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Review

AN OVERVIEW ON RIEMERELLOSIS: A WORLDWIDE EMERGING DISEASE OF DUCKS

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Summary

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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 septicaemia, 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

Locality	References	Findings
Germany	Köhler *et al*. (1995)	Demonstrated presence of *R. anatipestifer* as pathogen for geese in the northern and central parts of Germany
United States	Cooper (1989)	Detected presence of *Pasteurella anatipestifer* infections in California turkey flocks with evidence of a mosquito vector
Australia	Rosenfeld (1973)	Early identified *Pasteurella anatipestifer* infection in fowls
	Omaleki *et al*. (2021)	Molecularly and serologically characterised *Riemerella* isolates associated with different avian species
Hungary	Bitay *et al*. (1979)	Early detected anatipestifer syndrome of ducks (*Pasteurella anatipestifer* bacteria)
	Ivanics *et al*. (1996)	Demonstrated presence of anatipestifer disease in growing geese
	Gyuris *et al*. (2017)	Determined the antimicrobial susceptibility of *R. anatipestifer* strains isolated from geese and ducks
Japan	Baba *et al*. (1987)	Early identified *Moraxella* (*Pasteurella*) *anatipestifer* from an outbreak in ducklings
	Sakurai *et al*. (1987)	Demonstrated *Pasteurella anatipestifer* infection in duckling
	Tanaka *et al*. (1988)	Identified *Moraxella* (*Pasteurella*) *anatipestifer* infection in ducklings
	Chikuba *et al*. (2016)	Detected presence of *R. anatipestifer* infection in domestic ducks with specific clinical picture
India	Sarma *et al*. (1985)	Isolated *Pasteurella anatipestifer* and *P. haemolytica* from an outbreak of duck mortality
	Priya *et al*. (2008)	Detected *R. anatipestifer* from outbreaks of new duck disease
	Soman *et al*. (2014)	Identified *R. anatipestifer* from ducks using traditional methods and PCR.
	Ritam Hazarika *et al*. (2020)	Isolated and molecularly identified *R. anatipestifer* strains from ducks
Thailand	Panthansophon *et al*. (1994)	Identified serotypes 1, 2, 3, 5 and 15 of *R. anatipestifer* from ducks
	Panthansophon *et al*. (1995)	Detected new serotypes of *R. anatipestifer* strains from duck
	Panthansophon *et al*. (2002)	Demonstrated new serotypes of *R. anatipestifer* strains from duck

Table 1 (cont'd). The worldwide distribution of *R. anatipestifer* infections in different countries

Malaysia	Shome *et al*. (2004)	Identified *R. anatipestifer* from an outbreak in ducks
Taiwan	Chang *et al*. (2003)	Determined the antimicrobial susceptibility of *R. anatipestifer* isolates from ducks and the efficacy of ceftiofur treatment.
	Yu *et al*. (2008)	Detected the genomic diversity and molecular differentiation of *R. anatipestifer* associated with eight outbreaks in five farms
	Phonvisay *et al*. (2017)	Made surveillance studies on *R. anatipestifer* from outbreaks in duck farms
	Chang *et al*. (2019)	Made epidemiological and antibiogram studies on *R. anatipestifer* isolates from waterfowl slaughterhouses.
China	Li *et al*. (2011)	Isolated *R. anatipestifer* strains from chickens
	Sun *et al*. (2012)	Molecularly characterised *R. anatipestifer* isolates of Ducks and identified their antimicrobial resistance.
	Zhai *et al*. (2012)	Identified 11 novel immuno-reactive proteins of serotype 2 *R. anatipestifer* using immuno-proteomic technique.
	Zhai *et al*. (2013)	Prepared cross-protective vaccine candidates from *R. anatipestifer* serotypes 1 and 2 using immuno-proteomics.
	Li *et al*. (2016)	Detected the effects of two efflux pump inhibitors on the drug susceptibility of *R. anatipestifer*.
	Han *et al*. (2020)	Developed a colloidal gold immuno-chromatographic strip for detection of *R. anatipestifer* in ducks
Bangladesh	Sarker *et al*. (2017)	Confirmed presence of *R. anatipestifer* isolates at 421 bp fragment of ribonuclease Z gene
Greece	Tzora *et al*. (2021)	Identified *R. anatipestifer* isolates from broiler chickens using MALDI-TOF MS and detected the sensitivity pattern to different antibiotics.
Egypt	Heba *et al*. (2015)	Identified higher prevalence rate of *R. anatipestifer* among ducks (11.7%) than ducklings (5%). The specific *OmpA* gene was detected among all isolates using PCR.
	Abd El Hamid *et al*. (2019)	Found that the PCR with sequence analysis of Omp A gene of *R. anatipestifer* was highly sensitive and rapid for serotyping especially in case of unavailability of standard hyper immune serum of local duck isolates.
	Eman *et al*. (2020)	Successfully prepared and tested a single and combined local inactivated vaccine containing *R. anatipestifer* serotypes (A1 and A2) with *Pasteurella multocida* serotypes (A and D) to protect ducks till 6 months of age.

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;

Rosenfeld, 1973; Pascucci *et al*., 1989; Hinz *et al*., 1998; Li *et al*., 2011; Tzora *et al*., 2021). Young 2–3-week-old ducklings are more susceptible to *R. anatipestifer* infection than adult birds (Leibovitz, 1972; Ruiz & Sandhu, 2013; Chikuba *et al*., 2016). Moreover, 2–8-week-old goslings can be infected.

Some opinions regarded *R. anatipestifer* as a normal inhabitant microflora in the upper respiratory tract of some domestic and wild ducks (Ryll *et al*., 2001; Cha *et al*., 2015). The main routes of *R. anatipestifer* infection and transmission are either horizontally through the respiratory tract or mechanically via skin wounds (Ruiz & Sandhu, 2013). Vertical infection through ovaries or oviducts is controversial (Glünder & Hinz, 1989; Mavromatis *et al*., 2011; Hess *et al*., 2013).

CLINICAL SIGNS AND POST-MORTEM LESIONS

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, perihepatitis 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-

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; Panthansophon *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, Sarker *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 has been reported among *R. anatipestifer* strains (Tzora *et al*., 2021). Nearly similar resistance pattern of *R. anatipestifer* strains to tetracycline has been demonstrated in

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 levamisole (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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