Pasteurellosis: A significant bacterial disease in rabbit production

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Abstract
Rabbits are susceptible to various respiratory affections. Pasteurellosis, caused by *P. multocida*, is regarded as one of the most important bacterial diseases of rabbits. The disease is characterised by chronic mucopurulent respiratory affection (snuffles) or more acute and subacute bronchopneumonia leading to high mortality and severe devastating losses in rabbit production. Moreover, pasteurellosis is associated with septicaemia, abscesses, otitis media, and nervous and reproductive disorders. *P. multocida* is widely distributed worldwide and mostly affects rabbits from 4 to 8 weeks of life. Infection with *P. multocida* usually occurs by indirect or direct contact, chiefly via aerosol. Moreover, the presence of other stressors can aggravate the severity of infection. The gold standard for the diagnosis of pasteurellosis is traditional isolation and identification methods. However, molecular techniques are used now for rapid detection of *P. multocida* and its virulence genes. Prevention is based on the application of hygienic methods, vaccination, and treatment using various antimicrobials. This review article gives an overview of pasteurellosis in rabbits regarding disease incidence, susceptibility and transmission, signs and lesions, laboratory diagnosis, and the prevention and control methods.

Key words: *P. multocida*; rabbits; incidence; diagnosis; vaccination

Introduction
Rabbit production has a growing role in income improvement as a source of animal protein, especially in low-income countries (Abdel-Kafy et al., 2017). Rabbits are highly susceptible to many infections because they severely impact the rabbit industry and cause considerable economic losses (Eid and Ibraheem, 2006; Soriano-Vargas et al., 2012). One of these devastating infectious agents is pasteurellosis (Ismail et al., 2018; El-Jakee et al., 2020), a highly contagious bacterial disease of rabbits that causes severe epidemics and adverse significant economic losses in rabbit production worldwide (Nasser et al., 2013). Pasteurellosis is considered a predominant cause of death in rabbit fields (Soriano-Vargas et al., 2012). The disease is caused by *Pasteurella multocida* in rabbits (Takashima et al., 2001) and is characterised by chronic mucopurulent...

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respiratory affection (snuffles) or more acute and subacute bronchopneumonia leading to high mortality in rabbits (Stelian et al., 2011). However, epizootics and enzootics acute fatal pneumonia have been reported in cases of pasteurellosis in rabbits (Tinelli et al., 2020).

Pasteurellosis in rabbits is characterised by different clinical forms including septicaemia, rhinitis with purulent nasal discharge (snuffles), pneumonia, abscesses, otitis media, meningitis, pyometra, and orchitis (Wilson and Ho, 2013). Some cases of rabbit pasteurellosis did not show any clinical signs (Asran et al., 2016). It has been reported that more than 50% of adult rabbits either die or are culled due to pasteurellosis (Premalatha et al., 2009).

Therefore, this review article gives an overview of pasteurellosis in rabbits regarding disease incidence, susceptibility and transmission, signs and lesions, laboratory diagnosis, and the prevention and control methods.

Incidence and distribution

The prevalence of P. multocida infection in rabbits ranges from 7 to 100% (Stahel et al., 2009). A high incidence of P. multocida isolation (77.5%) was detected after examination of diseased and dead rabbits (Kawamoto et al., 1990). Sanchez et al. (2004) isolated P. multocida from apparent healthy rabbits and found a prevalence of 20–90%. Percy et al. (1984) isolated P. multocida from diseased rabbits in a rate at 51.3%. High incidences of P. multocida (55% - Mohamed et al., 2020; 47.5% - Suelam and Samie, 2011; 37.5% - Abd-Algawad et al., 2021) were also obtained in Egypt after examination of dead and diseased animals. Other Egyptian researchers such as Eid and Ibraheem (2006), Lee et al. (1990), Stelian et al. (2011), and Mazed et al. (2013) also demonstrated a range of incidences rates of P. multocida of 36.4%, 31%, 27%, and 27%, respectively. A recent study by Mahrous et al. (2022) revealed a total incidence rate of P. multocida in of 31.3%. Similar results were found by Takashima et al. (2001) who detected a 27% isolation rate of P. multocida in diseased rabbits. However, Youssef (2011) in Egypt detected the presence of P. multocida in the internal organs of diseased and dead rabbits at a rate of 20%. Additionally, Asran et al. (2016) reported a low prevalence rate of P. multocida (3.4 to 9.4%) in diseased rabbits. Likewise, Ehsan (2019) identified P. multocida isolates at rate of 10%. In Lithuania, Ruzauskas (2005) isolated P. multocida from rabbits with respiratory signs at a rate of 14.6%. Additionally, P. multocida was detected in environmental samples in Japan with a frequency of 31% (Kawamoto et al., 1990).

