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Efficacy of a Recombinant Vaccine Against Pasteurellosis in Chickens and Ducks

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Abstract | Pasteurellosis or fowl cholera (FC), is an economically-important poultry disease caused by *Pasteurella multocida* serotype A. In ducks, it is caused by *Riemerella anatipestifer*. Outbreaks of FC is frequently reported, suggestive of inefficacious vaccine. In this study, recombinant cells carrying OMP_{36} gene of *Pasteurella multocida* A:1 was prepared as a killed vaccine to test against pasteurellosis in chickens and ducks. A total of 325 chickens and 125 ducks were selected and divided to 4 groups based on the types of immunization. Groups 1, 2, 3, and 4 were administered with the recombinant vaccine, control *E. coli* without insert, commercial FC vaccine, and sterile PBS, respectively. A booster dose was administered after 2 weeks. Each group were then further divided to 4 subgroups based on birds and challenge strain. A challenge trial was conducted against *P. multocida* A:1, A:3, or A:1,3 for the chickens; and *P. multocida* A:1 and *R. anatipestifer* for the ducks. Clinical signs were observed and serum samples were collected weekly for determination of IgG antibody levels. Birds with severe clinical signs were euthanized for necropsy. Following immunization of chickens, IgG increased at week 1 and continued to increase after booster vaccination at week 2, and peaked at week 4 for both recombinant and commercial vaccines. The recombinant vaccine stimulated higher level of antibody in chickens. Following challenge, the recombinant vaccine provided excellent homologous (21/25; 84%) and cross-protection against *P. multocida* serotype A:1,3 (23/25; 92%) but provided low cross-protection against *P. multocida* serotype A:3 (11/25; 44%). In ducks, the recombinant vaccine provided moderate protection (14/25; 56%) compared to the excellent protection provided by the commercial vaccine (23/25; 92%). All euthanized birds showed typical clinical signs and gross pathology of FC. In conclusion, the recombinant vaccine is suitable against pasteurellosis in chicken, being more superior compared to the commercial vaccine. However, it provides poor protection against *R. anatipestifer* infection in ducks.

Keywords | Chickens, Ducks, OMP36, *Pasteurella multocida*, *Riemerella anatipestifer*

INTRODUCTION

Pasteurellosis, also known as fowl cholera (FC) in chickens is caused by *Pasteurella multocida*. All serotypes of *P. multocida* have been previously isolated from avian hosts (Christensen et al., 2008), except capsular types E and serotypes 8 and 13. The capsular type A has been observed to be the most frequently isolated from cases of severe FC (Christensen et al., 2008). In ducks, infections by *P. multocida* or *Riemerella antipestifer* (previously in the genus *Pasteurella*), or both are sometimes known as FC. The disease leads to great economic loss in poultry and duck industries, mainly due to the high mortality (Baksi et al., 2018). This high mortality is the result of acute septicaemia

or chronic respiratory infection by these pathogens (Homchampa et al., 1997). Sulpha drugs, semisynthetic penicillin, tetracycline, and erythromycin are administered in food or drinking water to control the disease (Huang et al., 2009). However, the usage of antimicrobials in animal production raises global concern pertaining to incidence of anti-microbial resistance (Stella et al., 2018).

Immunisation has also been used to control FC particularly in layers and breeders. Currently, commercially-available vaccines are in the form of bacterin, and only induce immunity to homologous serotypes (Homchompa et al., 1997). On the other hand, the use of live vaccine confers immunity against heterologous serotypes, but may revert to cause infection (Christensen et al., 2008). Furthermore, anaphylactic shock and short duration of protection are among the limitations of immunization using bacterin (Gershwin, 2015; Dellagostin et al., 2017). Hence, it is necessary to design a safe vaccine capable of conferring protection against homologous and heterologous challenges.

