
**UNIVERSITI PUTRA MALAYSIA**

# *DEVELOPMENT OF a RECOMBINANT (rOMP36) VACCINE FROM Pasteurella multocida A:1 AGAINST PASTEURELLOSIS IN CHICKENS AND DUCKS*

**DIDIK HANDIJATNO**

**FPV 2011 7**

# DEVELOPMENT OF a RECOMBINANT (rOMP36) VACCINE FROM *Pasteurella multocida* A:1 AGAINST PASTEURELLOSIS IN CHICKENS AND DUCKS

**DIDIK HANDIJATNO**

**DOCTOR OF PHILOSOPHY**
**UNIVERSITI PUTRA MALAYSIA**

**2011**

# DEVELOPMENT OF a RECOMBINANT (rOMP36) VACCINE FROM *Pasteurella multocida* A:1 AGAINST PASTEURELLOSIS IN CHICKENS AND DUCKS

**By**

**DIDIK HANDIJATNO**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Doctor of Philosophy**

**February 2011**

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**DEVELOPMENT OF a RECOMBINANT (rOMP36) VACCINE FROM *Pasteurella multocida* A:1 AGAINST PASTEURELLOSIS IN CHICKENS AND DUCKS**


By

**DIDIK HANDIJATNO**


**February 2011**


**Chairman : Professor. Mohd. Zamri Saad., DVM, PhD**

**Faculty : Veterinary Medicine**



Fowl cholera is one of the diseases that cause economic losses in poultry farms. It is caused by *Pasteurella multocida* serotype A in chickens but in ducks, it is caused by *Riemerella anatipestifer* or *P. multocida* serotype A or both. *P. multocida* serotype A:1 is highly virulent and is the common cause of the disease. Commercial vaccine is available for control but the vaccine provides homologous protection with limited cross-protection. Therefore, this study was conducted to identify suitable vaccine candidate for development of an improved vaccine against avian pasteurellosis. The study concentrated on the outer membrane proteins (OMPs) since OMPs play key roles in disease pathogenesis as well as in inducing immunity.

Characterisation study on the OMPs of the various isolates of *P. multocida* serotypes A:1, A:3, A:1,3 and *R. anatipestifer* revealed that the major protein of *P. multocida* was 36 kDa while *R. anatipestifer* was 34.5 kDa. There were some differences among the OMPs

of different serotypes of *P. multocida*, particularly in the minor bands where *P. multocida* serotype A:1,3 had more bands than other serotypes. Western blotting and immune-detection using antisera of rabbit against OMP 36 kDa and whole cell of *P. multocida* serotype A:1, revealed that the 36 kDa OMP of all serotypes of *P. multocida* and the 34.5 kDa OMP of *R. anatipestifer* appeared thick and dense. However, the 36 kDa OMP of *P. multocida* serotype A:1 appeared antigenic and provided cross-reaction with *P. multocida* serotypes A:3, A:1,3 and against *R. anatipestifer.*

Therefore, the gene encoding 36 kDa OMP of *P. multocida* serotype A:1 was amplified by PCR before it was cloned in pET32 KL/LIC vector. The recombinant was successfully transformed into *Escherichia coli* Nova Blue strain as cloning host. Furthermore, the product was successfully sequenced and transformed into *E. coli* strain BL21 (DE3) and Origami2 (DE3) as expression host cells, revealing a single band of 1250 bp that consisted of 1050 bp gene insert and 200 bp pET32 vector. The sequence showed 100% homology to *OMPH* gene of *P. multocida* serotype A:1 and 99% to OMP H gene of *P. multocida* strain 18 and the OMP H of *P. multocida* subsp *gallicida*. The expressed protein was successfully verified by SDS-PAGE revealing the expected 40 kDa band.

The recombinant of $OMP_{36}$ gene was eventually prepared as killed bacteria or inoculums and injected intramuscularly into chickens and ducks before being challenged. The chickens and ducks received 0.5 mL of the inoculums containing $1.2x10^7$CFU/mL. Following inoculation, antibody started to increase at week 1 and continued to increase after booster vaccination and reached peak at week 4 for both recombinant and

commercial vaccines although the recombinant stimulated higher level of antibody compared to the commercial vaccine. Following challenge, the recombinant vaccine provided excellent protection 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 chickens. In ducks, the recombinant provided moderate protection (14/25; 56%) compared to the excellent protection provided by the commercial vaccine (23/25; 92%).

In conclusion, the recombinant vaccine generally provided better protection against the various serotypes of *P. multocida* than the commercial vaccine in chickens but less protection in ducks compared to the commercial vaccine.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGHASILAN DARI PADA REKOMBINAN (rOMP36) VAKSIN DARI *Pasteurella multocida* JENIS A:1 MEMBERI PERLINDUNGAN TERHADAP JANGKITAN KOLERA PADA AYAM DAN ITIK**

Oleh

**DIDIK HANDIJATNO**

**Februari 2011**

**Pengerusi : Professor. Mohd. Zamri Saad, DVM, PhD**

**Fakulti : Perubatan Veterinar**

Kolera pada ayam dan sejenisnya adalah penyakit yang merugikan dari segi ekonomi peternakan ayam, punca penyakit kolera pada ayam dan itik disebabkan oleh *Pasteurella multocida* jenis A tetapi pada itik boleh disebabkan oleh *P. multocida* jenis A atau *Riemerella anatipestifer* atau keduanya. Vaksinasi pada penyakit ini telah dipraktikkan secara meluas tetapi nilai keupayaan dari segi perlindungan masih belum memuaskan. Dalam usaha untuk memperbaiki keberkesanan vaksin, telah dikaji bahawa protin selaput luar adalah calon yang khasnya menarik untuk pembangunan rekombinan vaksin kerana protin selaput luar adalah kukuh dari segi keimunogenannya dan seterusnya kajian ditujukan pada keupayaan dan kapasiti perlindungan rekombinan protin selaput luar pada ayam dan itik

Gambaran protin dan keantigenan pada isolat tempatan *P. multocida* telah menunjukkan kehadiran protin 36 kDa, yang mana 36 kDa ini diidentitikan sebagai protin yang besar dengan menggunakan "SDS-PAGE". Seterusnya, tempelan imun menggunakan samada serum arnab yang dibangkitkan pada jalur protin 36 kDa atau sel utuh *P. multocida* jenis A:1 menunjukkan bahawa kedua-dua serum didapati bertindakbalas dengan protin 36 kDa *P. multocida* A:1, A:3, A:1,3 dan dengan protin 34.5 kDa *R. anatipestifer*. Penemuan

ini mencadangkan bahawa protin selaput luar 36 kDa adalah antigenik, dan boleh meransang penghasilan antibodi dari pada haiwan.