This inconsistency in the prevalence frequency of P. multocida infections could be related to the age, the immunological and health status of animals at the sampling time (Deeb et al., 1990), the employed method of detection, the locality in which the study was done, or the environmental conditions (Stelian et al., 2011). The epidemiology of pasteurellosis also may differ according to the type of breeding conditions (small-scale or professional large-scale breeding and laboratory facilities). The results of El Tayeb et al. (2004) reported a prevalence of P. multocida infection in rabbits housed under laboratory conditions or on breeding farms without respiratory signs as 15.8% and 94%, respectively. Moreover, high temperature, malnutrition, and transportation may increase the incidence of P. multocida.
Susceptibility and mode of transmission

All ages of rabbits are susceptible to *P. multocida* infection and the bacterium colonises the sinus, middle ear, trachea, and lungs (Quinn et al., 1994). Rabbits can get infected with *P. multocida* soon after birth and after weaning more than 75% of rabbits nursed from infected dams were culture positive (Holmes et al., 1984). Moreover, the prevalence of *P. multocida* carries can increase to over 90% at the age of 5 months (Manning et al., 1994). Pasteurellosis commonly occurs at age of 1 to 2 months, but rabbits aged 8 months to one year exhibited low incidence (El-Ghawy, 1972).

*Pasteurella multocida* is a commensal pathogen that affects a wide host range including humans, animals, and birds (Hotchkiss et al., 2011). The bacterium is usually present as a normal microflora of the buccal cavity, nasopharynx, and upper respiratory tract of animals and birds (Wilson and Ho, 2013). Thus, it is regarded as an opportunistic or secondary bacterial pathogen of the respiratory tract of apparently healthy and diseased animals (Suelam and Samie, 2011). Stressors such as shipping and immunodeficiency of hosts play main roles in the development of pasteurellosis (Asran et al., 2016). Laboratory rabbits colonised with *P. multocida* often show clinical signs after being shipped to a research facility, however persistently colonised asymptomatic rabbits have been shown to produce unusual results when used in research (Richard et al., 1997). The disease is spread by indirect or direct contact, primarily by aerosol (Premalatha, 2009).

Regarding the zoonotic potential of *P. multocida*, a human can contract this infection after a rabbit lick or byte. A case of meningitis and epidural, subdural, and subgaleal empyema in a 15-year-old boy was recorded (Per et al., 2010). Also, a 68-year-old man showed an endovascular stent graft, after a bite by the patient’s household rabbit (Silberfein et al., 2006).

Signs and lesions

*P. multocida* has been demonstrated to show great differences in the pathogenicity in rabbits, varying from fatal acute septicaemia to chronic forms (snuffles) (Glavits and Magyar, 1990). Moreover, isolates of *P. multocida* differ in their abilities to produce disease conditions; some are mainly associated with inflammatory conditions in the upper respiratory tract, though others may cause septicaemia and pneumonia (DiGiacomo et al., 1991). Exposure to virulent invasive strains of *P. multocida* may result in rapid penetration of the respiratory mucosa, leading to per acute or acute disease conditions (Al-Haddawi et al., 2001). Zoran et al. (2008) demonstrated that infected rabbits with *P. multocida* showed fever and respiratory distress resulting in respiratory failure. The morbidity and mortality rates as a result of pasteurellosis reached 23% and 35–40%, respectively (Quesenberry and Carpenter, 2004; Premalatha et al., 2009).

Experimental infection of rabbits with different strains of *P. multocida* induced respiratory signs such as rhinitis, sneezing, conjunctivitis, dyspnea, and abdominal breathing (Al-Haddawi et al., 2001; Rameshkumar et al., 2006; Tinelli et al., 2020).