Recent vaccine development uses the outer membrane protein (OMP), which is part of the bacterial cell wall considered as a potent candidate for vaccine (Poolperm et al., 2018; Anun, 2001). The vaccine is prepared as recombinant cells that carry selected gene to provide better protection against challenge by *P. multocida* (Ibrahim et al., 2000). The objective of this study was conducted to compare the efficacy of a recombinant cell carrying OPM_{36} gene of *P. multocida* A:1 against FC in chickens and ducks.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS
A total of 325 Cobb chickens and 125 White Pekin ducks of 14 days old with no history of immunization against *P. multocida* and *R. anatipestifer* were selected. The chickens or the ducks were originated from one source. The birds were previously immunized against Marek's, Newcastle, and Gumboro diseases (Prabakaran, 2003). At the animal facility, they were kept in pens and were provided with commercial feed and drinking water. They were acclimatized for a week, while oral swabs were collected the following week for screening of *P. multocida*. The experiment was started when *P. multocida* was absent from all birds at which the birds were at 28 days old. All animal experiment was approved by an ethical review committee.

VACCINES
Recombinant OMP 36 kDa gene of *P. multocid*a serotype A:1 was previously prepared, inserted into pET 32 KL/LIC vector, and transformed into *Escherichia coli* BL21(DE3) strain before they were grown in Luria-Bertani broth with

induction using isopropyl-β-D-thiogalactoside (IPTG). The culture was incubated at 26°C for 24h. The culture was harvested for determination of bacterial concentration (Pommerville, 2014). It was then washed with PBS, and killed using 0.5% formalin at 4°C for 18h. The culture was washed three times and re-suspended in PBS to a concentration of $3.0x10^8$ cells/ml.

The control non-inserted vaccine was similarly prepared but without the OMP_{36} gene insert. Other control vaccine used for chickens was commercial alum-precipitated vaccine containing $3.0x10^8$ cfu/ml of *P. multocida* A:1 (MVP, Malaysia). For ducks, a commercial alum-precipitated vaccine containing $3.0x10^8$ cfu/ml of *P. multocida* serotype A:1 and *R. anatipestifer* (MVP, Malaysia) was used. Sterile PBS was used as negative control.

BACTERIAL INOCULUMS
Isolates of *P. multocida* serotypes A:1, A:3, A:1, 3 and *R. anatipestifer* from previous outbreaks of FC were obtained from stock cultures kept at -80°C. They were thawed at room temperature for 1h, and subsequently cultured onto blood agar and incubated at 37°C for 24h. Fifteen colonies were selected from each serotype and cultured in brain-heart infusion (BHI) broth, and incubated at 37°C for 24h. The culture was adjusted to a final bacterial concentration of $1.0x10^9$ cfu/ml.

EXPERIMENTAL DESIGN
IMMUNIZATION AND CHALLENGE
The chickens and ducks were divided to 4 groups based on the types of immunization. Group 1 were immunized with the recombinant vaccine, Group 2 were with *E. coli* without insert, Group 3 with commercial FC vaccine, and Group 4 with sterile PBS. The immunization was done at the dose of 0.5ml, intramuscular. Two weeks after the first immunization, booster dose of respective vaccine was administered with similar dose and route. Two weeks after the booster dose, the birds were then further divided into subgroups based on the challenge strains and were challenged with the inoculums prepared (Table 1).

BLOOD SAMPLING AND ELISA
Blood samples were collected via the brachial vein at weekly interval. The serum was subjected to determination of IgG level against *P. multocida* A:1 using ELISA as previously described, with minor modification (Sthitmatee et al., 2008; Solano et al., 1983). Briefly, microplates were coated with 50μl of antigen; $3.0x10^6$ cfu/ml *P. multocida* serotype A:1 in carbonate-bicarbonate buffer (pH 9.6), and incubated at 4°C overnight. The plates were then washed, filled with 200μl of blocking buffer [PBS-0.5% Tween 20(PBS-T)-3% BSA] and incubated at 37°C for 1h. After washing, 50μl of diluted serum samples in blocking buffer

(1:500) were added, and incubated at 37°C for 1 h. The plates were washed, and filled with 50μl of diluted goat anti-chicken IgG peroxidase conjugate (i-DNA, USA) in blocking buffer (1: 10,000) and incubated at 37°C for 1 h. Following washing, 100μl of TMB solution substrate buffer was added and incubated at 37°C for 30 min. The reaction was stopped by adding 2N sulphuric acid. Absorbance value was measured at 450 nm wavelength. The absorbance values were plotted to compare between different groups.