Klon protin selaput luar 36 kDa ke pET32 vektor telah berjaya, ditunjukkan adanya penggandaan DNA dengan berat molekul 1250 bp yang terdiri 1050 bp berasal dari protin 36 kDa gen dan 200 bp berasal dari gen vektor. Seterusnya klon ditransformasi ke *E. coli* jenis Nova Blue dan seterusnya dilakukan transformasi ke *E.coli* jenis BL21 dan Origami 2, juga dilakukan analisis jujukan gen. Analisis jujukan DNA menunjukkan jujukan gen yang terlibat berada pada kedudukan yang betul di dalam vektor. Ia mengesahkan bahawa gen yang terlibat adalah gen dari protin selaput luar 36 kDa *P. multocida* jenis A:1 dan mengandungi berat molekul 1050 bp. Analisis jujukan DNA dari gen protin selaput luar 36 kDa isolat lokal *P. multocida* jenis A:1 mendedahkan persamaan 100% dengan *P. multocida* jenis A:1, 99% dengan protin selaput luar H gen *P. multocida* strain 18 dan protin selaput luar H gen *P.multocida* subsp *gallicida*. Dalam proses membangunkan sel rekombinan vaksin yang telah mengekspresikan protin, dianalisis menggunakan tehnik "SDS-PAGE" dan pemblotan Western, mendedahkan bahawa protin lakuran pET32/LIC-36 kDa yang diekspresikan adalah lebih kurang 40 kDa terdiri 36 kDa adalah protin selaput luar dan 4 kDa dari pET32 vector selepas pengesanan menggunakan antibodi monoklon, His.Tag. Keputusan menunjukkan kejayaan dalam mengekspresikan gen selaput luar 36 kDa *P. multocida* jenis A;1

Kajian seterusnya adalah untuk menentukan paras antibodi dan perlindungan dari pada ayam dan itik dengan menggunakan rekombinan yang mengekspresikan protin selaput luar 36 kDa *P. multocida* jenis A:1 yang antigenik dan tidak aktif melalui suntikan dalam daging dengan takaran 0.5 mL yang mengandungi $10^7$CFU/mL. Secara keseluruhannya, didapati bahawa ayam dan itik yang diberi suntikan dengan rekombinan, telah membina paras antibodi IgG yang kuat dan meningkat secara bermakna ($p<0.05$) dalam serum apabila dibandingkan dengan ayam dan itik dari kumpulan yang tidak diberi suntikan. Pada awal minggu pertama selepas vaksinasi menunjukkan paras antibodi yang semakin meningkat pada minggu ke-2 selepas pemberian suntikan dan pengeluaran antibodi kekal meningkat secara yakin pada minggu ke-2 seterusnya iaitu minggu ke-3 dan minggu ke-4

selepas pemberian suntikan. Peningkatan paras antibodi, menunjukkan bahawa rekombinan yang digunakan di dalam percubaan ini telah merangsang penghasilan antibodi yang kuat ($p<0.05$) apabila dibandingkan dengan kumpulan ayam dan itik yang diberi vaksin *E. coli* dan kumpulan yang tidak diberi suntikan (kontrol). Pada cabaran keupayaan dengan menggunakan organisma hidup *P. multocida* A:1, A;3 dan A:1,3, kelompok ayam yang diberi suntikan dengan rekombinan memberikan perlindungan yang kuat ($p<0.05$) apabila dibandingkan dengan kelompok yang lain, sedangkan pada kelompok itik yang divaksin dengan rekombinan vaksin memberikan perlindungan terhadap cabaran keupayaan dengan orgainsma hidup campuran *P.multocida* jenis A:1 dengan *R. anatipestifer* yang cukup kuat ($p<0.05$) apabila dibandingkan dengan kumpulan itik yang diberi suntikan *E. coli* atau itik daripada kumpulan kontrol, tetapi lebih rendah apabila dibandingkan kumpulan itik yang diberi vaksin komersil.

## ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim

First and foremost praises to ALLAH, THE MOST COMPASSIONATE AND MERCIFUL for giving me the strength and courage to complete this thesis.

I would like to express my sincere gratitude especially to my supervisor, Professor Dr. Mohd Zamri Saad, and my co-supervisors, Dr. Md Sabri Mohd Yusoff, and Professor Dr. Mohd Hair Bejo from the Faculty of Veterinary Medicine, UPM for their continuous guidance, advices, ideas, and supervision on my work when things get tougher, and always be there for me with humor, patience and sense of perspective to help me get inspired which has been a great favor on my behalf.

Similarly, my utmost appreciation is extended to everybody who helped and contributed their efforts in making this thesis a success;

- Special thanks to and sincere appreciation are due to Associate Professor Dr. Zunita Zakaria, Associate Professor Dr. Siti Khairani Bejo and Dr. Shahiruddin Shamsuddin for the useful discussion and resourceful suggestions, and thanks goes to Mr. Arif Ahmad, Mr. Noraziman, Mr. Kamarzaman, Mr. Syamsudin, Mr. Apparau, Pak Malik, Pak Kudus, Aja and all staff of NaFish for their assistance and invaluable time spent.

- Special thanks go to staff of the Histopathology Laboratory, Faculty of Veterinary Medicine UPM, Mrs Jamilah, Mrs Latifah, Ms Darul Muqamah, Mr Jamil, Bacteriology Laboratory, Biologic Laboratory, Virology Laboratory and Molecular Biology Laboratory, Faculty of Veterinary Medicine, UPM. Not to forget, Prof. Dr. Abdul Rani Bahaman and Prof. Dr. Abdul Rahman Omar for allowing me to use their laboratory facilities.
- I have also been very fortunate in receiving assistance and support from Histopathology members, Ina Salwany, Shafarin, Siti Sarah, Khin, Trang, Nur Nazifah, Atyah, Aan, Dr Hani, Aini, Pak Sri, Firdaus, Amal, Parhom, Abu Bakar, Wawa, Wani, Sakinah, Dzarifah, Sharoom, Rafidah and Farid. Thanks for the help and support.
- All those who contributed directly or indirectly in sharing their knowledge, skill and assistance throughout the course of my study.

Finally yet importantly, I would like to express my deepest gratitude and I dedicate this thesis to these special persons;

- My beloved parents and my family. Thanks for your endless love and support.

I certify that a Thesis Examination Committee has met on 9th February 2011 to conduct the final examination of Didik Handijatno on thesis entitled "Development of Recombinant OMP 36 Gene of *Pasteurella multocida* serotype A:1 Vaccine Against Pasteurellosis in Chickens and Ducks" in accordance with the Universiti Putra Malaysia in 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy. Members of the Examination Committee are as follow:

**Abdul Rani Bahaman, PhD**
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

**Jasni Sabri, PhD**
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

**Siti Khariani Bejo, PhD**
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

**Priya Mohan Das, PhD**
Professor
Faculty of Veterinary Science
Bangladesh Agricultural University
(External Examiner)

_____

**NORITAH OMAR, PhD**
Professor and Deputy Dean
School of Graduate studies
Universiti Putra Malaysia

Date: 19 April 2011

The thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfilment of requirement for the degree of Doctor Philosophy. The members of Supervisory Committee were as follow:

**Mohd. Zamri Saad, PhD**
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

**Sabri Mohd Sabri Mohd Yussof, PhD**
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

**Mohd Hair Bejo. PhD**
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

_____

**HASANAH MOHD GHAZALI, PhD**

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

## DECLARATION

I declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that is has not been previously and is not concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

_____

**DIDIK HANDIJATNO**

Date: 9 February 2011

**Specially dedicated to:**

My Parents

MOHAMMAD TOHID (ALM)

SOEKARMI (ALM)