Post-mortem examination of dead rabbits with pasteurellosis revealed mucous exudate that accumulated in the trachea and lungs causing snuffling sounds during auscultation. Severe fibrinopurulent pleuropneumonia and
extensive fibrinous adhesions on the parietal pleura and lung surfaces were also observed (Zoran et al., 2008; Tinelli et al., 2020). Moreover, infected rabbits showed accumulation of mucopurulent exudate in the nasal cavity, congestion of the nasal mucosa, as well as catarrhal exudate mixed with yellowish threads of pus, consolidation of the lung, and adjacent emphysematous areas (Al-Haddawi et al., 2000).

Tracheal lesions due to *P. multocida* infection were characterised by hyperplasia and hypotrophy of goblet cells, infiltration of neutrophils, and desquamation of epithelial cells (El-Hendy et al., 2020). Carrillo et al. (2012) attributed the desquamation of epithelium to the migratory effect of inflammatory cells. The increase in the goblet cell number in the respiratory epithelium after *P. multocida* infection in rabbits was a logical reaction since goblet cells are involved in mucin production that is a defence mechanism against infections, especially in the respiratory tract (Scharfman et al., 1996). Lung lesions due to *P. multocida* infection in rabbits are characteristic (Patel et al., 2016; Alam et al., 2018; El-Hendy et al., 2020). Praveena et al. (2010) referred affection of lung lesions in pasteurellosis cases to the activation of macrophages by bacterial endotoxin, leading to an influx of other inflammatory cells and cascading injury. Moreover, the authors found that toxins of *P. multocida* alone or in combination with products of inflammatory cells induced necrosis and injuries of pulmonary blood vessel walls, leading to oedema and haemorrhages in the alveoli. Patel et al. (2016) demonstrated that the bacterial toxins caused necrosis of leukocytes with a production of oat cells that became marked by a streaming patterns of condensed chromatin material. The affection of the nervous system was also reported in rabbits after experimental infection with *P. multocida* (Kpodekon, 1983). Meningitis, vascular oedema, and neuronal degeneration were recorded (El-Hendy et al., 2020). Kpodekon (1983) proposed that affection of the nervous system triggered stagnation of neuronal lymph, provoking retrograde centripetal circulation of lymph up to the brain resulting in meningitis. Moreover, meningitis of experimentally infected rabbits with *P. multocida* was observed as a result of bacterial emboli in the blood vessels of the brain (Patel et al., 2016).

**Laboratory diagnosis**

Isolates of *P. multocida* appear as dew drop and non-haemolytic small yellowish-white colonies (1 mm in diameter) on 5% sheep blood agar and tiny white on tryptic soy agar (Ehsan, 2019). *Pasteurella multocida* is a member of the family *Pasteurellaceae*. These are facultative anaerobic, Gram-negative, capsulated, and non-motile or spore-forming coccobacilli (Kuhnert and Christensen, 2008). A characteristic bi-polar shape bacterium can be seen microscopically after staining blood smears in the acute stage of infection (Carter, 1987). Biochemical reactions of *P. multocida* indicate that the bacterium is positive for oxidase, catalase, and indole test but negative for urea hydrolysis. In addition, it ferments glucose and mannitol, but not lactose (Awad and Abd El-Hamid, 2019; Tinelli et al., 2020). Although culture is considered the gold standard for the detection of *P. multocida*, it is unreliable for screening, since up to 30% of infected rabbits may not be detected (Holmes et al., 1987). Moreover, techniques used for the isolation and identification of the bacterium are time-
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Nucleic acid-based assays allow for the detection of *P. multocida* directly from samples and small amounts of cultures, thus improving the sensitivity and decreasing the time required for bacterial identification (Dutta et al., 2005). Due to the great discriminatory power, the DNA-based *P. multocida* identification method has been recognised as an effective approach to characterisation (Blackall and Miflin, 2000). Accordingly, molecular identification of *P. multocida* isolates such as polymerase chain reaction (PCR) is now regarded as a basic method for the detection of infection. Stahel et al. (2009) demonstrated genetic heterogeneity among different *P. multocida* clones using PCR-typing techniques. Molecular fingerprinting of the pathogen will help to trace the sources and reservoirs of rabbit infections.

