AN OVERVIEW ON RIEMERELLOSIS: A WORLDWIDE EMERGING DISEASE OF DUCKS

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Summary


Riemerella anatipestifer (R. anatipestifer) is the bacterial cause of an economically important and serious disease of ducks and other poultry species. Extensive reports showed that this disease condition is widely distributed in different countries since 1904. Horizontal and mechanical transmissions are important routes of R. anatipestifer dissemination. The disease is characterised by respiratory, nervous and locomotor disturbance with high mortality rates especially in ducklings. Affected birds showed generalised polyserositis or localised lesions in different organs. The clinical picture of R. anatipestifer is similar to and confused with other bacterial infections, so diagnosis of the disease relies mainly on laboratory techniques. At least 21 serotypes of R. anatipestifer have been identified. Control of R. anatipestifer infection mainly depends on using of the suitable antibiotics according to the antibiogram results. Due to the extensive and hazardous uses of antibiotics, development of multi-drug resistance strains of R. anatipestifer is common. Prevention of the disease can be achieved though application of good management practice and vaccination. Different types of vaccines are commercially available. There are autogenous polyvalent live or inactivated bacterins as well as sub-unit and recombinant vaccines. The vaccines give protection only for the specific serotypes present in the used vaccines. Therefore, this review article gives an overview on R. anatipestifer infections regarding the distribution all over the world, susceptibility and infection, clinical picture, laboratory diagnosis as well as prevention and control methods.

Key words: diagnosis, distribution, poultry, prevention and control, R. anatipestifer

INTRODUCTION

Ducks are regarded as an important species of poultry that are susceptible to many important infectious diseases. New duck disease, duck septicemia, riemerellosis, anatipestifer septicaemia and infectious serositis are different synonyms for the infection of ducks with Riemerella anatipestifer (R. anatipestifer) (Leavitt & Ayroud, 1997). This bacterium is a Gram-negative rod-shaped, non-motile or spore former, and belongs to Flavobacteriaceae rRNA superfamily V (Subramaniam et al., 1997). The disease caused by R. anatipestifer is widely distributed among several
countries including Europe, South Asia, Africa and Oceania (Panthansophon et al., 2002; Chikuba et al., 2016; Gyuris et al., 2017; Abd El Hamid et al., 2019; Chang et al., 2019; Han et al., 2020; Ritam Hazarika et al., 2020; Omaleki et al., 2021; Tzora et al., 2021). Infection with R. anatipestifer causes severe economic losses in commercial ducks industry through high morbidity and mortality rates, reduced growth rate and increasing the costs of prevention and control as well as the condemnation rate (Leibovitz, 1972; Chikuba et al., 2016). Interestingly, the disease doesn’t affect ducks only, but there is a previous history of R. anatipestifer affections in other domestic and wild bird species (Saif et al., 2008).

Young birds are more susceptible and show high mortalities. The clinical picture of R. anatipestifer is represented as either an acute highly contagious septicaemic form or a chronic localised one. Affected birds displayed general respiratory, enteric, locomotor and nervous manifestations with generalised polyserositis, salpingitis and meningitis (Wobeser, 1997; Sandhu, 2008). Detection of R. anatipestifer infections in susceptible flocks depends on the use of conventional and recent techniques of laboratory diagnosis. There are at least 21 serotypes of R. anatipestifer that vary in virulence (Ruiz & Sandhu, 2013) and there is no cross protection among them. Although R. anatipestifer is sensitive to several antibiotics, it is highly susceptible to the development of drug resistance (Sun et al., 2019) in addition to the presence of drug residues in duck products (Sun et al., 2012). Accordingly, immunisation emerges as an effective way for prevention of such infection. Inactivated, living attenuated and subunit vaccines are currently used against R. anatipestifer infections in the field.

This review article gives an overview on R. anatipestifer infections regarding their distribution all over the world, susceptibility and infection, clinical picture, laboratory diagnosis as well as prevention and control methods.

**DISTRIBUTION OF THE DISEASE**

Infections with R. anatipestifer have been recorded worldwide since 1904. Although the disease was first described in geese by Riemer (1904), the taxonomy of the definitive cause remained undefined for several years (Hendrickson & Hilbert, 1932; Bruner & Fabricant, 1954; Breed et al., 1957). Further, the exact causative agent had been classified in a separate genus (Riemerella), family Flavobacteriaceae of the phylum Bacteroidetes and named as R. anatipestifer based on phynotypic and genotypic characterisations (Segers et al., 1993). The worldwide distribution of R. anatipestifer infections in different countries like United States, Germany, Australia, Hungary, Japan, India, Thailand, Taiwan, Malaysia, China, Bangladesh, Greece and Egypt is presented in Table 1.

