Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

SHORT COMMUNICATION

Optimization of Quality Control Factors for Indigenous *Mycoplasma Gallisepticum* Bacterin Preparation and Their Impact on Immunoprophylaxis in Broilers

Asim Raza[1], Arfan Ahmad[1], Masood Rabbani[1], Altaf Mahmood[2], Muhammad Younus[3], Zakir Ali[2] and Abdul Ahad[4],*

[1]Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan; [2]Livestock and Dairy Development Department, Govt. of Punjab, Pakistan; [3]Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan; [4]Department of Microbiology and Veterinary Public Health, Chittagong Veterinary and Animal Sciences University Chittagong, Khulshi–4225, Bangladesh
*Corresponding author:ahadvet1969@yahoo.co.uk

ARTICLE HISTORY (14-535)

Received: October 20, 2014
Revised: August 18, 2015
Accepted: September 01, 2015
Published online: December 31, 2015

Key words:
Adjuvants
Bacterins
Broilers
Inactivants
Mycoplasma gallisepticum (MG)

ABSTRACT

To inactivate locally isolated *Mycoplasma gallisepticum* (MG), phenol, formaldehyde and binary ethylenimine were optimized for their concentrations and exposure time. Their effect on immunoprophylaxis induction was then assessed by using different levels of immunogen/PCV and different adjuvants. 0.10M, 0.125% and 0.6% concentrations of binary ethylenimine, formaldehyde and phenol inactivated MG colonies within 24, 8 and 4 hours respectively. Formaldehyde inactivated vaccine with 1.5% Immunogen/PCV and montanide oil as adjuvant induced significantly ($P<0.05$) higher anti MG antibody titer than other locally prepared vaccines. Conclusion is that montanide oil adjuvated indigenous formalized bacterin can induce desired immune response.

To Cite This Article: Raza A, Ahmad A, Rabbani M, Mahmood A, Younus M, Ali Z and Ahad A, 2016. Optimization of quality control factors for indigenous *Mycoplasma gallisepticum* bacterin preparation and their impact on immunoprophylaxis in broilers. Pak Vet J, 36(2): 227-229.

INTRODUCTION

Mycoplasma gallisepticum (MG) is cause of chronic respiratory disease. Young birds are more susceptible than adults. The portals of entry of the pathogen are inhalation and conjunctiva whereas those of exit are respiratory tract, eggs and semen. How the organism overcomes bird's natural defense mechanism is still not clearly understood (Bradbury, 2001; Ahmad *et al.*, 2008). The pathogen is transmitted horizontally through contaminated dust, droplets and feathers and vertically through contaminated egg yolk. Economic losses occur due to high morbidity, reduced growth rate, poor feed conversion ratio, decreased egg production and poor hatchability (Sarkar *et al.*, 2005; Noel *et al*., 2012). Live and inactivated imported vaccines are mostly used in northern areas of Pakistan but the disease is not uncommon even in vaccinated flocks which may be due to subtle antigenic variation in immunogen of the vaccine. This situation necessitated the development of indigenous vaccine through present experiment in order to achieve desired immunoprophylaxis.

MATERIALS AND METHODS

Local MG isolate was obtained from University Diagnostic Lab. Different concentrations of inactivants including phenol (0.6, 0.30 & 0.125%); formaldehyde (0.50, 0.25 & 0.125%) and binary ethylenimine (0.05, 0.10 & 0.15M) were tested to determine the optimum concentration and exposure time for inactivation of MG culture. These mentioned concentrations were added in separate tubes containing viable MG isolates following the procedures of OIE (2015). Tubes were then incubated at room temperature. After exposure of 04, 08, 12, 24 and 48 hours, the viability of MG isolate was tested by culturing the MG containing broth on PPLO agar leading to incubation and observation under stereoscope microscope. Frey's agar plates were observed 10 days post streaking. The concentration of each inactivant with exposure time was recorded.

