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A Study on Bacterial Contamination of Poultry Vaccines

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ABSTRACT

A study was carried out to investigate the bacterial contamination in poultry vaccines. A total of 48 poultry vaccines, 18 local and 30 imported were examined for bacterial contamination. The vaccines were ND, ND+IB, IB, IBD, Hydro and A.I (local n=3 and imported n=5), respectively. Only 1 (2.08%) vaccine was found positive for bacterial contamination, while remaining 47 (97.92%) were exhibited no growth and recorded as negative for any sort of bacterial contamination. *Escherichia coli* was recognized from one local vaccine (5.56%) manufactured for Hydropercardium syndrome whereas imported vaccines were found free from bacterial contamination. Results of this study warrant immediate attention of the local manufactures to improve their vaccine quality. Such studies need to be continued on periodical basis. A number of biochemical and sugar fermentation tests were also carried out to confirm its species. *Escherichia coli* interacted with TSI agar and produced H_2S in the medium. The organism showed positive interaction with catalse, methyl red and nitrate, while negative for oxidase, indole, urease, Voges-Proskauer (VP), gelatin (GL) and citrate. The species fermented glucose, mannose, maltose, mannitol, xylose and inositol but did not ferment galactose, creatinin and dulcitol.

Key Words: Poultry; Vaccines; Contamination; Bacterial contaminants; *E. coli* in vaccine

INTRODUCTION

Vaccination is one of the more effective ways to prevent the poultry birds from the specific diseases. Disease producing microorganisms can be classified smallest to largest as viruses, bacteria, fungi, protozoa and parasites. All, except the viruses are sensitive to antibiotic, so drugs treatment against them is available when outbreaks occur. Vaccination is basically the introduction of a specific biological substance (antigen) into the bird to stimulate the formation of a resistance or immunity to a particular disease. Usually the biological substance is some of the live disease organisms, which are capable to protect the bird against the particular disease by producing an immune response. Presence of these organisms in the blood stimulates the body's defense mechanism to produce antibodies that attack the disease causing organisms when the bird is exposed to them (MSUES, 2006).

A danger of such type of live vaccines is that the live microbes can back mutate to a virulent form (MSUES, 2006). While, dead vaccines that contain whole killed (usually by formalin or phenol) microbes are safe. They may contain little or no extraneous material and therefore tend to produce fewer adverse effects (Tortora *et al.,* 2001). The vaccines that contain dead organisms are safe with respect to residual virulence and are easy to store, since organisms are already dead. While live vaccines may posses residual virulence not only for the animal for which vaccine is made but also for other animals by reversion of attenuated organisms into a fully virulent type or spread to non-vaccinated animals. Dead vaccines have very little risk of 'alive' contamination, while live vaccines always run the risk of contamination with unwanted organisms; for instance, out breaks of reticuloendotheliosis in chickens in Japan and Australia have been traced to contaminated Marek's disease vaccine (Tizard, 1995). Samad (2001) reported extraneous contaminants in different manufactured anthrax vaccines. He dected *Bacillus megaterium, B. cereus, B. mycoids* and *B. subtilis* from anthrax live-spore vaccines through the cultivation of vaccine batches on Brain Heart Infusion Agar (BHIA).

The present study thus was carried out to examine the bacterial contamination in poultry vaccines manufactured locally as well as being imported from different countries of the world. The findings of the study will not only be helpful in developing awareness regarding bacterial contamination of the vaccines and the causal organisms of such contamination among the poultry farmers, but they will also be more conscious for quality of the vaccines they use for vaccination their poultry birds.

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MATERIALS AND METHODS

Collection of vaccine samples. Forty eight samples of poultry vaccines (18 local & 30 imported) were collected from the market and vaccine production centers of the country and brought to the laboratory of the Department of Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam and Vaccine Production Unit Tando Jam, in the thermo flask with ice and then stored in the refrigerator at 4°C.

Isolation and identification. Vaccine samples were inoculated by streaking method on blood, nutrient, BHI (brain heart infusion) and MacConkey's agar media and incubated aerobically and anaerobically at 37°C for 24 h. Following 24 h of incubation, colonies from nutrient agar were picked-up by sterilized wire-loop and sub-cultured on nutrient and MacConkey's agar plates. The process of sub-culturing continued until pure growths were obtained. Purity of the isolated bacterial strains was determined on the basis of their morphological and cultural characteristics by making the smear, stained with Gram's stain and examined under microscope. The organisms were isolated and identified by adopting the method of Khalil and Gabbar(1992). The species of the organisms were confirmed by checking their biochemical and sugar fermentation properties.