**SUSCEPTIBILITY AND INFECTION**

R. anatipestifer can affect a wide variety of wild and domestic birds (Sandhu, 2003). Domestic ducks are highly susceptible (Jackson, 1972; Eleazer et al., 1973; Ruiz & Sandhu, 2013). The bacterium was also isolated from geese in Hungary (Ivanics et al., 1996; Gyuris et al., 2017) and Germany (Köhler et al., 1995). Some early reports showed infection of turkeys with R. anatipestifer (Helfer & Helmboldt, 1977; Smith et al., 1987; Cooper,
Table 1. The worldwide distribution of *R. anatipestifer* infections in different countries

<table>
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<tr>
<th>Locality</th>
<th>References</th>
<th>Findings</th>
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<tr>
<td>Germany</td>
<td>Köhler <em>et al.</em> (1995)</td>
<td>Demonstrated presence of <em>R. anatipestifer</em> as pathogen for geese in the northern and central parts of Germany</td>
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<td>United States</td>
<td>Cooper (1989)</td>
<td>Detected presence of <em>Pasteurella anatipestifer</em> infections in California turkey flocks with evidence of a mosquito vector</td>
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<td>Australia</td>
<td>Rosenfeld (1973)</td>
<td>Early identified <em>Pasteurella anatipestifer</em> infection in fowls</td>
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<td></td>
<td>Omaleki <em>et al.</em> (2021)</td>
<td>Molecularly and serologically characterised <em>Riemerella</em> isolates associated with different avian species</td>
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<tr>
<td>Hungary</td>
<td>Bitay <em>et al.</em> (1979)</td>
<td>Early detected anatipestifer syndrome of ducks (<em>Pasteurella anatipestifer</em> bacteria)</td>
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<tr>
<td></td>
<td>Ivanics <em>et al.</em> (1996)</td>
<td>Demonstrated presence of anatipestifer disease in growing geese</td>
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<td></td>
<td>Gyuris <em>et al.</em> (2017)</td>
<td>Determined the antimicrobial susceptibility of <em>R. anatipestifer</em> strains isolated from geese and ducks</td>
</tr>
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<td>Japan</td>
<td>Baba <em>et al.</em> (1987)</td>
<td>Early identified <em>Moraxella</em> (<em>Pasteurella</em>) <em>anatipestifer</em> from an outbreak in ducklings</td>
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<td>Sakurai <em>et al.</em> (1987)</td>
<td>Demonstrated <em>Pasteurella anatipestifer</em> infection in duckling</td>
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<td>Tanaka <em>et al.</em> (1988)</td>
<td>Identified <em>Moraxella</em> (<em>Pasteurella</em>) <em>anatipestifer</em> infection in ducklings</td>
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<td>Chikuba <em>et al.</em> (2016)</td>
<td>Detected presence of <em>R. anatipestifer</em> infection in domestic ducks with specific clinical picture</td>
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<td>India</td>
<td>Sarma <em>et al.</em> (1985)</td>
<td>Isolated <em>Pasteurella anatipestifer</em> and <em>P. haemolytica</em> from an outbreak of duck mortality</td>
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<td>Soman <em>et al.</em> (2014)</td>
<td>Identified <em>R. anatipestifer</em> from ducks using traditional methods and PCR.</td>
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<td>Ritam Hazarika <em>et al.</em> (2020)</td>
<td>Isolated and molecularly identified <em>R. anatipestifer</em> strains from ducks</td>
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<td>Thailand</td>
<td>Panthansophon <em>et al.</em> (1994)</td>
<td>Identified serotypes 1, 2, 3, 5 and 15 of <em>R. anatipestifer</em> from ducks</td>
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<td></td>
<td>Panthansophon <em>et al.</em> (1995)</td>
<td>Detected new serotypes of <em>R. anatipestifer</em> strains from duck</td>
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<td></td>
<td>Panthansophon <em>et al.</em> (2002)</td>
<td>Demonstrated new serotypes of <em>R. anatipestifer</em> strains from duck</td>
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Table 1 (cont’d). The worldwide distribution of *R. anatipestifer* infections in different countries