Extensive sub-culturing is required to obtain a pure culture of *P. multocida* required for serotyping (Rimler and Rhoades, 1989). Different serological tests such as the haemagglutination test, gel diffusion precipitin test, and enzyme-linked immunosorbent assay for detection of serum antibodies to *P. multocida* (Lukas et al., 1987; Zaoutis et al., 1991; Kawamoto et al., 1994; Asway et al., 2008). The bacterium could be serologically classified into five capsular serogroups (A, B, D, E, and F) and 16 somatic serotypes (1-16) using the latex agglutination test (Arumugam et al., 2011). However, pasteurellosis in rabbits is mainly caused by *P. multocida* strains containing capsular types A and D (El Tayeb et al., 2004), and to a lesser extent type F (Rimler and Rhoades, 1989; Jaglic et al., 2004). For instance, *P. multocida* serotypes 1, 3, and 12 with capsular type A were the predominant strains in Egyptian rabbit flocks (Mahrous, 2017; Ehsan, 2019; Mahrous et al., 2022). Serotype A:12 is the most common in rabbits in the USA, though A:3 and other A and D serotypes are present (Lu et al., 1988). Severe disease has been also associated with A:3 and D strains due to the production of toxins (Suckow et al., 1991). In Egypt, the majority of *P. multocida* isolates from dead rabbits belonged to serotypes A:3, A:12, B:2, and D:6, and to a lesser extent serotypes D:12 and A:4 (Youssef, 2011). Some cross-reaction may occur among *P. multocida* isolates such as A:3,5 and A:3 (Carter and Chengappa, 1981; Youssef, 2011). Serological monitoring might be not effective in identifying all *P. multocida* colonised rabbits because most of these tests use uncharacterised antigen mixtures that may not detect all serotypes that colonise rabbits (Delong et al., 1992).

**Virulence factors**

Lipopolysaccharides (endotoxins) and polysaccharide capsules are considered the main determinants of *P. multocida* virulence (Katsuda et al., 2013). The bacterium has been shown to adhere to the nasopharyngeal epithelium of rabbits by fimbriae (pili) (Glorioso et al., 1982). Accordingly, adhesion and colonisation factors, fimbriae, iron storage, regulatory proteins, exotoxins, plasmids, and extracellular enzymes are also putative virulence and pathogenicity factors of the pathogen (Katoch et al., 2014). Several studies proved the existence of virulence-associated genes of *P. multocida* in rabbits such as dermonecrototoxin production (toxA), fimbriae adhesions (ptfA), neuraminidase (nanH), neuraminidase (nanB), outer membrane protein synthesis (omA87), filamentous haemagglutinin consuming and often fail because some transport media do not maintain the organism’s viability for more than one day at room temperature (Kawamoto et al., 1997).

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(pfh\textit{A}), superoxide dismutase (\textit{sod}A), superoxide dismutase (\textit{sod}C), transferrin binding protein (\textit{tbp}A), haemoglobin-binding protein (\textit{hgb}A), and haemoglobin-binding proteins (\textit{hgb}B) (Shirzad Aski and Tabatabaei, 2016; Mohamed et al., 2020; Prajapati et al., 2020; Abd-Algawad et al., 2021; Mahrous et al., 2022). It has been reported that \textit{ptf}A gene is a critical factor in repairing the epithelial cell surfaces of the bacterium (Ferreira et al., 2012).