My Siblings,

TITIK SUHITA

YUDI ARIATI

LILIK AFIAH

ALEX HINDARTA

ANIK HANDAYANI

ELVI HANDIYAWATI

SONI OKTA HANAKA

My Family

RIZA AMBAR JULIANTI

YUDI AFRIANI DAMAYANTI

DIMAS DWI PRSETYO

**TABLE OF CONTENS**

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| % | Percentage |
| β | Beta |
| λ | Lambda |
| °C | degree celcius |
| µg | Microgram |
| µl | Microliter |
| µm | Micronmeter |
| µM | Micromolar |
| $Amp^R$ | ampicillin resistance |
| APS | ammonium persulfate |
| BLAST | basic local alignment search tool |
| BHI | brain heart infusion |
| bp | base pair |
| BSA | bovine serum albumin |
| cfu | colony forming unit |
| DMSO | Dimethylsulfoxide |
| DNA | deoxyribonucleic acid |
| dNTP | deoxynucleotide triphosphate |
| DTT | Dithiothreitol |
| *E. coli* | *Escherichia coli* |
| EDTA | Ethylene-diamine-tetraacetic acid (disodium salt) |

| | |
|---|---|
| ELISA | enzyme linked immunosorbent assay |
| G | Gram |
| $H_2O$ | Water |
| HS | haemorrhagic septicaemia |
| i.e. | in example |
| IgG | immunoglobulin G |
| *in vitro* | in an experimental situation outside the organism.. |
| *in vivo* | in a living cell or organism |
| IPTG | isopropyl-β-D-thiogalacosidase |
| IROMP | iron regulated outer membrane protein |
| kb | kilobase pair |
| kDa | kilo Dalton |
| LB | luria-bertani |
| LPS | Lipopolysaccharide |
| L | Liter |
| M | Molar |
| mM | mili molar |
| mA | Miliampere |
| mAB | monoclonal antibody |
| MCS | multiple cloning site |
| $MgCl_2$ | magnesium chloride |
| mg | Milligram |
| Min | Minutes |

| | |
|---|---|
| mL | Milliliter |
| mm | Milimeter |
| $MgCI_2$ | magnesium chloride |
| mRNA | messenger ribonucleic acid |
| MW | molecular weight |
| $Na_2HPO_4$ | di-sodium hydrogen phosphate |
| NaCl | natrium chloride |
| $NaH_2PO_4$ | sodium di-hydrogen phosphate |
| NaOH | sodium hydrogen peroxide |
| ng | nanogram |
| nm | Nanometer |
| OD | optical density |
| OMP | outer membrane protein |
| *ori* | Origin |
| PBS | phosphate buffer saline |
| PCR | polymerase chain reaction |
| pH | puissance hydrogen (Hydrogen-ion concentration) |
| *P. multocida* | *Pasteurella multocida* |
| pmol | pico-mol |
| PVDF | polyvinyl difluoride |
| *R. anatipestifer* | *Riemerella anatipestifer* |
| RBS | ribosome binding site |
| rpm | rotation per minute |

| | |
|---|---|
| RE | restriction enzyme |
| RT | room temperature |
| s | seconds |
| SDS | sodium dodecyl sulphate |
| SDS-PAGE | Sodiumdodecyl sulphate polyacrylamide gel electrophoresis |
| *Taq* | *Thermus aquaticus* YT-1 |
| TBE | tris-boric EDTA |
| Tbp | transferrin binding protein |
| TBS | Tris-buffer saline |
| TE | Tris-EDTA |
| TEMED | *N,N,N',N'*-tetramethylethylene diamine |
| $T_M$ | melting temperature |
| Tris-HCI | tris (hydroxymethyl) aminomethane hydrochloride |
| U | Unit |
| UV | ultra-violet |
| V | voltan/volt |
| v/v | volume per volume |
| w/v | weight per volume |

| **Amino acid** | **Single/Three letter** | **Amino Acid Code** |
|---|---|---|
| Alanine | A | Ala |
| Arginine | R | Arg |
| Asparagine | N | Asn |

| | | |
|---|---|---|
| Aspartic Acid | D | Asp |
| Glutamine | Q | Gln |
| Glutamic acid | E | Glu |
| Glycine | G | Gly |
| Isoleucine | I | Ile |
| Leucine | L | Leu |
| Lycine | K | Lys |
| Methionine | M | Met |
| Phenylalanine | F | Phe |
| Proline | P | Pro |
| Serine | S | Ser |
| Threonine | T | Thr |
| Tryptophan | W | Trp |
| Valine | V | Val |

# CHAPTER 1

# INTRODUCTION

*Pasteurella multocida* causes a wide range of diseases in animals such as haemorrhagic septicemia in cattle and buffalo, atrophic rhinitis in pigs, pneumonia in sheep and goat, snuffles in rabbits and fowl cholera in avian, including in chickens and ducks. Fowl cholera in chickens is caused particularly by *P. multocida* serotype A but pasteurellosis in duck is caused either *P. multocida* serotype A or *P. anatipestifer* (*Riemerella. anatipestifer*) or both (Adlam and Rutter, 1989). Pasteurellosis in chickens and ducks causes great economic loses to poultry and duck industries through decrease in egg production between 10% to 40% and high mortality, usually between 23% and 60% and occasionally over 90% (Partadireja *et al*., 1979; Glunder and Hinz, 1989).

*P. multocida* type A that causes pasteurellosis in chickens involves several serotypes, which include A:1; A:3; A:1,3; A:3,4; A:4 and A:9. However, studies revealed that serotype A:1 is the most virulent and most common cause of the disease (Curtis, 1979). Other than *P. multocida* type A, *P. multocida* types B, D and F have also been isolated from chickens, but they are less associated with the disease (Rimler and Rhoades, 1989).

Based on the 16S ribosomal primers, *P. multocida* can be differentiated into 5 groups known as Group A, B, D, E and F. Group A has prominent 1044 base pairs (bp) band, Group B with 760 bp, Group D with 657 bp, Group E with 511 bp and Group F with 854 bp (Townsend *et al*., 2001; Shivachandra *et al*., 2006). For serotype B2, Townsend *et al*.

(2001) revealed the 460 bp and 512 bp bands. Based on *Enterobacter Repetitive Intergenic Consensus* (ERIC) primers, *R. anatipestifer* showed band of approximately 670 bp – 700 bp (Kardos *et al*., 2006).

Currently, control of pasteurellosis is by vaccination. The commercially available vaccines against *P. multocida* are made of whole bacteria, known as bacterins, killed vaccine prepared either by using high temperature such as 100$^{o}$C for 30 minutes or 60-80$^{o}$C for 1 hour or by using chemicals such as 0.5% formalin or 0.5% phenol. The vaccine is usually administered by injection, inducing antibody with limited cross-protection against heterologous *P. multocida* strains and short duration of protection. Later, improved vaccines were prepared from micro-organisms grown *in vivo*, particularly in embryonic eggs (Mariana and Hirst, 2000) before alum precipitation or gel alum precipitation vaccines were prepared. These vaccines provided protection for up to 6 months with cross-protection against heterologous *P. multocida* strains (Adlam and Rutter, 1989).

In recent years, experimental vaccines were prepared using parts of the cells of *P. multocida* such as lipopolysaccharide, ribosome, capsular, outer membrane proteins and the others part of the bacterium. These vaccines are known as the sub-unit vaccines. Among the most commonly studied parts is the outer membrane protein (OMP), considered a potent vaccine candidate. This is because OMP is part of the cell wall of bacteria that is exposed to the environment, enabling it to induce strong protective immunity in poultry and other animals (Lu *et al*., 1991a; Lu *et al*., 1991b; Zhao *et al*.,

1995; Ruffolo *et al*., 1996). The most recent method of vaccine preparation is the incorporation of the outer membrane protein gene into non-pathogenic bacteria such as *Escerichia coli*, known as recombinant vaccine (Manoha *et al*., 1993).

