF MS technology.

RESULTS

Two hundred fifteen (48.3%) rabbits showed middle ear inflammation and empyema of one or both tympanic bullae. Thirty-five were pre-weaning rabbits (47.3%), 135 were post-weaning rabbits (47.9%) and 45 were breeders (51.7%). Three out the 40 live animals showed head tilt as clinical sign. Histological findings were consistent with severe middle heterophilic otitis with the presence of bacterial colonies and bone remodeling of the tympanic bulla.
In 203 out of the 215 rabbits with middle ear otitis, additional pathological findings were observed: catarrhal or catarrhal-purulent conjunctivitis (1.9%), rhinitis (29.8%), catarrhal/fibrin-purulent

pneumonia (48.8%), pleuritis (17.7%), thoracic empyema (33%), splenomegaly (11.6%), cutaneous abscesses (14%), liver necrotic lesions (12.1%), mastitis (3.3%) and pyometra (1.9%). Twelve animals (8 fattening rabbits, 3 breeders, 1 pre-weaning rabbit) showed purulent otitis without additional lesions.

Pasteurella multocida was isolated in 83/110 samples, *Staphylococcus aureus* in 19/110; *P. multocida* and *S. aureus* coinfection was detected in 13/110 animals. In a small number of bullae, other bacterial species were isolated: *Streptococcus pyogenes* (4/110), *Pseudomonas aeruginosa* (2/110), *Bordetella bronchiseptica* (1/110) and *Klebsiella pneumoniae* (1/110) (Figure 1).

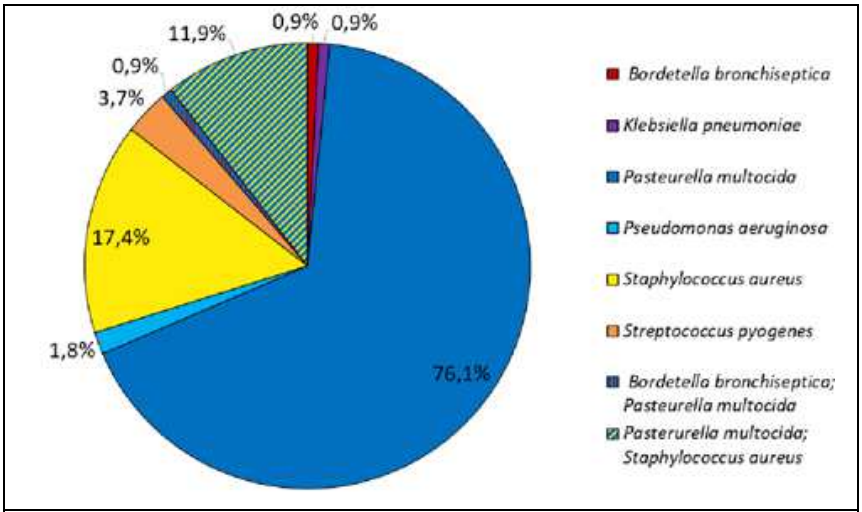

Figure 1. Percentage of the bacterial species isolated from the Tympanic bullae.

DISCUSSION

Rabbits with respiratory diseases usually show purulent nasal discharge, sneeze, cough and sometimes dyspnea, but they may also present no specific signs such as anorexia, weight loss and depression. If acute septicemia occurs, animals may die suddenly without clinical signs (Johnson-Delaney, 2011; Varga, 2013).

In our report, almost half of the examined animals with respiratory signs or lesions, showed tympanic bullae empyema, with a widespread distribution among the three groups classified on the base of the age.

Only three out of the 40 live rabbits examined, showed torticollis and vestibular syndrome, suggesting that the inner ear involvement is not a recurring complication and that the middle ear infection could be underestimated on the clinical basis (Chow, 2011).

Twelve animals (5.6%) had only an infection of the middle ear, supporting that the inspection of the tympanic bulla should be always performed.

In pet rabbits, advanced imaging diagnostic techniques and surgery are required to diagnose and solve otitis media (King *et al*., 2007; Chow, 2011; Mancinelli *et al*., 2017), however this approach cannot be applied in intensive farming systems.

Although *P. multocida* is the most frequent bacterial pathogen isolated, a wide range of different bacterial species was detected in the tympanic bullae. This finding suggests that the bacteriological examination should be always performed when a purulent exudate is detected in the middle ear. In bovine, *Mycoplasma bovis* is the main bacterial pathogen responsible for the otitis media (Maunsell *et al*., 2012). In future studies, *Mycoplasma* spp. should be investigated also in this anatomic district of rabbit.