Table 1: Summary of the chicken and duck groupings, immunization, and challenge

Groups	Immunization[1]	Birds	Challenge strain
Group 1	Recombinant	Chicken	*P. multocida* A:1
		Chicken	*P. multocida* A:3
		Chicken	*P. multocida* A:1,3
		Duck	*P. multocida* A:1 + *R. anatipestifer*
Group 2	*E. coli* no insert	Chicken	*P. multocida* A:1
		Chicken	*P. multocida* A:3
		Chicken	*P. multocida* A:1,3
		Duck	*P. multocida* A:1 + *R. anatipestifer*
Group 3	Commercial	Chicken	*P. multocida* A:1
		Chicken	*P. multocida* A:3
		Chicken	*P. multocida* A:1,3
		Duck	*P. multocida* A:1 + *R. anatipestifer*
Group 4	PBS	Chicken	*P. multocida* A:1
		Chicken	*P. multocida* A:3
		Chicken	*P. multocida* A:1,3
		Chicken	none
		Duck	*P. multocida* A:1 + *R. anatipestifer*
		Duck	none
Group 5	PBS	Chicken	none
		Duck	none

[1]Birds were immunized 2 weeks apart with respective vaccine (0.5ml, IM).

CLINICAL SIGNS, SURVIVAL RATE, AND POST-MORTEM EXAMINATION

All birds were observed for a period of 2 weeks. Birds with severe clinical signs of FC were euthanized by slaughtering (Grandin, 1994), and were considered succumbed to the infection. Any surviving birds were euthanized at the end of the experiment. Survival rate was calculated for each group. Immediately after euthanasia, liver, lung, pericardial fluid, heart and spleen samples were collected for bacterial isolation.

BACTERIAL ISOLATION

The abovementioned samples were cultured on blood agar and incubated at 37°C for 24h. *Pasteurella multocida* and *R. anatipestifer* was identified using Gram stain and biochemical tests (Carter and Cole, 2012; Peter et al., 1996), and subsequent confirmation of *P. multocida* (Shivachandra et al. 2006) and *R. anatipestifer* (Kardos et al., 2006) were made with PCR.

STATISTICAL ANALYSIS

Data of IgG antibody levels in the serum samples were tabulated and analyzed using One Way ANOVA and comparison of mean by Duncan test. Data on percentage of animal that died after challenge were tabulated and analyzed using Chi-square.

RESULTS

SURVIVAL RATE IN CHICKENS

Chickens immunized with the recombinant vaccine showed 84% (21/25), 44% (11/25) and 92% (23/25) survival rates following challenged by *P. multocida* A:1, A:3 and A:1,3, respectively. The recombinant vaccine provided significantly ($p<0.05$) lower protection against *P. multocida* A:3 compared to *P. multocida* A:1 and A:1,3. In comparison, chickens immunized by the commercial vaccine showed significantly ($p<0.05$) lower level of protection at 64% (16/25) and 44% (11/25) against *P. multocida* A:1 and A:3, respectively but higher ($p>0.05$) level of protection at 80% (20/25) against *P. multocida* 1,3. The *E. coli* with no insert provided significantly ($p<0.05$) low homologous and heterologous protections at 40% (10/25), 32% (8/25) and 44% (11/25) against *P. multocida* A:1, A:3 and A:1,3, respectively. Only few chicken administered with PBS and challenged with *P. multocida* A:1, A:3, or A:1,3 survived the challenge trial (Table 2).