Moreover, the sialidases (\textit{nan}B) gene plays a role in the colonisation of bacterium on the surface epithelium and enhances the virulence of bacteria by unmasking key receptors and reducing mucin effectiveness (Sarangi et al., 2014). After the colonisation of \textit{P. multocida} in the respiratory system, it produces sialidase which is important for the removal of sialic acid from mucus and gives access to sialic acid as an energy source (Mizan et al., 2000). The gene \textit{tad}D has been detected as a putative non-specific tight adherence protein D in \textit{P. multocida} (May et al., 2001). The gene encoding dermonecrotic toxin is \textit{toxA} which is more common in sheep and swine (Ewers \textit{et al.}, 2006). Ferreira et al. (2012) could not detect \textit{toxA} gene in a study performed on 46 \textit{P. multocida} isolates. Other researchers demonstrated that this gene was uncommonly found in \textit{P. multocida} isolated from rabbits. Pullinger et al. (2004) supposed that the \textit{toxA} gene was not inserted into the bacterial chromosome but inserted into the lysogenic phage that infects the pathogen (Garcia-Alvarez \textit{et al.}, 2015; Massacci \textit{et al.}, 2018). Unlikely, Ahmed \textit{et al.} (2016), Mohamed \textit{et al.} (2020), and Abd-Algawad \textit{et al.} (2021) detected the \textit{toxA} gene from rabbits in Egypt. It is important to note that the \textit{toxA} gene has been found to differentiate non-toxinogenic from toxinogenic strains of \textit{P. multocida} (Lichtensteiger \textit{et al.}, 1996).

**Prevention and treatment**

Detection of antibiotic resistance and virulence properties of circulating \textit{P. multocida} isolates would facilitate in the implementation of appropriate prevention strategies.

**Vaccination**

The ability of rabbits to counteract \textit{P. multocida} infection depends on the health of the exposed mucosa and the rapid production of mucosal immunoglobulins (IgA) that inhibit bacterial growth. High IgG levels in the blood are not associated with the elimination of infection but rather with a chronic process (Zimmerman \textit{et al.}, 1992). Induction of immunity and protection against \textit{P. multocida} using bacterins, potassium thiocyanate extracts, or attenuated live bacteria have failed to prevent pasteurellosis over time (Deeb \textit{et al.}, 1990). It is recommended to use autovaccines against rabbit pasteurellosis to avoid problems associated with differences in the antigenic structure of prepared vaccines and those antigens circulating in the field (Peshev and Christova, 2003). Borkowska-Opaka \textit{et al.} (1996) also recommended the preparation of a pasteurellosis vaccine from the most frequently isolated and immunogenic strains. Al-Lebban \textit{et al.} (1989) demonstrated that intravenous immunisation of rabbits with vaccines containing \textit{P. multocida} or a cross-protective core lipopolysaccharide mutant of \textit{Escherichia coli} induced severe purulent bronchopneumonia and pleuropneumonia as well as kidney lesions, while no lesions were detected in animals vaccinated by the mucosal (aerosol, conjunctival) route. Youssef (2011) demonstrated that subcutaneous double vaccination of rabbits with a monovalent formalised inactivated \textit{P. multocida}
multocida vaccine (at a 2-week interval) induced good protection after challenging the homologous serotypes rather than with the heterologous serotypes. This may be referred to as a lack of cross-protection among P. multocida serotypes. Ruzauskas (2005) prepared an oil-in-water adjuvant inactive P. multocida vaccine containing serotypes A and D to vaccinate infected rabbits. The results showed that subcutaneous double vaccinations of infected rabbits with the vaccine were safe, highly protective (100% survival), immunogenic, and reduced the severity of the respiratory signs. However, some animals remained carriers after recovery which was normal as a result of survival of the pathogen in macrophages with the resistance to neutrophil phagocytosis. Inactivated formalised P. multocida vaccine has been commercially used for the vaccination of rabbits (Nasser et al., 2013; Ismail et al., 2018). The efficacy of a bivalent inactivated vaccine against pasteurellosis and rabbit haemorrhagic disease virus (RHDV) was evaluated in rabbits (Tian, 1989; El-Jakee et al., 2020). In the study of Peshev and Christova (2003) in Bulgaria, the results proved the safety of the vaccine for all age groups including pregnant animals with a high antibody titre for 9 months post-vaccination. Moreover, in rabbits taken from vaccinated dams, the vaccine protected the young from RHDV up to 30 days of age. El-Jakee et al. (2020) in Egypt evaluated the efficacy of rabbit vaccinations with a bivalent oil adjuvant vaccine (ISA70 adjuvant) against P. multocida and RHDV in comparison with monovalent preparations and commercially available monovalent vaccines. Serological monitoring revealed that rabbits that received a bivalent vaccine showed the highest antibody titres against P. multocida and RHDV with a protection rate of 90%. Moreover, neither pathogen was detected in the liver of vaccinated animals. The bivalent vaccine candidate was fully protective. Immunisation against both pathogens can be achieved by a single vaccination.