**1.1. The objectives of this study** were:

1. to determine the antigenicity and cross-reactivity of 36 kDa OMP of *P. multocida* serotypes A:1, A:3, A:1,3 and *R. anatipestifer.*
2. to clone, sequence and express the $OMP_{36}$ gene of *P. multocida* serotype A:1 as a recombinant (rOMP36) vaccine material.
3. to determine the efficacy of the newly recombinant (rOMP36) vaccine against pasteurellosis of chickens and ducks.

## REFERENCES


Adamus, G., Mulezyk, M., Witkowska, D. and Romanowska, E. 1980. Protection against keratoconjunctivitis shigellosa induced by immunization with outer membrane proteins of *Shigella* spp. Infect. Immun. 30: 321-324.

Adlam, C. and Rutter, J.M. 1989. Pasteurella and Pasteurellosis. Academic Press. London San Diego New York Berkeley. pp 37-73

Adler, B., Bulach, D., Chung, J., Doughty, S., Hunt, M., Rajakumar, K., Serrano, M., van Zanden, A., Zhang, Y. and Ruffolo, C. 1999. Candidate vaccine antigens and genes in *Pasteurella multocida*. J. Biotechnol. 20; 73(2-3): 83-90.

Adler, B., Mitchison, M., Wei, L., Kwang, J. and Wilkie, I. 2000. Overexpression and immunogenicity of the Oma87 outer membrane protein of *Pasteurella multocida*. Vet Microbiol. 72: 91-96.

Afzal, M., Muneer, R. and Akhtar, S. 1992. Serological evaluation of *Pasteurella multocida* antigens associated with protection in buffalo calves. Rev. Sci. Tech. 11: 917-923.

Alcamo, I.E. 1997. In: Fundamental of Microbiology. ($5^{th}$ ed). (Addison Wesley Longman, Inc., California), pp 766-767.

Anum, B.M. 2001. Molecular characterisation and pathogencity of *Pasteurella multocida* isolated in chickens with fowl cholera. Master's Thesis. University Putra Malaysia. pp 83-95

Baba, T., Odagiri, Y., Morimoto, T., Horimoto, T. and Yamamoto, S. 1987. An Outbreak of *Moraxella (Pasteurella) anatipestifer* infection in ducklings in Japan, J. Vet. Sci 49: 939-941.

Babiuk, L.A. 1999. Broadening the approaches to developing more effective vaccines. Vaccine. 17: 1587-1595.

Bain, R.V.S. and Jones, R.F. 1955. Studies on haemorrhagic septicaemia of cattle. III. production of adjuvant vaccine. Brit. Vet. J. III, 30-34.

Bain, R.V.S., De Alwis, M.C.L., Carter, G.R. and Gupta, B. K. 1982. Haemorrhagic septicaemia. FAO Animal production and health paper No. 3. Food and Agriculture Organisation of the United nations, Rome. pp 132-145

Bain, R.V.S. and Knox, K.W. 1961. The antigens of *P. multocida* type I. II. Lipopolysaccharides. Immunology. 4: 122-129.

Bain, R.V.S., De Alwis, M.C.I., Carter, G.R and Gupta, B.K. 1982. Haemorrhagic septicemia animal production and health Paper 33. Food and Agriculture Organization of the United Nation, Rome,Italy : 11-33.

Baneyx, F. 1999. Recombinant protein expression in *Escherichia coli*. Curr. Opin. Biotechnol. 10: 411-421.

Bardakci, F. 2001. Random amplified polymorphic DNA (RAPD) markers. *Turkish J. Biol*. 25:185-196.

Bergey's Manual of Determinative Bacteriology. 1994. 9th ed. Williams and Wilkins. Co. p 96

Bolin, C.A. and Jensen, A.E. 1987. Passive immunization with antibodies against iron-regulated outer membrane proteins protects turkeys from *Escherichia coli* septicaemia. Infect. Immun. 55: 1239-1242.

Bording, A., Nymark, K. and Smidt, E. 1994. Field trial new genetically engineered Vaccine for protection against progressive atrophic rhinitis in pigs. Acta. Vet Scand 35: 155-163.

Bowden, R.A., Estein, S.M., Zygmunt, M.S., Dubray, G. and Cloeckaert, A. 2000. Identification of protective outer membrane antigens of *Brucella ovis* by passive immunization of mice with monoclonal antibodies. Microbes Infect. 2: 481-488.

Bowe, F., O'Gaora, P., Maskell, D., Cafferkey, M. and Dougan, G. 1989. Virulence, persistence, and immunogenicity of *Yersinia enterocolitica* O:8 aroA mutants. Infect Immun. 57: 3234-3236.

Boyce, J.D and Adler, B. 2001. Acapsular *Pasteurella multocida* B:2 can stimulate protective immunity against pasteurellosis. Infect and Immun.: 1943-1946.

Brogden, K.A ., Rhoades, R.A. and Rimler, R.B. 1982. Serologic types and physiologic characteristics of 46 avian Pasteurella anatipestifer cultures. Avian Dis. 26: 891-896.

Brock, T.D. and Madigan, M.T. 1988. Biology of Microorganism. (5th ed.). Prentice-Hall International Inc., New Jersey. pp: 72-77.

Brown, T.A. 1998. Gene cloning and introduction. (3rd ed.). Stanley Thornes (Publishers) Ltd. pp 3-12.

Carpenter, T.E., Snipes, K.P., Wallis, D. and. McCapes, R.H. 1988. Epidemiology and financial impact of fowl cholera in turkeys: A retrospective analysis. Avian Dis. 32:16-23.

Carpenter, T.E., Snipes K.P., Wallis, D. and McCapes, R.H. 1991. Epidemiology and financial impact of fowl cholera in turkeys: A retrospective analysis. Avian Dis. 32:16-23.

Carter, G.R. 1955. 1957, 1961. *Pasteurella.* In: Pathogenesis of bacterial infection in animals. Edited by Carlton. L. Gyles and Charles, O. Thoen. (1st Ed.). Iowa State University Press. 147-150.

Carter, G.R. 1955. Studies on *Pasteurella multocida* I. A haemagglutination test for the identification of serological type. Am. J. Vet. Res. 16: 481-484.

Carter, G.R. 1963. Contribute of Capsular Antigen of Pasteurella Vet. Rec. 75: 1264

Carter, G. R. 1967. Pasteurellosis- *Pasteurella multocida* and *Pasteurella haemolytica*. Adv. Vet. Sci.. 11: 321-379.

Carter, G.R. 1973. (2nd ed). Diagnostic procedure in Veterinary Microbiology Academic Press Limited, London. pp 61-65.

Cassataro, J., Velikovsky, C.A., Giambartolomei, G.H., Estein, S., Bruno, L., Cloeckaert, A., Bowden, R.A., Spitz, M. and Fossati, C.A. 2002. Immunogenicity of the *Brucella melitensis* recombinant ribosome recyling factor-homologous protein and its cDNA. Vaccine. 20: 1660-1669.

Chaudhuri, P. and Goswami P.P. 2001. Cloning of 87 kDa outer membrane protein gene of *Pasteurella multocida* P52. Res. Vet. Sci. 70: 255-256.

Chengappa, M.M., Carter, G.R and Bailie, W.E. 1986. Identification of Type D *Pasteurella multocida* by Counterimmunoelectrophoresis. J Clin Microbiol. 34: 721-723

Chevalier, G., Duclohier, H., Thomas, D., Shechter, E. and Wroblewski, H. 1993. Purification and characterization of protein H, the major porin of *Pasteurella multocida*. J. Bacteriol. 175: 266-276.