SURVIVAL RATE IN DUCKS

Following immunization with the recombinant vaccine, 56% (14/25) of the ducks were protected against a challenge by the combination of *P. multocida* A:1 and *R. anatipestifer*. In contrast, the commercial vaccine provided significantly ($p<0.05$) higher rate of protection at 92% (23/25). Immunization by *E. coli* failed to provide protection when significantly ($p<0.05$) low (20%; 5/25) number of the ducks survived. None of the duck administered with PBS and challenged with *P. multocida* A:1 and *R. anatipestifer* survived the challenge (Table 2).

BACTERIAL ISOLATION AND IDENTIFICATION

The respective *P. multocida* was isolated from all organ samples of chickens, while *R. anatipestifer* was isolated from the lungs of ducks that succumbed to the challenge. All isolates of *P. multocida* were confirmed by PCR with the

1044 bp band while *R. anatipestifer* with the 700bp band (Figure 1). All organ samples from surviving chickens and ducks yielded no isolation of *P. multocida* or *R. anatipestifer*.

Table 2: Summary of immunization and survival rate after challenge trial in chickens and ducks.

| Groups	Immuni-zation[1]	Birds	Challenge strain	Survival rate %
Group 1	Recombi-nant	Chicken	*P. multocida* A:1	84[a]
		Chicken	*P. multocida* A:3	44[b]
		Chicken	*P. multocida* A:1,3	92[a]
		Duck	*P. multocida* A:1 + *R. anatipestifer*	56[b]
Group 2	*E. coli* no insert	Chicken	*P. multocida* A:1	40[c]
		Chicken	*P. multocida* A:3	32[c]
		Chicken	*P. multocida* A:1,3	44[b]
		Duck	*P. multocida* A:1 + *R. anatipestifer*	20[c]
Group 3	Commer-cial	Chicken	*P. multocida* A:1	64[b]
		Chicken	*P. multocida* A:3	44[b]
		Chicken	*P. multocida* A:1,3	80[a]
		Duck	*P. multocida* A:1 + *R. anatipestifer*	92[a]
Group 4	PBS	Chicken	*P. multocida* A:1	8[d]
		Chicken	*P. multocida* A:3	4[d]
		Chicken	*P. multocida* A:1,3	16[d]
		Chicken	none	100[a]
		Duck	*P. multocida* A:1 + *R. anatipestifer*	0[d]
		Duck	none	100[a]

[1]Birds were immunized 2 weeks apart with respective vaccine (0.5ml, IM); [a,b,c,d]Different superscript letters signify significant ($p<0.05$) difference.
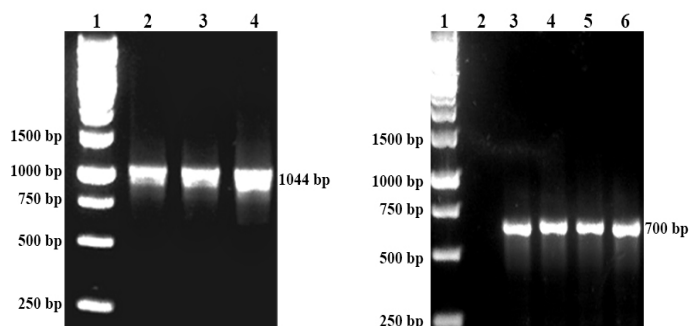
Figure 1: Left: DNA detection of *P. multocida* by PCR. Lane 1: Protein marker, Lane 2: *P. multocida* A:1, Lane 3: *P. multocida* A:3 Lane 4: *P. multocida* A:1,3. Right: DNA detection of *R. anatipestifer* by PCR. Lane 1 Protein marker. Lane 3,4,5 and 6: *R. anatipestifer*.
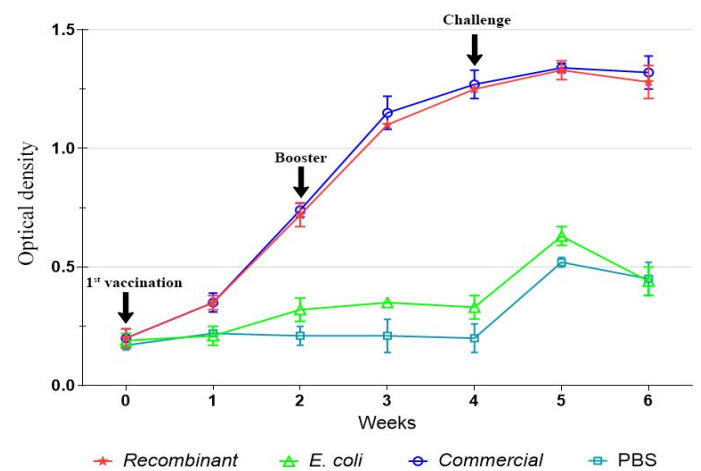
SERUM IgG ANTIBODY RESPONSE

