

Bacteriological Study on Local and Imported Livestock Vaccines Used in Sindh, Pakistan

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ABSTRACT

A total of 40 (28 local & 12 imported) livestock vaccines used in Sindh province of Pakistan were tested for bacterial contaminants. Four different bacterial species were identified from local vaccine samples. The species were *Escherichia coli*, *Pasteurella multocida*, *Bacillus cereus* and *B. subtilis*. Imported vaccines were found free from bacterial contaminants. One of the total eight local Haemorrhagic septicaemia (H.S.) vaccines examined was found contaminated with *E. coli* and *P. multocida*, whereas one of the two local anthrax vaccines examined was positive for *B. cereus* and the other with *B. subtilis*. None of the enterotoxaemia (n=4), black quarter (n=3), foot and mouth disease (3 local & 4 imported), contagious caprine pleuropneumonia (n=3) and rabies (5 local & 3 imported) vaccines were contaminated with bacteria. All the 12 imported vaccines (2 HS, 3 anthrax, 4 FMD & 3 rabies), were free of bacterial contamination. Results of this study warrant immediate attention of the manufactures to the improvement of quality production of vaccines. Such studies need to be continued on periodical basis.

Key Words: Livestock; Vaccines; Contamination

INTRODUCTION

Vaccines are the biological products designed to prevent infections of various kinds by promoting an immune response termed as “active immunity”. Typically vaccines work by enhancing the immune system of the body. This is done by using the vaccines, containing live or dead whole microorganisms or their microscopic parts (Levinson, 2000). This leads to the production of memory cells within the host, so that on a second encounter with the microbe the immune system can generate a rapid antibody response thereby preventing infection (Nicklin *et al.*, 1999).

A danger of such vaccines is that live microbes can back mutate to a virulent form, 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 possess 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, outbreaks 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 detected *Bacillus megaterium*, *B. cereus*, *B. mycoides* and *B. subtilis* from anthrax live-spore vaccines through the cultivation of vaccine batches on Brain Heart Infusion Agar (BHIA). Feeling the gravity of the situation, bacterial contamination of livestock vaccines, currently used in Sindh province of Pakistan was investigated.

MATERIALS AND METHODS

Collection of vaccine samples. Forty livestock vaccines (both live & killed) were collected from the market and vaccine production centres 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 blood, MacConkey’s and nutrient agars were picked-up by sterilized wire-loop and 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. This was done by making the smear, stained with Gram’s stain and

examined under microscope. The organisms were isolated and identified by adopting the method as prescribes by Khalial and Gabbar (1992). The species of the organisms were confirmed by checking their biochemical and sugar fermentation properties.

RESULTS AND DISCUSSION

The number and percentage prevalence of bacterial species as contaminants recognized from local and imported livestock vaccines are presented in Fig. 1. A total of 40 livestock vaccines were examined (Table I), from which 3 (7.5%) vaccines 1 Haemorrhagic Septicaemia (H.S) and 2 Anthrax possessing batch numbers 057, 079 and 010, respectively were found positive for various bacterial isolates, while 37 (92.5%) exhibited no growth and recorded as negative for any bacterial contaminants.

During the present investigation of livestock vaccines, total 28 local vaccine samples (8 HS, 4 ETV, 3 BQ, 2 anthrax, 3 FMD, 3 CCPP & 5 rabies), were examined, from which 3 were positive. The prevalence of bacterial contaminants in local vaccines was 10.71%. Among 28 local vaccine samples, 4 were live vaccines and 24 were dead vaccines from which 2 (50%) live Anthrax vaccines and 1 (4.71%) dead H.S vaccine was found positive. While, 12 samples of imported vaccines (2 HS, 3 anthrax, 4 FMD & 3 rabies), were examined, from which no vaccine were found positive for bacterial contaminants. Hence, the percentage prevalence of bacterial contaminants in imported vaccines was 0% (Fig. 2 & 3).

The percent prevalence of bacterial contaminants in live and dead livestock vaccines indicated that out of 40 examined samples of vaccines 13 were live; of which 2 Anthrax vaccines were found positive, while 27 were formaldehyde treated (dead) vaccines, of which 1 H.S vaccine was found positive for bacterial contaminants (Fig. 4). Therefore, the percentage prevalence of bacterial contaminants in live vaccines was 15.38%, while in dead vaccines was 3.70%. Furthermore, contamination in the dead vaccine clearly indicates that vaccine contains poor quality or less percentage of preservative than the recommended dose.