RESULTS AND DISCUSSION

A total of 48 examined samples of different poultry vaccines (both local & imported), only 1 (2.08%) was found positive for bacterial isolate, while remaining 47 (97.92%) exhibited no growth and were recorded as negative for any sort of bacterial contamination. Among the 48 samples, 18 vaccines were locally manufactured, while remaining 30 were imported. These vaccines were ND, ND+IB, IB, IBD, Hydro and A.I (Table I). The contaminated vaccine sample (Batch No: BA.01045) was a local vaccine, prepared for Hydropercardium Syndrome. Kamboh *et al.* (2007) reorded the 7.5% bacterial contamination in livestock vaccines, while 10.71% in local and 0% in imported livestock vaccines used in Sindh province of Pakistan. While, Yu *et al*. (2003) reported 8.69% contamination of pneumococcal surface protein A (PspA) in 23-valent polysaccharide pneumococcal vaccines. Similar prevalence of vaccine contamination was recorded by Bruschke *et al.* (2001) who tested the 82 batches of bovine herpesvirus vaccines for bovine virus diarrhea virus and found 7 (8.53%) batches positive and 75 (91.47%) without any extraneous contamination.

A somewhat higher prevalence of vaccine contamination was recorded by Giangaspero *et al*. (2001) who analysed 38 different human live virus vaccines for Pestivirus RNA. They found 5 (13.1%) samples positive and 33 (86.9%) were found to be negative for Pestivirus RNA. Similar prevalence was recorded by Sasaki *et al.* (1996), who recorded 28.0% contamination of Pestivirus RNA in live viral vaccines.

Table I. Prevalence of bacterial contaminants in the poultry vaccines

Vaccines	Local/ imported	Number of samples examined	Number of positive samples
ND	Local	3	0
(Newcastle Disease)	Imported	5	0
ND+IB	Local	3	0
(Newcastle+Infectious Bronchitis)	Imported	5	0
IB	Local	3	0
(Infectious Bronchitis)	Imported	5	0
IBD	Local	3	0
(Infectious Bursal Disease /Gumboro)	Imported	5	0
Hydro	Local	3	1
(Hydropericardium)	Imported	5	0
A-I	Local	3	0
(Avian Influenza)	Imported	5	0

Table II. Biochemical properties of *Escherichia coli* recognized from poultry vaccines

Biochemical tests	*Escherichia coli*
Catalase	+v
Oxidase	-ve
Coagulase	-
Indole	-ve
TSI	A/A
H2S	+v
Urease	-ve
M R	+v
VP	-ve
GL	-ve
Citrate	-ve
NR	+v
Aesculin	-

Where: -ve = negative - = not done +ve = positive
K/A= alkaline slant and acidic butt TSI = triple sugar iron.
A/A= acidic slant and acidic butt H2S = Hydrogen sulphide gas
MR = methyl red VP = Voges Proskauer
GL = gelatin liquefaction NR = Nitrate reduction

Table III. Sugar fermentation properties of bacterial species isolated from poultry vaccines

Name of sugar	*Escherichia coli*
Mannose	+ve
Xylose	+ve
Inositol	+ve
Galactose	-ve
Mannitol	+ve
Glucose	+ve
Maltose	+ve
Creatinin	-ve
Dulcitol	-ve

+ve = Positive -ve = Negative

The results clearly indicate that the imported poultry vaccines were free of any type of contamination and not a single imported product examined was observed to be positive for bacterial contamination, while locally manufactured poultry vaccines are still observed not completely free from bacterial contamination. Local poultry vaccines might be getting this contamination due to use of poor quality preservative, use of poor instruments and old

techniques, use of poor sterilized packing material, unhygienic condition at laboratory and poor management at laboratory especially in the culture room and unsound technical staff at vaccine production units/centers.

While examining the poultry vaccines for bacterial contamination, the *E. coli* was only bacterial species that recognized from a vaccine against Hydropericardium syndrome (Batch number: BA.01045). Kamboh (2007) reported *Escherichia coli, Pasteurella multocida, Bacillus cereus* and *B. subtilis* as extraneous contaminants in local livestock vaccine samples. While, Samad (2001) isolated *B. megaterium, B. cereus, B. mycoids* and *B. subtilis* from anthrax live-spore vaccines. Whereas, Kojima *et al*. (1997) reported the contamination of avian *Mycoplasma* DNA in the avian live virus vaccines. The specificity of the primers showed 34 strains belonging to nine species of avian *Mycoplasma,* from which *M. synoviae* and *M. gallisepticum* were predominant. Landman *et al*. (2000) examined Marek's disease vaccine and observed the pure contamination by *Enterococcus faecalis*.