Prior to immunization, there was significantly ($p<0.05$) low level of IgG in both chickens and ducks of all groups. However, following the first immunization, the IgG level increased significant ($p<0.05$) among the chickens and ducks immunized with the recombinant and commercial vaccines, but not following immunization with *E. coli* (Figure 2). The antibody levels increased until week 4 post-immunization, particularly after the administration of booster dose on week 2. Further increase of the IgG level was observed on week 5 following challenge but started to decrease on week 6.
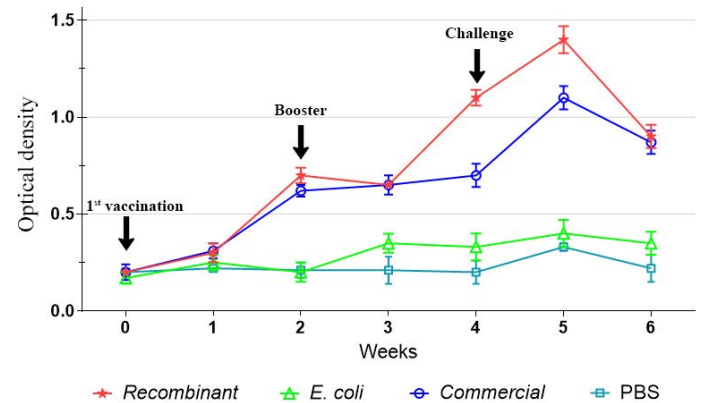
Figure 2: IgG antibody against *P. multocida* A:1 response in chickens during the immunization and challenge trial.

The IgG level was not significantly ($p>0.05$) increased following immunization with *E. coli* and in the control unimmunized groups. However, slight increase in the levels of IgG was observed on week 5 following challenge (Figures 2, 3).


Figure 3: IgG antibody against *P. multocida* A:1 response in ducks during the immunization and challenge trial.

CLINICAL SIGNS AND GROSS PATHOLOGY

All challenged chickens and ducks that were euthanized due to severe clinical signs of FC had abnormal respiratory sounds, greenish diarrhea, weakness, and anorexia.