<table>
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<tr>
<th>Country</th>
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<tr>
<td>Malaysia</td>
<td>Shome <em>et al.</em> (2004)</td>
<td>Identified <em>R. anatipestifer</em> from an outbreak in ducks</td>
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<td></td>
<td>Yu <em>et al.</em> (2008)</td>
<td>Detected the genomic diversity and molecular differentiation of <em>R. anatipestifer</em> associated with eight outbreaks in five farms</td>
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<td></td>
<td>Phonvisay <em>et al.</em> (2017)</td>
<td>Made surveillance studies on <em>R. anatipestifer</em> from outbreaks in duck farms</td>
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<td>China</td>
<td>Li <em>et al.</em> (2011)</td>
<td>Isolated <em>R. anatipestifer</em> strains from chickens</td>
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<tr>
<td></td>
<td>Sun <em>et al.</em> (2012)</td>
<td>Molecularly characterised <em>R. anatipestifer</em> isolates of Ducks and identified their antimicrobial resistance.</td>
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<tr>
<td></td>
<td>Li <em>et al.</em> (2016)</td>
<td>Detected the effects of two efflux pump inhibitors on the drug susceptibility of <em>R. anatipestifer</em>.</td>
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<td></td>
<td>Han <em>et al.</em> (2020)</td>
<td>Developed a colloidal gold immuno-chromatographic strip for detection of <em>R. anatipestifer</em> in ducks</td>
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<tr>
<td>Bangladesh</td>
<td>Sarker <em>et al.</em> (2017)</td>
<td>Confirmed presence of <em>R. anatipestifer</em> isolates at 421 bp fragment of ribonuclease Z gene</td>
</tr>
<tr>
<td>Greece</td>
<td>Tzora <em>et al.</em> (2021)</td>
<td>Identified <em>R. anatipestifer</em> isolates from broiler chickens using MALDI-TOF MS and detected the sensitivity pattern to different antibiotics.</td>
</tr>
<tr>
<td>Egypt</td>
<td>Heba <em>et al.</em> (2015)</td>
<td>Identified higher prevalence rate of <em>R. anatipestifer</em> among ducks (11.7%) than ducklings (5%). The specific OmpA gene was detected among all isolates using PCR.</td>
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<tr>
<td></td>
<td>Abd El Hamid <em>et al.</em> (2019)</td>
<td>Found that the PCR with sequence analysis of Omp A gene of <em>R. anatipestifer</em> was highly sensitive and rapid for serotyping especially in case of unavailability of standard hyper immune serum of local duck isolates.</td>
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<tr>
<td></td>
<td>Eman <em>et al.</em> (2020)</td>
<td>Successfully prepared and tested a single and combined local inactivated vaccine containing <em>R. anatipestifer</em> serotypes (A1 and A2) with <em>Pasteurella multocida</em> serotypes (A and D) to protect ducks till 6 months of age.</td>
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1989; Frommer *et al.*, 1990; Metzner *et al.*, 2008). Chickens, quails, pheasants, guinea fowl, quails, gulls, budgerigars, and wild waterfowl are also frequently susceptible to *R. anatipestifer* infection (Bruner *et al.*, 1970; Karstad *et al.*, 1970;
Infections caused by *R. anatipestifer* may be represented as an acute septicaemic form in young birds or chronic localised form in older birds. Infected birds with *R. anatipestifer* manifested signs of nasal discharge, sinusitis, coughing, diarrhoea, lameness, abnormal gait, head tremors and torticollis (Bisgaard et al., 2008; Fulton & Rimler, 2010). Affected 3–4-week-old ducklings showed a characteristic reduced movement, dorsal recumbency, ataxia and leg paddling (Chikuba et al., 2016). Stress factors as moving the birds and environmental variations increase the severity of the disease condition. The disease course may extend to 2 weeks and the mortality rate varies from 10–75% (Ruiz & Sandhu, 2013).

The post-mortem lesions of *R. anatipestifer* are characterised by septicaemia, fibrinous pericarditis, perirehepatitis and airsacculitis, pneumonia, catarrhal rhinitis and enteritis, enlarged spleen and liver, caseous arthritis and salpingitis, skin necrosis as well as serous-fibrinous meningitis (Dougherty et al., 1955; Leibovitz, 1972; Smith et al., 1987; Bisgaard et al., 2008; Ruiz & Sandhu, 2013; Chikuba et al., 2016; Tzora et al., 2021).