**Treatment**

Treating P. multocida infected animals only alleviates the severity of the clinical signs and slows the development of the disease, but it does not eliminate the infection (Kehrenberg et al., 2001). Treatment could be considered effective only for a short time (Mahler et al., 1995). The sensitivity of P. multocida to different antimicrobials varies geographically (Awad and Abd El-Hamid, 2019). For instance, a recent study by Mahrous et al. (2022) revealed that Egyptian isolates of P. multocida in rabbits showed predominant resistance to erythromycin, oxytetracycline, and kanamycin, while Cucco et al. (2017) revealed that Italian isolates were sensitive to tetracycline. Other studies indicated a higher resistance of P. multocida isolates to ampicillin, amoxicillin, neomycin, and tetracycline (Balakrishnan et al., 2012; Awad and Abd El-Hamid, 2019). El-Sayed et al. (2018) demonstrated that all Egyptian P. multocida isolates of rabbits with pasteurellosis showed resistance to neomycin, penicillin, and ampicillin. Moreover, Mohamed et al. (2020) demonstrated resistance of P. multocida isolates to erythromycin, amoxicillin, colistin sulphate, neomycin, and streptomycin. The development of multi-drug resistance phenomenon among P. multocida isolates in rabbits is common (El-Sayed et al., 2018). In Brazil, Ferreira et al. (2012) showed that 47.8% of P. multocida strains were resistant to at least one of the tested drugs, however, the isolates were
sensitive to cephalosporins, florfenicol, tetracyclines, and fluoroquinolones. Antibiotic resistance is a growing problem in the rabbit industry, possibly due to the extensive and indiscriminate use of antibiotics to prevent or control pasteurellosis in rabbitries (Kehrenberg et al., 2001; Oh et al., 2019).

Conclusion
Rabbit pasteurellosis is regarded as a serious bacterial disease that affects large rabbit farms worldwide. Therefore, adoption of strict hygienic measures and application of autogenous \textit{P. multocida} bacterin are considered proper preventive measures against the disease. In addition, there is an urgent need for the judicious use of antibiotics in rabbit treatment to successfully mitigate the propagation of drug resistance across \textit{P. multocida} species.

References
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Pastereloza: značajna bakterijska bolest u uzgoju kunića


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Pastereloza: značajna bakterijska bolest u uzgoju kunića

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Kunići su osjetljivi na razna respiratorna oboljenja. Pastereloza, koju prouzroči *P. multocida*, smatra se jednom od najvažnijih bakterijskih bolesti kunića. Bolest je okarakterizirana kroničnim mukupurulentnim respiratornim oboljenjem (hunjavica) ili akutnim i subakutnim bronhopneumonijom koja dovodi do visoke smrtnosti i ozbiljnih razornih gubitaka u uzgoju kunića; pastereloza je povezana i sa sepsom, apsesima, infekcijom srednjeg uha i živčanim te reproduktivnim poremećajima. Pastereloza je široko rasprostranjena diljem svijeta i uglavnom pogađa kuniće u dobi od 4 do 8 tjedana. Do infekcije bakterijom *P. multocida* obično dolazi neizravnim ili izravnim kontaktom, uglavnom preko aerosola. Nadalje, prisutnost drugih uzročnika stresa može pogoršati ozbiljnost infekcije. Zlatni standard za dijagnozu pastereloze jesu tradicionalne metode izolacije i identifikacije. Međutim, sada se rabe molekularne tehnike za brzo otkrivanje *P. multocida* i njezinih virulentnih gena. Prevencija se temelji na primjeni higijenskih metoda, cijepljenju i liječenju uporabom različitih antimikrobnih lijekova. Cilj ovog preglednog članka jest dati pregled pastereloze u kunića s obzirom na pojavnost bolesti, osjetljivost i prijenos, znakove i lezije, laboratorijsku dijagnozu i metode prevencije i kontrole.

**Ključne riječi:** *P. multocida*, kunići, pojavnost, dijagnoza, cijepljenje