Chiang, W. L., Wilkie, I. A., Townsend, K. Mand Forst, A. J., 2000. The Demonstration of Pasteurella multocida in the Alimentary tract of chickens after experimental oral Infection. Vet. Microbiology 72: 47-55

Choi, Kim-K., Maheswaran, S.K., Felice, L.J. and Molitoi, T.W. 1991. Relationship between the iron regulated outer membrane proteins and the outer membrane proteins of *in vivo* grown *Pasteurella multocida.* Vet. Microbiol. 28:75-92.

Chung, J.Y., Wilkie, I., Boyce, J.D. and Adler, B. 2005. Vaccination against fowl cholera with acapsular *Pasteurella multocida* A:1. Vaccine. 23: 2751-2755.

Confer, A.W. 1993. Immunogens of pasteurella. Vet. Microbiol. 37: 353-368.

Cossart, P., Engleberg, N.C., Eisenstein, B.I and Medof, G. 2000. Cellular Microbiology. Washington D. C. 91-103.

Curtis, P.E., 1979. Observation on avian pasteurellosis in Britain. Vet. Rec. 104: 471-474.

Dabo, S.M., Confer, A., Montelongo, M., York, P., Wyckoff III, J.H. 2008. Vaccination with *Pasteurella multicida* recombinant OmpA induces strong but non-protective and deleterious Th2-type immune response in mice. Vaccine. 26: 4345-4351

Dalrymple, B. and Mattick, J.S. 1987. An analysis of the organization and evolution of type 4 fimbrial (MePhe) subunit proteins. J. Mol. Evol. 25: 261-269.

Davies, R.L., MacCorquodale, R. and Cafrey, B. 2003. Diversity of avian *Pasteurella multocida* strain base on capsular typing and variation of the OMP A and OMP H outer membrane protein. Vet. Microbiol., 91: 169-182.

Davies, R.L., MacCorquodale, R. and Reilly, S. 2004. Characterization of bovine with isolates of avian and procine origin. Vet. Microbiol. 99: 145-158.

Davis, G.D., Elisee, C., Newham, D.M. and Harrison, R.G. 1999. New fusion protein systems designed to give soluble expression in *Escherichia coli*. Biotecnol. Bioeng. 65: 382-388.

Davis, R.B. 1987. Cholera and broiler breeder. Poult. Dig: 430-434.

De Alwis, M.C.L. 1992. Haemorrhagic septicaemia. A general review. Br. Vet. J. 148: 108.

Dhanda, M.R. 1959. Immunization of cattle against haemorrhagic septicaemia with purified capsular antigens-A preliminary communication. The Indian Vet. J. 36: 6-8.

Dick, J.W. and Avakian, A.P. 1991. Response of broiler chickens to fowl cholera vaccination at 1 to 6 weeks of age. Avian Dis. 35: 761-766.

Doughty, S.W., Ruffolo, L.G. and Adler, B. 2000. The type 4 fimbrial subunit gene of *Pasteurella multocida*. Vet. Microbiol. 72: 79-90.

Dzarifah, Z. 2008. Development of an avirulent *Pasteurella multocida* B:2 by distruption of the ABA392 DNA fragment. Master's Thesis, Faculty of Veterinary Medicine, University., Schőherr, W., Heilmann, P., Kielstein, P., Műller, G., Feist, H. and Floss-mann, K.D. Putra Malaysia. pp 1-85.

Erler, W., Schőherr, W., Heilmann, P., Kielstein, P., Műller, G., Feist, H. and Floss-mann, K.D. 1983. Zur Imunogen wirkung von extrakten aus Pasteurella multocida im Kalb gegen die Patseurella infection. Mh. Vet. Med. 38: 87-92.

Fleiss, J.L., Levin, B. and Cho-Paik, M. 2003. 3rd ed. Statistical methods for rates and proportions. John Wiley & Sons, Inc. Hoboken, New Jersey. pp 855- 869

Gatto, N.T., Dabo, S.M., Hancook,R.E. and Confer, A.W. 2002. Characterization of, and immune responses of mice to, the purified OMPA- equivalent outer membrane protein of *Pasteurella multocida* serotype A:3 ( OMP 28). Vet. Microbiol. 87: 221-235).

Gautam, R., Kumar, R.R., Singh,V.P., Singh, V.P., Dutta, T.K and Shivachandra, S.B. 2004. Specific identfication of *Pasteurella multocida* serogroup-A isolates by PCR. Res. in Vet. Jour. 76: 179-185

Gilleland, L.B. and Gilleland, E. Jr. 1995. Synthetic peptide representing two protective, linear B-cell epitopes of outer membrane protein F of *Pseudomonas aeruginosa* elicit whole-cell reactive antibodies that are functionally pseudomonand specific. Infect. Immun. 63: 2347-2351.

Glisson, J.R., Contreras, M.D., Cheng, I.H.N and Wang, C. 1993. Cross protective studies with *Pasteurella multocida* bacterins prepared from bacteria propagated in iron-depleted medium. Avian Dis. 37: 1047-1079.

Glorioso, J.C., Jone, G.W., Rush, H.G., Rentler, C.J., Darif, C.A. and Coward, J.E. 1982. Adhesion of type A *Pasteurella multocida* to rabbit pharyngeal cells and its possible role in rabbit respiratory tract infection. Infect. Immun. 35: 1103-1109.

Glunder, G. and Hinz, K.H. 1989. Isolation of *Moraxella anatipestifer* from embryonated goose eggs. Avian Pathol. 18: 351-355.

Goswami, P.P., Chaudhuri, P., Tiwari,V., Parihar, N.S. and Harbola, P.C. 2004. Cloning and sequencing of a 16 kDa outer membrane protein gene of *Pasteurella multocida* P52. Vet. Res. Com. 28: 17-25.

Gray, H. 1913. In System of Veterinary Medicine (E.W.Hoare ed). Alexander Eger. Chicago. pp 420-423

Gray-Owen, S.D., Schryvers, A.B. 1996. Bacterial transferrin and lactoferrin receptors. Trends. Microbiol. 4: 185-91.

Gurunathan, S., Wu, C.Y., Freidag, B.L. and Seder, R.A. 2000. DNA vaccines: a key for inducing long-term cellular immunity. Curr. Opin. Immunol. 12: 442-447.

Hancock, R.E.W. 1987. Minireviews: Role of porins in outer membrane permeability. J. Bacteriol. 169: 929-933.

Hannig, G. and Makrides, S.C. 1998. Strategies for optimizing heterologous protein expresión in *Escherichia coli*. Trends Biotechnol. 16: 54-60.

Heckels, J.E. 1989. Structure and function of pili of pathogenic *Neisseria* species. Clin. Microbiol. Rev. 2: S66-S73.

Heddleston, K.L., Rhoades, K.R. and Rebers, P.A. 1972. Fowl cholera. Gel diffusion precipitation test for serotyping *Pasteurella multocida* from avian species. Avian Dis. 16: 925-936.

Hodgson, J.C., Finucane, A., Dagleish, M.P., Ataei, S., Parton, R. and Coote, J.G. 2005. Efficacy of vaccination of calves against haemorrhagic septicaemia with aroA derivative of *Pasteurella multocida* B:2 by two different routes of administration. Infect. Immun. 73: 1475-1481.

Hofacre**,** C.L**.** 1986. A serotypic survey of *Pasteurella multocida* isolated frompoultry. Avian Dis. 30:632–633.