The clinical picture of *R. anatipestifer* in ducks is similar to other bacterial infections like *Pasteurella multocida*, *Escherichia coli* and *Salmonella enterica*. Therefore, it is difficult to diagnose *R. anatipestifer* infection through the pathological features.

**LABORATORY DIAGNOSIS**

Confirmative diagnosis of *R. anatipestifer* infection is based on laboratory methods. Suspected samples could be collected from organs with lesions. After enrichment in the broth media, *R. anatipestifer* grows on the selective blood agar under micro-aerophilic conditions to produce dew drop, small (1–2 mm in diameter), transparent, glistening and non-haemolytic colonies (Brogden et al., 1982; Markey et al., 2013). However, the haemolytic character of the organism has been also detected (Surya et al., 2016). The bacterium is now belonging to the genus *Riemerella*, however, it has previously belonged to the genera *Pfeifferella*, *Pasteurella* and *Moraxella* (Segers et al., 1993). Microscopically, *R. anatipestifer* appears as Gram negative short bacillus, non-motile or spore forming bacterium with a bipolar staining reaction (Ruiz & Sandhu, 2013). Biochemically, isolates of *R. anatipestifer* are positive for catalase, oxidase, urease and gelatinase tests, but negative for indole, methyl red, citrate utilisation, nitrate reduction tests. Besides, the organism shows negative reactions to dextrose, ga-
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lactose, lactose, fructose, mannose, maltose, mannitol, sorbitol, inositol, trehalose and sucrose fermentation tests.

Phenotypic characterisation of *R. anatipestifer* using conventional isolation and identification techniques proved to be not accurate, labourious and time-consuming as there are difficulties in differentiating this organism from other similar bacteria as *Pasteurella multocida* (Hinz et al., 1998; Rubbenstroth et al., 2013).

Serotyping of *R. anatipestifer* isolates is very important for epidemiological and vaccination studies (Pathanasophon et al., 2002; Fulton & Rimler, 2010; Rubbenstroth et al., 2013). Due to the high antigenic diversity of the bacterium, an increase in the number of serotypes and presence of at least 21 types have been recorded (Bisgaard, 1982; Sandhu & Leister, 1991; Ruiz & Sandhu, 2013; Chikuba et al., 2016). Unfortunately, for many years ago, *R. anatipestifer* isolates were regarded as un-typable during routine surveys (Sandhu & Leister, 1991; Metzner et al., 2008). There is no cross protection between the different serotypes and the same flock could be infected by more than one serotype (Ruiz & Sandhu, 2013). Serotypes 1, 2, 3, 5 and 15 of *R. anatipestifer* have been frequently detected in ducks (Sandhu & Leister, 1991; Panthanosophon et al., 1994). It has been demonstrated that serotypes 1, 2 and 5 are the most common in the United States; 1, 10 and 15 in Thailand (Pathanasophon et al., 1995), 1, 2, and 10 in China (Zhai et al., 2013), 2 and 6 in Taiwan (Phonvisay et al., 2017) and 1 and 2 in Egypt (Abd El Hamid et al., 2019; Eman et al., 2020).

It has been found that outer membrane protein (Omp) A is a major immunogenic protein for *R. anatipestifer* (Subramaniam et al., 2000) and it is important for the organism virulence even after mutation or attenuation (Hu et al., 2011). This protein could be used as an antigen for preparation of new vaccines against *R. anatipestifer* in ducks (Subramaniam et al., 2000). Furthermore, it has been developed for serological detection of all *R. anatipestifer* serotypes (Heba et al., 2015; Abd El Hamid et al., 2019). In addition to Omp A, there are other types of *R. anatipestifer* virulence factors as VapD and CAMP cohemolysin (Chang et al., 1998; Hu et al., 2011). Lately, a colloidal gold immuno-chromatographic strip based on monoclonal antibodies against *R. anatipestifer* in ducks has been successfully used (Han et al., 2020). This test has been regarded as a rapid, easy, reliable and economic diagnostic test.

Specific primers for *R. anatipestifer* have been developed and used for proper detection of the organism using polymerase chain reaction (PCR) (Kardos et al., 2007). Real-time PCR (Zhang et al., 2017) and multiplex PCR (Wei et al., 2013) have been used to detect these bacterial strains. Shancy et al. (2018) identified 546 bp PCR amplicon size in *R. anatipestifer* isolates while in Bangladesh, Sarkar et al. (2017) confirmed presence of *R. anatipestifer* isolates at 421 bp fragment of ribonuclease Z gene after using the primer sequence of Kardos et al. (2007). The Egyptian study of Abd El Hamid et al. (2019) used a partial coding sequence 608 bp of *R. anatipestifer* Omp A for serotyping of the local isolates. However, Christensen & Bisgaard (2010) demonstrated that most of PCR assays for *R. anatipestifer* failed in detection of all the strains of the bacterium.