These birds were observed with gross lesions including haemorrhage musculature, hepatic congestion (Figure 4), haemorrhagic trachea, air sacculitis, ventricular congestion, and hydropericardium.
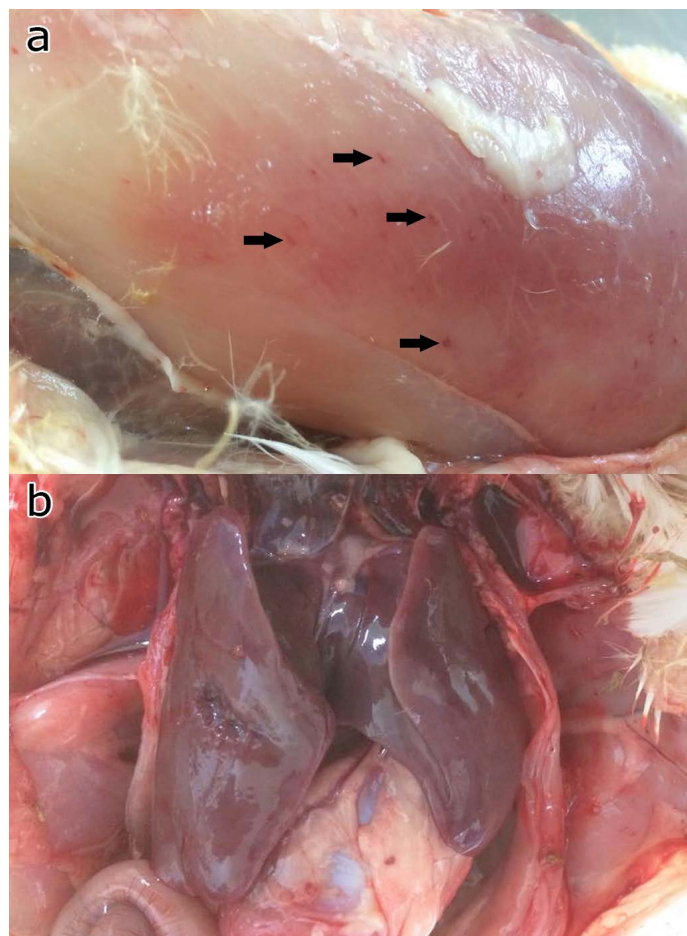
Figure 4: Gross pathology of the chicken and ducks showing petechia haemorrhage (arrows) of the pectoral muscle (a), and hepatic congestion (b).

DISCUSSION

Despite the availability of vaccines against FC, outbreaks were commonly reported globally, affecting both domestic and wild birds (Srinivasan et al., 2011; Hassan et al., 2017; Sellyei et al., 2017; Jaeger et al., 2018). This might be due to the poor usage of the currently available vaccines. This study investigates the attempts to use the OMP_{36} gene of *P. multocida* serotype A:1, expressed in *E. coli* strain BL21 through pET 32 plasmid as recombinant vaccine against FC in chicken and duck.

Following immunizations, the antibody titers increased significantly until week 4 following activation of memory cells (Dick and Avakian, 1991; Tizard, 2018). It seemed that both recombinant and commercial vaccines produced similar antibody pattern in chickens and ducks. Nevertheless, the antibody pattern following booster with the recombinant vaccine in chicken was significantly

(p<0.05) higher than that of the commercial vaccine, possibly due to the superior antigenicity of recombinant OMP_{36} that was expressed more after induction with IPTG than the whole cell *P. multocida* in the commercial vaccine. On the other hand, antibody titers in ducks following exposures to recombinant and commercial vaccines showed no significant difference (p>0.05).

The impact of antibody response could be observed following challenge. The recombinant vaccine that produced higher antibody level was capable of providing better protection in chickens, particularly after challenged by *P. multocida* A:1 and A:1,3. However, the recombinant vaccine that did not significantly different from the commercial vaccine did poorly in ducks upon challenged by *P. multocida* A:1 and *R. anatipestifer*. The significant finding of this study is that the recombinant cells carrying OMP_{36} gene were capable of providing cross protection against homologous and heterologous *P. multocida* infection in chicken. Similar observations were previously reported (Chung et al., 2005; Sthitmatee et al., 2008), particularly due to the fact that vaccines made from bacteria that were cultured *in vivo* or *in vitro* under iron limited condition (Ibrahim et al., 2000; Kedrak and Opacka, 2003).

The low protection provided by the recombinant vaccine in the ducks suggested that the IgG antibody against *P. multocida* A:1 conferred were not capable of providing cross protection against infection by *R. anatipestifer*. Hence, further research should focus to improve this aspect. Detection and insertion of antigenic gene from *R. anatipestifer* into the existing recombinant cells to produce bivalent recombinant vaccine should be considered. However, this may be a challenge since previous study utilizing OMPA and P45 genes of *R. anatipestifer* had failed to provide effective protection in ducks against *R. anatipestifer* infection (Huang et al., 2002).