Three optimum concentrations (one for each inactivant) with least exposure time were selected and nine types of vaccines were prepared using 1% PCV with montanide oil as adjuvant, i.e., F-Vac: Formaldehyde inactivated; P-Vac: Phenol inactivated; B-Vac: Binary ethylenimine inactivated; Using formaldehyde and montanide oil (4:1 ratio) with different PCV concentrations adjusted according to procedure of Ghany (2011); PCV1-Vac: 0.5% PCV; PCV2-Vac: 1% PCV; PCV3-Vac: 1.5% PCV; Using formaldehyde as inactivant with 1% PCV but different adjuvants M-Vac: Montanide oil as adjuvant; A-Vac: Aluminium hydroxide gel; W-Vac: Water

Day old commercial broilers (n=110) were randomly divided into 11 groups with 10 birds in each group. Each of the bacterin was injected to respective group on 10th day of their age @ 0.5ml per bird intramuscularly. One group was administered with commercial/imported bacterin and kept as positive control whereas another one was maintained as non-vaccinated group. Blood samples from birds of each group were collected on 15th, 30th, 45th and 60th days post vaccination, sera were separated and stored at -20ºC until analysis. All sera were subjected to ProFLOK. MG ELISA kit (Synbiotics Corporation, USA) for titration of antibodies against MG as per manufacturer recommendations. Sample to positive ratio (SP) was calculated for each sample using the formula:

SP= Sample absorbance – Negative control absorbance / Positive control absorbance – Negative control absorbance.
Titers were calculated using the formula: Titer = Anti Log of Log10 titer
Log10 Titer = (1.464 × Log10 SP + 3.197)

A sample with SP ratio of <149 was considered as no titer, 149–743 as probable whereas SP ratio of ≥744 was taken as positive. Comparative potency of each vaccine was determined by comparing the serological titers by two way factorial ANOVA considering variation between vaccines and post vaccination days using SPSS version 17.

RESULTS

Various concentrations of phenol (0.6%), formaldehyde (0.125%) and binary ethylenimine (0.10M) inactivated MG colonies in 4, 8 and 24 hrs, respectively. These least concentrations of each inactivant with minimum exposure times were taken as optimum ones for further experimentation. Local formalized bacterin (F-Vac) induced significantly (P<0.05) increased antibody titer as compared to phenol (P-Vac) and binary ethylenimine (B-Vac) inactivated vaccines. Non-significant difference with respect to antibody titer was recorded between imported and local formalized bacterins from 30th to 60th days post vaccination (Table 1). The vaccine containing 1.5% level of bacterial mass/PCV (PCV-3 Vac) exhibited significantly (P<0.05) higher antibody titer as compared to all other vaccines (PCV-1 Vac, PCV-2 Vac, imported & control) from 15th to 60th days post vaccination (Table 2). Vaccine with montanide oil as adjuvant (M-Vac) induced significantly (P<0.05) increased antibody titer than other two locally prepared vaccines i.e. A-Vac and W-Vac from 15th to 60th days post vaccination. Non-significant difference was recorded between M-Vac and imported bacterin (Table 3).

DISCUSSION

Formaldehyde (0.5%) and phenol (0.6%) inactivated MG culture in 4 hours post exposure. Phenol causes inactivation by disruption of membranes, precipitation of proteins and inactivation of intracytoplasmic enzymes by forming unstable complexes. Previous reports state that formaldehyde concentration above 0.02% could inactivate MG colonies (Christian *et al*., 2004; Hassan *et al*., 2014). Formaldehyde acts on proteins by denaturation and alkylation of nucleic acids. For binary ethylenimine, which mainly acts on nucleic acid of microorganisms, results showed that 0.1 M and 0.15M concentrations of BEI inactivated MG colonies just after 24 hrs of inactivation without second time inactivation whereas; 0.05M concentration caused inactivation over 48 hours of second time inactivation. This is in accordance with the findings of Christian *et al*. (2004) who reported that various *Mycoplasma* species are inactivated by 0.1M concentration of BEI with 24 hrs of inactivation. Regarding effect of inactivant on the potency of bacterins, formaldehyde provoked maximum immune response compared to phenol and BEI throughout the trial period which is in accordance with the findings of Cryz *et al.* (1982) who recorded that immunoglobulin G titers to intact *V. cholerae* cells measured by ELISA were 100-fold higher in rabbits immunized with the formaldehyde-inactivated preparation as compared to the classical phenol-inactivated vaccine however divergent results were reported by Hussein *et al.* (2007) that binary ethylenimine inactivated vaccine titers peaked two weeks post vaccination and remained at the end while least immunizing effect was observed in the formaldehyde inactivated MG bacterins immunized chicken. The titers