The full length of bacterial 16S ribosomal RNA sequencing and Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight (MALDI-TOF) mass
spectrometry are also used for genetic characterization of *R. anatipestifer* (Tsai *et al.*, 2005; Christensen & Bisgaard, 2010; Hess *et al.*, 2013; Rubbenstroth *et al.*, 2013; Chikuba *et al.*, 2016; Tzora *et al.*, 2021). The latter method is highly efficient and superior for identification of *Riemerella* species (Rubbenstroth *et al.*, 2012; Philipp *et al.*, 2013). Although MALDI-TOF mass spectrometry method is considered as a fast, cost-effective and reliable manner for detection of *R. anatipestifer* (Seng *et al.*, 2009; Hu *et al.*, 2012), it is based on a limited proportion of the target bacterial proteins that cover only a small proportion of the proteome (Huang *et al.*, 2002; Zhai *et al.*, 2012). Pulsed-field gel electrophoresis is also regarded as a highly discriminating molecular typing method of *R. anatipestifer* isolates (Kiss *et al.*, 2007; Yu *et al.*, 2008; Rubbenstroth *et al.*, 2012) as it can detect the total genome of the bacterium (Bizzini *et al.*, 2010).

Wang *et al.* (2012) and Udayan *et al.* (2019) defined a type II DNA topoisomerase [gyrase B-encoding gene (gyrB)] based-PCR as a more accurate, consistent, sensitive and specific marker than 16S rRNA based PCR for the detection of *R. anatipestifer*. Recently, Ritam Hazarika *et al.* (2020) used specific PCR assay (564 bp) and gyrB based-PCR (162 bp) and found that both genes were suitable as molecular markers for identification of *R. anatipestifer* isolates.

Loop-mediated isothermal amplification (Han *et al.*, 2011), enzyme-linked immunosorbent assay (ELISA) (Lobbedey & Schlatterer, 2003; Huang *et al.*, 2011), gel diffusion precipitin and slide agglutination tests (Pathanasophon *et al.*, 2002) are other techniques that are used for diagnosis of *R. anatipestifer* infection.

**PREVENTION AND CONTROL**

Maintaining a high level of good biosecurity management and hygienic practices may be effective in prevention and elimination of *R. anatipestifer* infection (Ono & Tanaka, 1988). However, eradication of the disease is difficult as repeated infections with *R. anatipestifer* in the same farm can occur (Tsai *et al.*, 2005).

Some *R. anatipestifer* isolates resist treatment with antimicrobials (Ono & Tanaka, 1988) and persist in the environment for a long time forming biofilms (Hu *et al.*, 2010). Therefore, *in vitro* antibiotic sensitivity test is extremely important to select the suitable antibiotic before any treatment. The results of sensitivity test are variable and differ according to the locality and the time.

Recent study of Tzora *et al.* (2021) revealed that strains of *R. anatipestifer* isolated from broiler chickens in Greece were sensitive to amoxicillin, ceftiofur and sulphamethoxazole-trimethoprim. Similar results were found in Taiwan as 97.4% of *R. anatipestifer* strains from water fowl were sensitive to amoxicillin, ceftiofur and 57% were susceptible to sulphamethoxazole-trimethoprim (Chang *et al.*, 2019). Chikuba *et al.* (2016) demonstrated high sensitivity of *R. anatipestifer* strains of ducks to amoxicillin. In addition, most of Chinese (Sun *et al.*, 2012), Japanese (Chikuba *et al.*, 2016) and Hungarian (Gyuris *et al.*, 2017) duck strains of *R. anatipestifer* displayed sensitivity to sulphamethoxazole-trimethoprim.