From the available literature, it is manifest that most of the workers have carried-out their investigation on vaccine samples by advance diagnostic techniques like, PCR, Pulsed-field electrophoresis, ELISA and IFA for the presence of surface proteins, antigens and nucleic acids. Most of the work has been done for the detection of viral genomes and viral antigens. Therefore, it is very difficult to compare our findings to other workers, regarding bacterial organisms in the local and imported livestock vaccines. Furthermore, irrespective of the number of samples examined and techniques used by them, the results of the present investigation of vaccine contamination are very much close to the findings of Yu *et al.* (2003) who tested the 23-valent polysaccharide pneumococcal vaccines for

Table I. Prevalence of bacterial contaminants in livestock vaccines

Sr#	Vaccines	Local/imported	Number of samples examined	Number of positive samples
1.	H.S. (Hemorrhagic septicemia)	Local	08	01
		Imported	02	00
2.	E.T.V. (Enterotoxaemia Vaccine)	Local	04	00
		Imported	00	0
3.	B.Q. (Black quarter)	Local	03	00
		Imported	00	00
4.	Anthrax	Local	02	02
		Imported	03	00
5.	FMD (Foot and mouth disease)	Local	03	00
		Imported	04	00
6.	C.C.P.P. (Contagious Caprine Pleuropneumonia)	Local	03	00
		Imported	00	00
7.	Rabies	Local	05	00
		Imported	03	00

Fig. 1. Number and percentage prevalence of bacterial contaminants in livestock vaccines

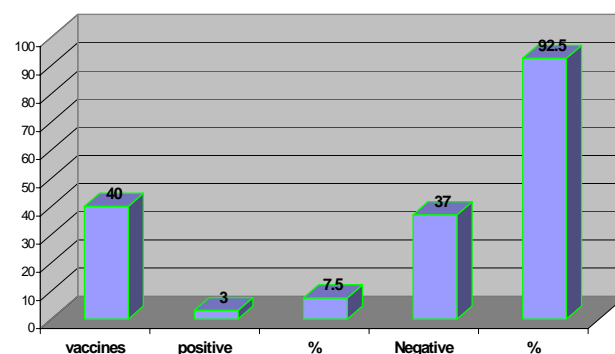
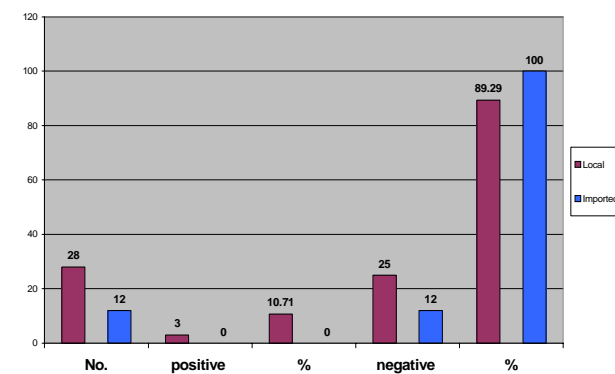


Fig. 2. Number and percentage prevalence of bacterial contaminants in local and imported livestock vaccines



contamination by pneumococcal surface protein A (PspA) and pneumococcal surface adhesin A (PsaA). The PspA was detected in 2 (8.69%) vaccines, while 21 (93.31%) vaccine samples were found negative. Similar prevalence of vaccine contamination was recorded by Brusckhe *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.

Fig. 3. Number and percentage prevalence of bacterial contaminants live and dead livestock vaccines

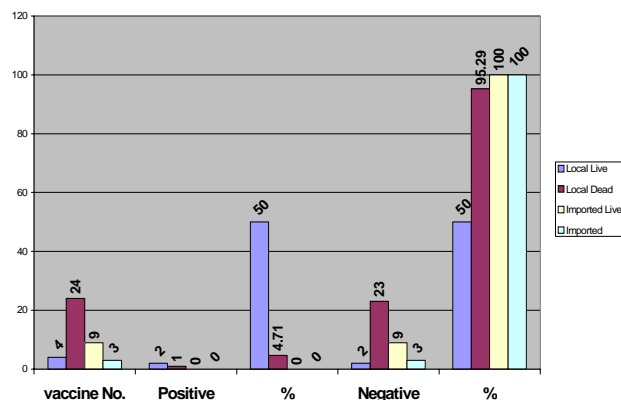
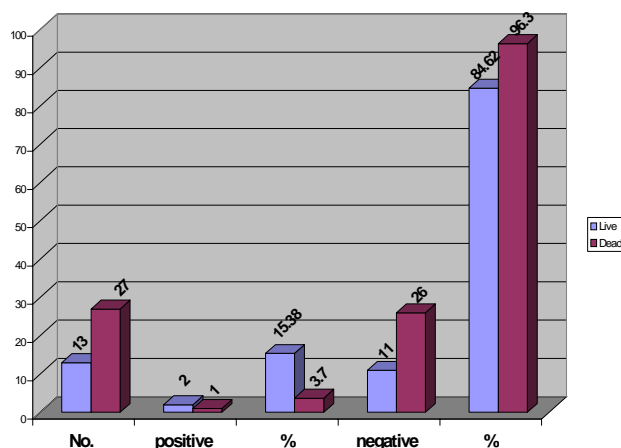