Table 1: Response of broilers to oil based vaccine containing *Mycoplasma gallisepticum* inactivated by different chemicals

Post Vaccination Elisa Antibody Titer	Vaccine Type					Sig.
	F-Vac	P-Vac	B-Vac	Imported	Control	
0	1017±80.60	997±94.02	1010±80.30	1002±80.11	985±70.01	0.9 21
15	3067±290.80	2953±198.70	1300±100.50	975±135.40	848±142.50	0.00
30	3364±315.50	3073±187.50	1490±110.64	3175±270.45	16.06±3.40	0.00
45	2133±170.85	1799±140.60	585±55.80	1931±190.40	4.05±0.57	0.00
60	1432±112.50	967±92.60	213±25.70	1325±157.50	1±0.00	0.00

Values at P<0.05 are statistically significant; P-VAC: Phenol Inactivated vaccine; F-VAC: Formalized vaccine; B-VAC: Binary ethylenimine inactivated vaccine; IMPORTED: Imported vaccine of FORTE- DODGE; CONTROL: Non vaccinated group.

Table 2: Response of broilers to oil based killed vaccine containing different levels of *Mycoplasma gallisepticum* immunogen

Post Vaccination Elisa Antibody Titer	Vaccine Groups					Sig.
	PCV1-Vac	PCV2-Vac	PCV3-Vac	Imported	Control	
DAY 0	950±165.89	1017±80.60	1011±102.60	1002±80.11	985±70.01	0.631
DAY 15	2224±315.64	3067±290.80	3257±250.70	975±135.40	848±142.50	0.00
DAY 30	1615±155.75	3364±315.50	4993±375.60	3175±270.60	16.06±3.40	0.00
DAY 45	1169±155.60	2133±170.85	3080±260.65	1931±190.40	4.05±0.57	0.00
DAY 60	412±75.60	1432±112.50	2768±235.40	1325±157.50	1±0.00	0.00

Values at P<0.05 are statistically significant; PCV 1-VAC: Formalized vaccine having 0.5% packed cell volume; PCV 2-VAC: Formalized vaccine having 1% packed cell volume; PCV 3-VAC: Formalized vaccine having 1.5% packed cell volume; IMPORTED: FORTE- DODGE; CONTROL: Non vaccinated group.

Table 3: Effect of adjuvants on antibody response of broilers to killed *Mycoplasma gallisepticum* vaccine

Post Vaccination Elisa Antibody Titer	Vaccine Groups					Sig.
	M-Vac	A-Vac	W-Vac	Imported	Control	
DAY 0	1017±80.60	980±75.02	1011±85.90	1002±80.11	985±70.01	0.793
DAY 15	3067±290.80	1536±230.40	789±87.85	975±135.40	848±142.50	0.00
DAY 30	3364±315.50	1261±110.30	912±100.90	3175±270.45	16.06±3.40	0.00
DAY 45	2133±170.85	543±70.60	354±45.50	1931±190.40	4.05±0.57	0.00
DAY 60	1432±112.50	157±25.02	1±0.00	1325±157.50	1±0.00	0.00