During the species confirmation of isolated contaminant it was noted that *Escherichia coli* interacted positively with TSI agar and changed the medium in A/A (acidic slant & acidic butt) with production of H2S (Hydrogen sulphide gas). Further tests indicated that species was catalase positive and oxidase negative. It also interacted positively with methyl red and nitrate reduction but negatively to indole, urea, Voges-Proskauer (VP), gelatin (GL) and Citrate (Table II). The species produced acid by fermentation of glucose, mannose, maltose, mannitol, xylose and inositol but did not ferment galactose, creatinin and dulcitol (Table III). Khalial and Gabbar (1992); Nazamani (1999); Deverjani (2005); Dewani (2000) and Kamboh (2007) recorded similar biochemical and sugar fermentation properties of *Escherichia coli* as demonstrated in the present investigation.

CONCLUSION AND RECOMMENDATIONS

Some locally manufactured vaccines possess extraneous bacterial contaminants, while imported vaccines are found free from any bacterial contamination. Generally, 2.08% of bacterial contamination was found in poultry vaccines, while in local vaccines, this contamination was observed as 5.55%. *Escherichia coli* was recognized as a common contaminant of vaccines.

REFERENCES

Bruschke, C.J., H.A. Paal and K. Weerdmeester, 2001. Detection of bovine virus diarrhea virus in a live bovine herpes virus 1 marker vaccine. *Tijdschr Diergeneeskd.*, 126: 189–190

Devrajani, K., 2005. Bacteriological study on camel wounds. *M. Sc. (Hons.) Thesis*, Department of Microbiology, Sindh Agriculture, University, Tandojam

Dewani, P., 2000. Bacteriological studies on mastitis in ewes and goats. *M. Sc. (Hons) Thesis*, Department of Micro. Sindh Agriculture, University Tandojam

Giangaspero, M., G. Vacirca, R. Harasawa, M. Buttner, A. Panuccio, D. Giuli C. Morghen, A. Zanetti, A. Belloli and A. Verhulst, 2001. Genotypes of pestivirus RNA detected in live virus vaccines for human use. *Vet. Med. Sci*., 63: 723–733

Khalial, M.A. and A. Gabbar, 1992. *Procedures in Veterinary Microbiology*, *Field Document No. 6*, 2nd edition, p: 93. C.V.D.L. Tandojam, Sindh, Pakistan

Kamboh, A.A., R. Rind, A.H. Shah, J.A. Gandahi and N. Rajput, 2007. Bacteriological study on local and imported livestock vaccines used in Sindh, Pakistan. *J. Agric. Soc. Sci.,* 3: 125–128

Kojima, A., T. Takahashi, M. Kijima, Y. Ogikubo, M. Nishimura, S. Nishimura, R. Harasawa and Y. Tamura, 1997. Detection of Mycoplasma in avian live virus vaccines by polymerase chain reaction. *Biologicals,* 25: 365–371

Landman, W.J.M., K.T. Veldman, D.J. Mevius and P. Doornenbal, 2000. Contamination of Marek's disease vaccine suspensions with enterococcus faecalis and its possible role in amyloid arthropathy. *Avain Pathol.,* 29: 21–25

MSUES, 2006. *Poultry Health Basics*, pp: 1–5. Coordinated Access to the Research and Extension System, Mississippi Agril. and Forest Exp. Stat

Nizamani, A.W., 1999. Studies on the Bacterial flora of uteri of slaughter sheep. *M. Sc. (Hons) Thesis*, Department of Microbiology, Sindh Agriculture University, Tandojam

Samad, A., 2001. Use of antimicrobial susceptibility pattern as an identification marker of vaccinal and wild type strains of Bacillus anthracis in vaccine manufacturing process. *M. Sc. Thesis*, Department of Veterinary Microbiology, Sindh Agriculture University, Tandojam

Sasaki, T., R. Harasawa, M. Shintani, H. Fujiwara, Y. Sasaki, A. Horino, T. Kenri, K. Asada, I. Kato and F. Chino, 1996. Application of PCR for detection of mycoplasma DNA and pestivirus RNA in human live viral vaccines. *Biologicals*, 24: 371–375

Tizard, I.R., 1995. Immunoprophylaxis: General Principles of Vaccination and Vaccines. *In*: *An Introduction to Veterinary Immunology*, 4th edition, pp: 178–191. Philadelphia: W.B. Saunders

Tortora, J. Gerard, Berdell R. Funke and Christine L. Case, 2001. *Microbiology: An Introduction,* 7th edition, pp: 501–505. Benjamin Cummings, An imprint of Addison Wesley Longman, Inc. San Francisco

Yu, J., D. Briles, J. Englund, S. Hollingshead, W. Glezen and M. Nahm, 2003. Immunogenic protein contaminants in pneumococcal vaccines. *Infect Dis*., 187: 1019–1023

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