CONCLUSIONS

The study showed that the usage of recombinant vaccine derived from OMP 36 kDa gene of *P. multocida* serotype A:1 insert into pET 32 plasmid and *E. coli* strain BL21 as expression host cell able to provide high protection against homologous (*P. multocida* A:1) and heterologous *P. multocida* A:1,3, but provided moderate cross protection against heterologous *P. multocida* serotype A:3 in chickens. This is more superior compared to the tested commercial vaccine. In ducks, the recombinant vaccine able to provide partial protection against *P. multocida* A:1 and *R. anatipestifer*, but significantly lower than the tested commercial vaccine.

AUTHORS CONTRIBUTIONS

DH conducted the study, data analysis and writing of ma-

uscript, NAA and AS involved in revision of manuscript, MZS and SMY designed and supervised the study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

• Anun M (2001). Molecular characterisation and pathogenicity of Pasteurella multocida isolated in chickens with fowl cholera (Master of Science Thesis). Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

• Baksi S, Rao N, Khan M, Chauhan P, Chauhan A (2018). Efficacy of Inactivated Fowl Cholera Vaccine in Chickens. PSM Veterinary Research, 3(2): 32-35.

• Carter GR, Cole JR (2012). Diagnostic procedures in veterinary microbiology. London, England: Academic Press Limited.

• Choi-Kim K, Maheswaran SK, Felice LJ, Molitor TW (1991). Relationship between the iron regulated outer membrane proteins and the outer membrane proteins of in vivo grown Pasteurella multocida. Vet. Microbiol., 28(1): 75-92. https://doi.org/10.1016/0378-1135(91)90100-T

• Christensen JP, Bojesen AM, Bisgaard M (2008). Fowl cholera. In Poultry Diseases (Pattison, McMullin, Bradbury, Alexander, eds): Elsevier, Philadelphia, USA, pp: 149-154. https://doi.org/10.1016/B978-0-7020-2862-5.50015-5

• Chung JY, Wilkie I, Boyce JD, Adler B (2005). Vaccination against fowl cholera with acapsular Pasteurella multocida A:1. Vaccine, 23(21): 2751-2755. https://doi.org/10.1016/j.vaccine.2004.11.036

• Dellagostin O, Grassmann A, Rizzi C, Schuch R, Jorge S, Oliveira T, McBride A, Hartwig D (2017). Reverse vaccinology: an approach for identifying leptospiral vaccine candidates. Int. J. Mol. Sci., 18(1): 158. https://doi.org/10.3390/ijms18010158

• Dick JW, Avakian AP (1991). Response of broiler chickens to fowl cholera vaccination at 1 to 6 weeks of age. Avian Dis., 35(4): 761-766. https://doi.org/10.2307/1591608

• Gershwin LJ (2015). Comparative immunology of allergic responses. Annu. Rev. Anim. Biosci., 3(1): 327-346. https://doi.org/10.1146/annurev-animal-022114-110930

• Grandin T (1994). Euthanasia and slaughter of livestock. J. Amn. Vet. Med. A., 204: 1354-1354.

• Hassan N, Hamadani H, Zargar UR (2017). Rare outbreak of fowl cholera in waterfowls in Dal Lake area of Kashmir, with isolation, antibiogram and successful treatment. Rep. Int. J. Curr. Microbiol. App. Sci., 6(5): 481-484. https://doi.org/10.20546/ijcmas.2017.605.056

• Homchampa P, Strugnell RA, Adler B (1997). Cross protective immunity conferred by a marker-free aroA mutant of Pasteurella multocida. Vaccine, 15(2): 203-208. https://doi.org/10.1016/S0264-410X(96)00139-9

• Huang B, Subramaniam S, Frey J, Loh H, Tan HM, Fernandez CJ, Kwang J, Chua KL (2002). Vaccination of ducks with recombinant outer membrane protein (OmpA) and a 41 kDa partial protein (P45N′) of Riemerella anatipestifer. Vet. Microbiol., 84(3): 219-230. https://doi.org/10.1016/S0378-1135(01)00456-4

• Huang TM, Lin TL, Wu CC (2009). Antimicrobial susceptibility and resistance of chicken Escherichia coli, Salmonella spp., and Pasteurella multocida isolates. Avian Dis., 53(1): 89-93. https://doi.org/10.1637/8268-021608-Reg.1