Hofacre, C.L., Glisson , J.R. and Kleven, S.H. 1987. Comparison of vaccination protocols of broiler breeder hens for *Pasteurella multocida* utilizing enzyme-linked immunosorbent assay and virulent challenge. Avian Dis. 31: 260-263.

Hofacre, C.L., Glisson, J.R. and Kleven, S.H. 1989a. Evaluation of *Pasteurella multocida* mutants of low virulence. II. Immunologic response of turkeys. Avian Dis. 33: 275-278.

Hofacre, C.L., Glisson, J.R., Kleven, S.H., Brown, J. and Rowland, G.N. 1989b. Evaluation of *Pasteurella multocida* mutants of low virulence. 1. Development and pathogenicity. Avian Dis. 33: 270-274.

Hofstad, M.S., Calneck, B.W., Helmboldst, C.F., Reid, W.M. and Yorder, Jr, H.W. 1982. ($8^{th}$ ed.) Diseases of Poultry. The Iowa State University Press. Ames, Iowa. pp: 181-203.

Hoiseth, S.K**.** and Stocker, B.A. 1981. Aromatic-dependent Salmonella typhimurium are non-virulent and effective as live vaccine. Nature: 238-239.

Homchampa, P., Strugnell, R.A. and Adler, B. 1992. Molecular analysis of the aroA gene of *Pasteurella multocida* and vaccine potential of a constructed aroA mutant. Mol Microbiol. 6: 3582-3593.

Homchampa, P., Strugnell, R.A. and Adler, B. 1997. Cross protective immunity conferred by a marker-free aroA mutant of *Pasteurella multocida* Vaccine. 15: 203-208.

Hooley, P. and Burns, A.T.H. 1993. Strategies in the engineering of *Escherichia coli*. In strategies for engineering organisms, ed. Open Universities and University of Greenwich

Hungerford, T.G. 1968. A Clinical note on avian cholera. The effect of age on the susceptibility of fowls. Austr. Vet. J., 44: 32

Ibrahim, R.S., Sawada, T., Shahata, M., Ibrahim, A. 2000. Cross-protection and antigen expression by chicken embryo-grown *Pasteurella multocida* in chickens. J. Comp. Pathol. 123: 278-284.

Ikeda, J.S. and Hirsh, D.C. 1988. Antigenically related iron-regulated outer membrane proteins produced by different somatic serotypes of *Pasteurella multocida*. Inf. Immun. 56: 2499-2502.

Inouye, M. 1979. Bacterial outer membranes: Biogenesis and function. New York: John Wiley and Son Inc. pp 25-32

Isibasi, A., Ortiz-Navarrate, V., Paniagua, J., Pelayo, R., Gonzalez, C.R., Garcia, J.A. and Kumate, J. 1992. Active protection of mice against *Salmonella typhi* by immunization with strain-specific porins. Vaccine. 10: 811-813.

Issacson, R.E. and Trigo, E. 1995. Pili of *Pasteurella multocida* of porcine origin. FEMS Microbiol. Lett. 132: 247-251.

Jain, A., Roy, A., Rank, D. N., Joshi, C.G. and Purohit, J.H. 2005. Indian J. compo Microbiol. Immunol. Infect. Dis. 26: 63-65.

Jawetz, E., Melnick , J, L. and Aldelberg, E.A. 1987. (17th ed.) Review o Medical Microbiology Appleton and Lange, Connecticut. pp: 14-20.

Jenteur, D., Lackey, J.H. and Pattus, F. 1991. The bacterial porin superfamily sequence alignment and structure prediction, Mol. Microbiol. 5: 2153-2164

Jin Kim, Kyung-Jin Kim,[1] Han-Eol Kim, Kwang-Hoon Lee, Wondeok Han, Min-Ju Yi, Jinseok Jeong, and Byung-Ha Oh. 2004. Two-promoter vector is highly efficient for overproduction of protein complexes. Prot. Sci. 13: 1698-1703

Jonasson, P., Liljeqvist, S., Nygren, P.A. and Stahl, S. 2002. Genetic design for facilitated production and recovery of recombinant proteins in *Escherichia coli*. Biotechnol. Appl. Biochem. 35: 91-105.

Kardos, G., Nagy, J., Antal, M., Bistyak, A., Tenk, M. and Kiss, I. 2006. Development of a Novel PCR assay specific for *Riemerella anatipestifer*. J. App. Microbiol. 44: 145-148.

Kasten, R.W., Hansen, L.M., Hinojoza, J., Bieber, D., Ruehl, W.W. and Hirsh, D.C. 1995. *Pasteurella multocida* produces a protein with homology to the P6 outer membrane protein of *Haemophilus influenzae*. Infect. Immun. 63: 989-993

Kedrack, A. and Opacka, B. 2003. Immunological response to outer membrane proteins of *Pasteurella multocida* serotype A:3 in calves. Bull. Vet. Inst. Pulawy. 47: 387-394

Kennett, L., Muniandy, N. and Mukur, T.K.S. 1993. Comparative protective potential of nonliving intact cells and purified outer membrane and associated protein of *Pasteurella multocida* type 6:B grown under iron-regulated conditions. In: B. E. Pattern *et al*., (eds), Pasteurellosis in production animals, (ACIAR Press, Canberra). pp: 144-149.

Laemmli, U.K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature. 277: 680-685.

Lee, R.W.H., Strommer, J., Hodgins, D., Shewen, P.E., Niu, Y. and Lo, R.Y.C. 2001. Towards development of edible vaccine against bovine pneumonic pasteurelllosis using transgenic white clover expressing a *Mannheimia haemolytica* A1 leukotoxin 50 fusion protein. Infect. Immun. 69: 5786-5793.

Lee, J., Kim, Y.B. and Kwon, M. 2007. Outer membrane protein H for protective immunity against *Pasteurella multocid*a. The J of Microbiol., 45: 179-184.

Levine, M.M., Giron, J. And Noriega, F. 1994. Fimbrial Vaccine. In Fimbriae, Adhesion, Genetics, Biogenesis, and Vaccine. pp: 271-286. Edited by Klemm. P. Boca Raton, FL: CRC Press.

Levinson, W. E. and Jawetz, E. 1994. Medical microbiology and immunology: Examination and board review. Pp. 5-8. (3rd Ed.). Prentice- Hall International Inc. New Jersey. pp 73-75

Lin, J., Huang, S. and Zhang, Q. 2002. Outer membrane protein key player for bacterial adaptation in host niches. Microb. Infect 4: 325-331

Lu, Y.S., Aguila, H.N., Lai, W.C. and Pakes, S.P. 1991a. Antibodies to outer membrane proteins but not to lipopolysaccharides inhibit pulmonary proliferation of A *Pasteurella multocida* in mice. Infect. Immun. 59: 1470-1475.

Lu, Y.S., Lai W.C., Pakes, S.P. and Stefance, C. 1991b. A monoclonal antibody against a *Pasteurella multocida* outer membrane protein protects rabbits and mice against pasteurellosis. Infect. Immun. 59: 172-180.

Lűbke, A., Hartmann, L., Schröder, W. and Hellmann, E. 1994. Isolation and partial characterization of the major protein of the outer membrane of *Pasteurella haemolytica* and *Pasteurella multocida.* Zentralbl. Bakteriol. 281: 45-54.