Values at P<0.05 are statistically significant; M-VAC: Formalized vaccine having montanide oil as adjuvant; A -VAC: Formalized vaccine having aluminium hydroxide gel as adjuvant; W –VAC: Formalized vaccine having water as adjuvant; IMPORTED: FORTE – DODGE; CONTROL = Non vaccinated group.

induced by all vaccines were unusually dropped in short period of time which may be due to the reasons that this trial was conducted in summer months of the year when there was lot of humidity leading to high toxin level in the feed. MG bacterin containing 1.5% PCV provoked significantly (P<0.05) higher titer as compared to other locally prepared vaccines. It might be due to the reason that PCV3 VAC contained more number of bacterial colonies that provoked maximum titer by slow release and presentation on antigen presenting cells for long duration of time compared to other two vaccines containing less number of bacterial biomass. Oil based vaccine showed significantly (P<0.05) higher anit-MG antibody titer as compared to other bacterins which is in line with the observations of Barbour and Newman (2002) who recorded significant immunoglobulin (Ig) response specific to MG in sera of chickens collected 3 weeks after the first and second vaccination with MG adjuvanted with or oil-emulsion as compare to other adjuvants. It may be due to more antigen retention and slow degradation power of oil as compared to gel leading to maximum prolonged effect and slow decrease in titer.

Conclusions: Significantly (P<0.05) higher desired antibody titer induced by indigenous formalized bacterin revealed its prevailing impact for effective prevention.

Acknowledgements: We are grateful to Dr. Mazhar I Khan, Department of Pathobiology, University of Connecticut, USA and Dr. Khushi Muhammad. Department of Microbiology, UVAS, Lahore for designing the experiment; HEC for financial support and University diagnostic laboratory staff for help in lab work.

REFERENCES

Ahmad A, Rabbani M, Yaqub T, Ahmad A, Shabbir MZ *et al*., 2008. Status of IgG antibodies against *Mycoplasma gallisepticum* in non-vaccinated commercial poultry breeder flocks. J Anim Pl Sci, 18: 61-63.

Barbour Ek and Newman JA, 2002. Efficacy of Mycoplasma gallisepticum vaccines containing different adjuvants in laying hens. Vet Immunol Immunop, 26: 115-123.

Bradbury JM, 2001, Avian Mycoplasmosis. In: Poultry Diseases 5th ed. WB Saunders, USA,pp: 178-193.

Christian DLF, Assuncão P, Ramírez AS and Poveda JB, 2004. Inactivation of Mycoplasma species involved in contagious agalactia. Berl Munch Tierarztl Wochenschr, 117: 1-5.

Cryz JrSJ, Furer E and Germanier R, 1982. Effect of chemical and heat inactivation on the antigenicity and immunogenicity of Vibrio cholera. Infect Immun, 38: 21-26.

Ghany WAAE, 2011. Evaluation of autogenous Avibacterium paragallinarum bacterins in chickens. Int J Poult Sci, 10:56-61.

Hassan S, Mukhtar N, Rahman SU and Mian AA, 2014. Molecular epidemiology of *Mycoplasma gallisepticum* in different types of chickens. Int J Agric Biol, 16: 165-170.

Hussein Ael-D, EL-Shahib TM, Saou SM, Shalaby NA, Sultan H *et al*., 2007. Protective immune response of Mycoplasma gallisepticum vaccines in poultry. Egypt J Immunol, 14: 93-99.

Noel F, Laibinis VA, and Farrar M, 2012. Influence of swab material on the detection of Mycoplasma gallisepticum and Mycoplasma synoviae by real-time PCR. Avian Dis 56:310-314

OIE, 2015. OIE Terrestrial Manual: Avian Mycoplasmosis (Mycoplasma gallisepticum, M synoviae) Chapter 2.3.3, pp: 482-496.

Sarkar SK, Rahman MB, Rahman M, Amin KMR, Khan MFR, *et al*., 2005. Sero-Prevalence of Mycoplasma gallisepticum infection of chickens in model breeder poultry farms of Bangladesh. Int J Poult Sci, 4: 32-35.