• Ibrahim RS, Sawada T, Shahata M, Ibrahim A (2000). Cross-protection and antigen expression by chicken embryo-grown Pasteurella multocida in chickens. J. Comp. Path., 123(4): 278-284. https://doi.org/10.1053/jcpa.2000.0419

• Jaeger A, Lebarbenchon C, Bourret V, Bastien M, Lagadec E, Thiebot JB, Boulinier T, Dellagi K (2018). Avian cholera outbreaks threaten seabird species on Amsterdam Island. PloS One, 13(5): e0197291. https://doi.org/10.1371/journal.pone.0197291

• Kardos G, Nagy J, Antal M, Bistyak A, Tenk M, Kiss I (2006). Development of a novel PCR assay specific for Riemerella anatipestifer. J. App. Microbiol., 44(2): 145-148. https://doi.org/10.1111/j.1472-765X.2006.02053.x

• Kedrak A, Opacka B (2003). Immunological response to outer membrane proteins of Pasteurella multocida serotype A:3 in calves. B. Vet. I. Pulawy., 47(2): 387-394.

• Peter EJ, Martha J, Carolyn JH (1996). Animal medium for growth of Pasteurella multocida. FEMS Microbiol Lett, 140(2-3): 165-169. https://doi.org/10.1016/0378-1097(96)00174-7

• Pommerville JC (2014). Fundamental of Microbiology (10th edition): Jones and Bartnett Publishers, Massachusetts, USA.

• Poolperm P, Apinda N, Kataoka Y, Suriyasathaporn W, Tragoolpua K, Sawada T, Sthitmatee N (2018). Protection against Pasteurella multocida conferred by an intranasal fowl cholera vaccine in Khaki Campbell ducks. Jpn. J. Vet. Res., 66(4): 239-250.

• Prabakaran R (2003). Chicken: broiler production. In: Good practices in planning and management of integrated commercial poultry production in South Asia (No. 159): FAO of the United Nations, Viale delle Terme di Caracalla, Rome, pp. 9-24.

• Sellyei B, Thuma A, Volokhov D, Varga Z (2017). Comparative analysis of Pasteurella multocida isolates from acute and chronic fowl cholera cases in Hungary during the period 2005 through 2010. Avian Dis., 61(4): 457-465. https://doi.org/10.1637/11674-051817-Reg.1

• Shivachandra SB, Kumar AA, Gautam R, Singh VP, Saxena MK, Srivastava SK (2006). Identification of avian strains of Pasteurella multocida in India by conventional and PCR assays. Vet. J. 172(3): 561-564. https://doi.org/10.1016/j.tvjl.2005.05.007

• Stella P, Beloeil PA, Guerra B, Hugas M, Liebana E (2018). The Role of the European Food Safety Authority (EFSA) in the Fight against Antimicrobial Resistance (AMR). Food Prot. Trends. 38(1): 72-80.

• Solano W, Giambrone JJ, Panangala VS (1983). Comparison of enzyme-linked immunoabsorbent assay and indirect hemagglutinin test for quantitating antibody responses in chickens against Pasteurella multocida. Avian Dis., 27(4): 1034-1043. https://doi.org/10.2307/1590204

• Srinivasan P, Gopalakrishnamurthy TR, Mohan B, Saravanan S (2011). Occurrence of sub-acute fowl cholera in a broiler flock. Tamil Nadu J. Vet. Anim. Sci., 7: 45-47.

• Sthitmatee N, Numee S, Kawanmoto E, Sasaki H, Yamashita K, Takahashi N, Kataoka Y, Sawada T (2008). Protection of chickens from fowl cholera by vaccination with recombinant adhesive protein of Pasteurella multocida. Vaccine, 26(19): 2398-2407. https://doi.org/10.1016/j.vaccine.2008.02.051

• Tizard I (2018). Veterinary Immunology-E-book. Elsevier Health Sciences: Missouri, USA.