Luderitz, O., Fredenberg, A., Galanos, C., Lehmann, V., Rietschel, E. and Shaw, D.H. 1982. LPS of gram negative bacteria. In: S. Razin and S. Rottem (Editors), Current Topics in Membrane and Transport, Volume 17, Membrane Lipids of Prokaryotes. Academic Press, New York. pp: 79-151.

Lugtenberg, B., Van Boxtel, R., Evenberg, D., De Jong, M., Storm, P. and Frik, J. 1986. Biochemical and immunological characterization of cell surface proteins of *Pasteurella multocida*. Infect. Immun. 52: 175-182.

Luo, Y., Zeng, Q., Glisson, J.R., Jackwood, M.W., Cheng, I.H.N. and Wang, C.L. 1999. Sequence analysis of *Pasteurella multocida* major outer membrane protein (OmpH) and application of synthetic peptides in Vaccination of chickens against homologous strain challenge. Vaccine. 17: 821-831.

Mannings, P.J.S., Haasz, M.A., Pelong, D. and Leary, S.L. 1986. Pasteurellosis in laboratory rabbits: Characterizations of LPS of *Pasteurella multocida* by polyacrylamide gel electrophoresis immunoblot technique and enzyme-link immunosorbant assay. Infect. Immun. 53: 460-463.

Manoha F., Chevaliar, G., Wroblewski, H. and Delamarche, C. 1993. Cloning and expression of two *Pasteurella multocida* genes in *Escherichia coli*. Biochemie 76: 9-14.

Mariana, S. and Hirst, R. 2000. The immunogenicity and pathogenicity of Pasteurella multocida isolated from poultry in Indonesia. Vet. Microbiol. 72: 27-36.

Merchant, I.A. and Packer, R.A. 1967. Veterinary Bacteriology and Virology. (7th ed.) Iowa State University Press. Ames, Iowa. pp 413-435

Moon, H.W. and Bunn, T.O. 1993. Vaccine for preventing enterotoxigenic *Escherichia coli* infections in farm animals. Vaccine. 11: 213-219.

Moore, L.J. and Rutter, J.M. 1989. Attachment of *Moraxella bovis* to calf corneal cells and inhibition by antiserum. Aust. Vet. J. 66: 39-42.

Mosier, P.A. 1992. Prevention and control of pasteurellosis. In: Pasteurellosis in production animals. ACIAR proceeding no. 43. pp. 121-134. International Workshop in pasteurellosis in production animals hold in Bali, Indonesia. 10-13 August. Workshop and Abstract.

Mukkur, T.K.S., Pyliotis, N.A. and Banes, A. 1982. Possible immunological synergism among protective antigens of *Pasteurella multocida* type A. J. Comp. pathol. 92: 249-260.

Muniandy, N. 1993. Immunochemical studies on protective antigens of *Pasteurella multocida* type 6:B. Doctor of Philosophy Thesis, The University of Sydney, Australia.

Nagy, L.K. and Penn, S.W. 1976. Protection of cattle against experimental haemorrhagic septicaemia by the capsular antigens of *Pasteurella multocida* types B and E. *Res. Vet. Sc*. 20: 249-253.

Namioka, M. and Murata, S. 1961a. Serological studies on *Pasteurella multocida* I: A simplified methods for capsular typing of the organisms. Cornell Vet. 51: 498-507.

Namioka, M. and Murata, S. 1961b. Serological studies on *Pasteurella multocida* II: Characteristics of the somatic "O" antigen of the organism. Cornell Vet. 51: 507-521.

Partadiredja, J.A., Pasaribu, H.F. and Utami, C.S. 1979. Kejadian fowl cholera pada sebuah peternakan itik di daerah Bogor. Media Vet.

Pati, U.S., Srivastava, S. K., Roy, S.C. and More, T., 1996. Immunogenicity of outer membrane protein of *Pasteurella multocida* in buffalo calves. Veterinary Microbiology, 52: 301-311.

Penn, C.W. and Nagy, L.K. 1974. Capsular and somatic antigens of *Pasteurella multocida* types B and E. Res. Vet. Sc. 16: 251-259.

Pearay, L.O., Faden, H. and Welliver, R.C. 2001. Vaccination strategies for mucosal immune responses. Clin. Microbiol. Rev. pp. 430-445.

Peter, E.J., Martha, J. and Carolyn, J.H. 1996. Animal medium for Growh of *Pasteurella multocida*. FEMS Microbiol. Lett.. 140: 165-169.

Potter, A.A., Schryvers, A. B., Ogunariwo, J.A., Lo, R.Y.C. and Watts, T. 1999. Protective capacity of *Pasteurella haemolytica* transferrin-binding proteins TbpA and TbpB in cattle. Microbial Pathogenesis, 27: 197-206.

Prokop, A., Bajpai, R.K. and Ho, C. 1991. Recombinant DNA Technology and Applications. New Cork: McGrill-Hill, Inc. pp 27-32

Purnomo, S. 1980. Case pasteurellosis in ducks. Buletin Investigation report. BPPH19: 42-56.

Rebers, P.A. and Heddleston, K.L. 1974. Immunologic comparison of westphal type lipopolysaccharide and free endotoxin from an encapsulated and a nonencapsulated avian strain of *Pasteurella multocida*. Am. J. Vet. Res. 35: 555-560.

Rebers, P.A. and Heddleston, K.A. 1977. Fowl cholera. Induction of cross protection in turkey with bacterins prepared from host passaged *Pasteurella multocida*. Avian Dis 21: 50-56

Rimler, R.B. and Phillips, M. 1984. Lipopolysaccharides of the Heddleston serotypes of *Pasteurella multocida.* Am. J. Vet. Res. 45: 759-63.

Rimler, R.B., Rebers, P.A. and Rhoades, K.R. 1979. Fowl cholera: cross-protection induced by *Pasteurella multocida* separated from infected turkey blood. 23: 730-741.

Rimler, R.B. and Rhoades, K.R. 1989. *Pasteurella multocida*. In Adlam, C. and J. M Rutter (Eds.). Pasteurella and Pasteurellosis. London: Academic Press. pp. 37-73.

Robert, R.S. 1947. An immunological study of *Pasteurella septica*. J. Comp. Pathol. 57: 261-278

Rocke, T.E., Smith, S.R., Myamoto, A. and Shadduck, D.J. 2002. A Serotype specific polymerase chain reaction for identification of *Pasteurella multocida* serotype A:1. Avian Dis. 46: 370-377.

Rossi-Campos, A., Anderson, C., Gerlach, G.F., Klashinisky, S., Potter, A.A. and Wilson, P.J. 1992. Immunization of pigs against *Actinobacillus pleuropneumoniae* with two recombinant protein preparations. Vaccine. 10: 512-518.

Ruffolo, C.G., Jost, B.H. and Adler, B. 1996. Cloning, sequencing, expression and protective capacity of oma87 gene encoding the *Pasteurella multocida* 87 kilodalton outer membrane antigen. Infect. Immun. pp 3161-3167.

Ruffolo, C.G., Jost, B.H. and Adler, B. 1998. Iron-regulated outer membrane proteins of *Pasteurella multocida* and their role in immunity. Vet. Microbiol. 59: 123-137.

Ruffolo, C.G., Tennet, J.M., Michalski, W.P., Adler, B. 1997. Identification, purification, and characterization of the type 4 fimbriae of *Pasteurella multocida*. Infect.Immun. 65: 339-343.

Saharee, A.A., Salim, N.B., Rasedee, A. and Jainudeen, M.R. 1993. Haemorrhagic septicaemia carries among cattle and buffalo in Malaysia. Pasteurellosis in production animals. ACIAR Proceedings no. 43, 89-91.