The control of pasteurellosis is mainly based on antimicrobial therapy that should be guided by the results of an *in vitro* sensibility test (Cucco *et al*., 2017). *In vitro*, *P. multocida* isolates are fully susceptible to a wide range of antimicrobials (Ferreira *et al*., 2012; Cucco *et al*., 2017) but its effectiveness in the middle ear is poorly studied. In this site, *P. multocida* can persist and work as source of re-colonization of the upper respiratory tract (Mähler *et al*., 1995).

Prevention throw biosecurity and husbandry implementations should be considered. Autogenous and commercial dead vaccines have been used to prevent pasteurellosis in rabbit but their effectiveness in the containment of otitis media caused by *P. multocida* should be investigated.

CONCLUSIONS

Middle ear infection is a common and frequently underestimated disease of rabbits with or without respiratory lesions and it can be diagnosed only with an in deep necropsy. Our bacteriological results demonstrate that *Pasteurella multocida* is the most frequent bacterial species isolated, but other microorganisms could play a crucial role in the tympanic bullae empyema.
The efficacy of antimicrobial treatments should be investigated by means of pharmacokinetic studies of antimicrobials in the middle ear. If the microorganism is not eliminated in this niche, it could re-infect the respiratory tract after an antibiotic treatment. Indeed, inappropriate antimicrobial therapy could create chronic infected animals and maintain the disease in the farm.

REFERENCES

Bortolami R. , Callegari E. 1999. *Neurologia ed estesiologia degli animali domestici*. Edagricole.

Carter G. R., 1952. The type specific capsular antigen of *Pasteurella multocida*. *Canadian journal of medical sciences, 30(1), 48-53.*

Chow E. P. 2011. Surgical management of rabbit ear disease. *Journal of Exotic Pet Medicine, 20(3), 182-187.*

Cucco L., Massacci F. R., Sebastiani C., Mangili P., Bano,L., Cocchi M., Magistrali, C. F. 2017. Molecular characterization and antimicrobial susceptibility of *Pasteurella multocida* strains isolated from hosts affected by various diseases in Italy. *Vet Ital, 53(1), 21-27.*

Deeb B. J. 2004. Respiratory disease and pasteurellosis. *Ferrets, Rabbits, and Rodents, 172.*

Elvang H. 2011. *Necropsy: a handbook and atlas*. Samfundslitteratur.

Ferreira T. S. P., Felizardo M. R., Sena de Gobbi D. D., Gomes C. R., Nogueira Filsner P. H. D. L., Moreno M., Micke Moreno A. 2012. Virulence genes and antimicrobial resistance profiles of *Pasteurella multocida* strains isolated from rabbits in Brazil. *The Scientific World Journal. Volume 2012, Article ID 685028*

Heddleston K. L., Gallagher J. E., Rebers P. A. 1972. Fowl cholera: gel diffusion precipitin test for serotyping *Pasteurella multocida* from avian species. *Avian diseases, 16 (4):925-936.*

Johnson-Delaney C. A., Orosz S. E. 2011. Rabbit respiratory system: clinical anatomy, physiology and disease. *Veterinary Clinics: Exotic Animal Practice, 14(2), 257-266.*

King A. M., Hall J., Cranfield F., Sullivan M. 2007. Anatomy and ultrasonographic appearance of the tympanic bulla and associated structures in the rabbit. *The Veterinary Journal, 173(3), 512-521.*

Mähler M., Stünkel S., Ziegowski C., Kunstýř I. 1995. Inefficacy of enrofloxacin in the elimination of *Pasteurella multocida* in rabbits. *Laboratory animals, 29(2), 192-199.*

Mancinelli E., Lennox A. M. 2017. Management of otitis in rabbits. *Journal of Exotic Pet Medicine, 26(1), 63-73.*

Massacci F. R., Magistrali C. F., Cucco L., Curcio L., Bano L., MangiliP., Christensen H. 2018. Characterization of *Pasteurella multocida* involved in rabbit infections. *Veterinary microbiology, 213, 66-72.*

Maunsel F., Brown, M. B., Powe J., Ivey J., Woolard,M., LoveW., Simecka J. W. 2012. Oral inoculation of young dairy calves with *Mycoplasma bovis* results in colonization of tonsils, development of otitis media and local immunity. *PLoS One, 7*(9)*, e44523.*

Varga, M. 2013. *Textbook of Rabbit Medicine*. Elsevier Health Sciences.

Wang J., Sang L., Sun S., Chen Y., Chen D., Xie X. 2019. Characterization of *Pasteurella multocida* isolated from dead rabbits with respiratory disease in Fujian, China. *BMC Veterinary Research, 15(1), 438.*