However, resistance to colistin sulfate, spectinomycin, gentamicin, lincomycin, neomycin, oxytetracycline, spectinomycin, tetracycline and tylosin have been reported among *R. anatipestifer* strains (Tzora *et al.*, 2021). Nearly similar resistance pattern of *R. anatipestifer* strains to tetracycline has been demonstrated in
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Hungary (Gyuris et al., 2017), in Taiwan (Yu et al., 2008) and in China (Zhong et al., 2009). Several studies showed that R. anatipestifer isolates were resistant to gentamicin (Zhong et al., 2009; Surya et al., 2016; Gyuris et al., 2017; Ritam Hazarika et al., 2020; Tzora et al., 2021). High resistance to colistin was also detected among R. anatipestifer strains (Chang et al., 2019; Tzora et al., 2021). In addition, more than 70% of the isolates were resistant to lincomycin as well (Luo et al., 2018; Chang et al., 2019; Tzora et al., 2021). The resistance rate to erythromycin was up to 75.1% in Hungary (Gyuris et al., 2017), 64% in Taiwan (Yu et al., 2008) and 32.7% in China (Zhong et al., 2009). Sensitivity of R. anatipestifer isolates from ducklings to enrofloxacin was variable (Turbahn et al., 1997; Soman et al., 2014; Tzora et al., 2021).

In Hungary, the average rate of the extensive multi-drug resistance among geese and duck R. anatipestifer strains was 30.3% and the percentage can be increased over time (Gyuris et al., 2017). This finding was explained by the overuse and improper application of antibiotics in ducks (Köhler et al., 1995; Zhong et al., 2009; Sun et al., 2012).

As a result of increasing the emergence of drug-resistant strains of R. anatipestifer (Yang et al., 2012; Li et al., 2016; 2017), alternative measures such as vaccination has been encouraged (Higgins et al., 2000; Hu et al., 2011; Li et al., 2012). Inactivated, living attenuated and subunit vaccines are currently used to prevent R. anatipestifer infections in ducks farms. Inactivated vaccines have been used to prevent or reduce ducks' mortalities and to develop serotype specific immunity (Layton & Sandhu, 1984). Proper autogenous vaccines can protect ducks from infection (Layton & Sandhu, 1984; Floren & Kaleta, 1988; Huang et al., 2002; Liu et al., 2013). The protective efficacy of the vaccine depends mainly on the used strains and the protection developed only against the homologous challenge (Panthansophon et al., 1996; Huang et al., 2002). The serotypes of R. anatipestifer present in any vaccine showed no cross-protection with other serotypes. The frequent changes of serotypes in the farms and the presence of more than one serotype in one farm make problems in application of vaccines against R. anatipestifer. Therefore, the vaccines should contain all the predominant R. anatipestifer serotypes to provide effective broad spectrum protection (Timms & Marshall, 1989). Multivalent inactivated vaccines have been used for the prevention of R. anatipestifer in ducks, especially against serotypes 1 and 2 (Eman et al., 2020) and 1, 2 and 6 (Wu et al., 2020). Moreover, inactivated R. anatipestifer vaccine containing levmisole (Zhang et al., 2014) and chaperonin GroEL (Han et al., 2012; Haiwen, 2013) as adjuvants were successfully protected ducks from the infection.

It is important to note that the immune response of vaccinated ducklings at very young age can interfere with the maternal immunity. Sandhu & Leister (1991) observed a good immune response after vaccination of 2–3 weeks old ducklings with inactivated trivalent R. anatipestifer vaccine. The recent Egyptian study of Eman et al. (2020) demonstrated that vaccination of ducklings (priming at 2–3-week-old and booster at 4–6-week-old) with single or combined local inactivated bacterin containing R. anatipestifer serotypes (A1 and A2) and Pasteurella multocida serotypes (A and D) induced strong immune response as detected by indirect haemagglutination and ELISA tests.

Subunit vaccine containing recombi-
nant *R. anatipestifer* Omp A plus CpG oligo-deoxy-nucleotides as an adjuvant has been successfully developed (Chu et al., 2015). Recently, Wu et al. (2020) vaccinated breeder ducks with DNA and subunit combination vaccine containing serotypes 1, 2 and 6 of *R. anatipestifer*. The results revealed that the prime-boost regimens elicited deeper immune responses with stronger humoral and cellular immunity when compared with the conventional inactivated vaccine. The authors also suggested using of the subunit with inactivated regimen to reduce the cost of preparation of such type of vaccines and also to elicit a strong immune response.

**CONCLUSION**

Infection with *R. anatipestifer* creates great losses for duck industry worldwide. Although extensive studies have been conducted on such infection, the disease is still present and circulating among flocks. There are great difficulties in the treatment of *R. anatipestifer* due to the development of drug resistance. Moreover, the available vaccines induce homologous immunity and there is no cross protection between the different serotypes. So, treatment and vaccination protocols against *R. anatipestifer* need further investigations and research work to eradicate such serious infection.

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