Salton, M.R.J. 1963. The relationship between the nature of the cell wall and the Gram stain. J. Gen. Microbiol. 30: 223-235.

Schmerr, M.J. and Rebers, P.A. 1979. Studies of immunogenicity of westphal lipopolysaccharides from *Pasteurella multocida* in mice. J. Comp. Pathol. 89

Schwab, H. 1993. Principles of genetic engineering for *Escherichia coli*. In: genetics engineering of microorgsnisms, ed. Puhler, A. VCH. Verlagsgesellschaft. pp 73-82

Shivachandra, S.B., Kumar, A.A., Gautam, R., Singh, V.P., Saxena, M.K. and Srivastava, S.K. 2006. Identication of Avian strains of *Pasteurella multocida* in India by conventional and PCR assays. The Vet. Journ.172: 561-564

Snipes, K.P., Hansen, L.M. and Hirsh, D.C. 1988. Plasma and iron-regulated expression of high molecular weight outer membrane proteins by *Pasteurella multocida*. Am. J. Vet. Res. 49: 1336-1338.

Solano, W., Giambrone, J.J. and Panangala, V.S. 1983. Comparison enzyme-linked immunoabsorbent assay and indirect hemagglutinin test for quantitating antibody response in chickens against *Pasteurella multocida*. Avian Dis. 27: 1034-1043.

Sokol, P.A. and Woods, D.E. 1986. Characterization of antibody to the ferripyochelin-binding protein of *Pseudomonas aeruginosa*. Infect. Immun. 51: 896-900.

Sørenson, H.P., Laursen, B.S., Mortenson, K.K. and Sperling Petersen, H.U. 2002. Bacterial translation initiation-mechanism and regulation. Recent Res. Dev. Biophys. Biochem. 2: 243-270.

Sørenson, H.P. and Mortenson, K.K. 2005. Advanced genetic strategies for recombinant protein expression in *Escherichia coli*. J. Biotechnol. 115: 113-128.

Sørenson, H.P., Sperling-Petersen, H.U. and Mortensen, K.K. 2003. Dialysis strategies for protein refolding. Preparative streptavidin production. Protein Expr. Purif. 32: 252-259.

Srivastava, S.K. 1998. Immunogenicity of *Pasteurella multocida* grown in iron restricted medium. J of Appl Animal Res. 136: 137-144.

Sthitmatee, N., Numee, S., Kawanmoto, E., Sasaki, H., Yamashita, K., Takahashi, N., Kataoka, Y. and Sawada, T. 2008. Protection of chickens from fowl cholera by vaccination with recombinant adhesive protein of *Pasteurella multocida*. Vaccine. 26: 2398-2407.

Strom, M.S., and Lory, S. 1993. Structure-function and biogenesis of the type IV pili. Annu. Rev. Microbiol. 47: 565-595.

Tabatabaei, M., Liu, Z., Finucane, A., Parton, R. and Coote, J. 2002. Protective immunity conferred by attenuated aroA derivatives of *Pasteurella multocida* B:2 strains in a mouse model of haemorrhagic septicaemia. Infect. Immun. 70: 3355-3362.

Tabatabai, L.B. and Zehr, E.S. 2004. Identification of five outer membrane-associated proteins among cross-protective factor proteins of *Pasteurella multocida*. Infect. Immun. 72: 1195-1198.

Tennet, J. M., and Mattick, J. S. 1994. Type 4 fimbriae. pp. 127-146. In P. Klemm (ed.), Fimbriae: adhesion, genetics, biogenesis, and vaccines. CRC Press Inc., Boca Raton, Fla.

Teo, T.P., Tan, H.C. and Loh, H. 1992. Protective efficacy of bivalent *Pasteurella anatipestifer* broth grown bacterin in ducklings. Singaps. J. Prim.Ind. 20: 53-60.

Tizard, I. 1992. (4th ed.) Veterinary Immunology. W. B. Saunders. USA. pp: 121-125.

Tomer, P., Chaturvedi, G.C, Minakshi Malik, P. and Monga, D.P. 2002. Comparative analysis of the outer membrane protein profiles of isolates of the *Pasteurella multocida* B:2 associated with haemorrhagic septicaemia. Vet. Res. Commun. 26: 513-522.

Towbin H**.,** Stachilin, T. and Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets procedure and applications. Proc. Nati. Acad. Sci. USA. 76: 4350-4354

Townsend, K.M., Boyce J.D., Chung, J.Y., Frost, A.J. and Adler, B. 2001. Genetic Organization of *Pasteurella multocida* cap loci and development of multiplex capsular PCR typing system. J. Clin. Microbiol. 39: 924-929.

Towsend, K.M., Frost, A.J., Lee, C.W., Papaditmitrious, J.M. and Dawkins, H.J. 1998. Development of PCR assay for species and type specific identification of *Pasteurella multocida* isolates. J. Clin. Microbiol. 36: 1096-1100.

Truscott, W.M. and Hirsh, D.C. 1988. Demonstration of an outer membrane protein with antiphagocytic activity from *Pasteurella multocida* of avian origin. Infect. Immun. 56: 1538-1544.

Veken, J.W., Oudega, B., Luirink, J. and de Graaf, F.K. 1994. Binding of bovine transferrin by *Pasteurella multocida* serotype B: 2, 5 a strain which causes haemorrhagic septicaemia in buffalo and cattle. FEMS Microbiol. Lett. 115: 253-257.

Ventura, S. and Villaverde, A. 2006. Protein quality in bacterial inclusion bodies. Trends Biotechnol. 24: 179-184.

Versalovic, J., Koeuth, T. and Lupski, J.R. 1991. Distribution ofrepetitive DNA sequences in eubacteria and application of fingerprinting of bacterial genomes. Nucleic Acids Research, 19: 6823 - 6831.

Virji, M., Saunders, J.R., Sims, G., Makepeace, K., Maskell, D. and Ferguson, D.P.J. 1993. Pilus-facilitated adherence of *Neisseria meningitidis* to human epithelial and endothelial cells: modulation of adherence phenotype occurs concurrently with changes in primary amino acid sequence and the glycosylation status of pilin. Mol. Microbiol. 10: 1013-1028.

Watts, T.H., Sastry, P.A., Hodges, R.S. and Paranchych, W. 1983. Mapping of the antigenic determinants of Pseudomonas aeruginosa PAK polar pili. Infect. Immun. 42: 113-121.

Zamirah, Z.A. 2002. Purification and characterization of the 33 kilodalton outer membrane protein of *Pasteurella multocida* type 6:B. Master's Thesis. Universiti Putra Malaysia. pp 1-105.

Zamri-Saad, M. 2005. Attempts to develop vaccines against Haemorrhagic septicaemia: A review. In Regional Symposium on Haemorrhagic Septicaemia, 1st – 2nd September. 2005. Palm Garden Hotel, Putrajaya. pp 1-8.

Zhang, H., Ainsworth, A. J. and Montgomery, R. D. 1994. Use of a 35.5 kDa cell membrane composition of *Pasteurella multocida* and an anti-idiotype antibody to induce protective immunity in Leghorn chickens. Vet. Immunol. Immunopathol. 41: 89-100.

Zhao, G., Pijoan, C., Choi, K., Maheswaran S. K, and Trigo, E. 1995. Expression of iron-regulated outer membrane proteins by porcine strains of *Pasteurella multocida*. Can. J. Vet. Res. 59: 46–50.