Fig. 4. Number and percentage prevalence of bacterial contaminants in live and dead vaccines



A somewhat higher prevalence of vaccine contamination was recorded by Giangaspero *et al.* (2001) who analyzed 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.

The incidence of pure and combined bacterial contaminants in local and imported livestock vaccines.

The bacterial contaminants identified from local livestock vaccines are given in Fig. 5 and 6. A total of 40 samples of livestock vaccines were examined, 1 (33.33%) with batch number 079 and 2 (66.67%) with batch numbers 057 and 010 were determined having pure and combined bacterial species, respectively. The pure bacterial contaminant was *Bacillus cereus* from Anthrax Vaccine with batch number 079, while the mixed bacterial contaminants in H.S vaccine sample with batch number 057 were *P. multocida* + *E. coli* and Anthrax vaccine with batch number 010 were *B. cereus* + *B. subtilis* (Fig. 6). Samad (2001) reported the mixed contaminants i.e. *B. megaterium*, *B. cereus*, *B. mycoides* and

Fig. 5. Number and percentage prevalence of pure and mixed bacterial contaminants in livestock vaccines

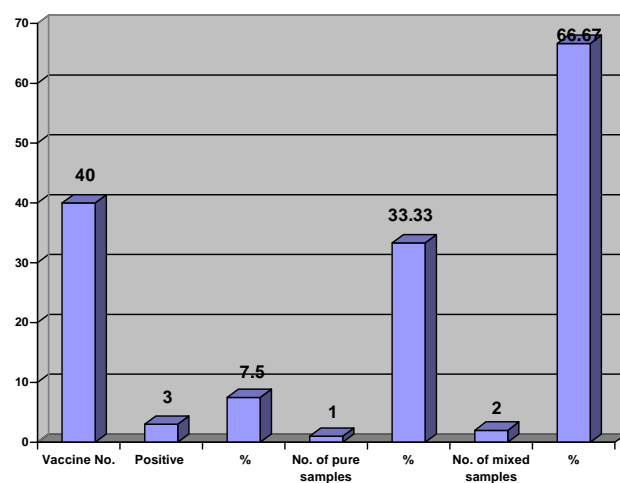
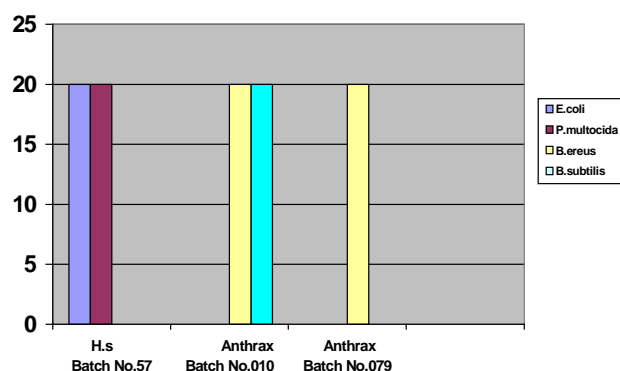


Fig. 6. The incidence of individual bacterial species in livestock vaccines



B. subtilis in the local 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. Mbulu *et al.* (2004) reported *M. mycoides* subsp. *mycoides* in cattle due to use of contaminated contagious bovine pleuropneumonia (CBPP) vaccine. Landman *et al.* (2000) examined Marek's disease vaccine and found the pure contamination by *Enterococcus faecalis*.

Although, the findings of some workers are not in close agreement to the bacterial contaminants identified during the present study. They also determined some other bacterial species and viruses that had contaminated livestock and human vaccines. The bacterial species recognized in our study were more or less same as recorded by Samad (2001), while Mbulu *et al.* (2004), Landman *et al.* (2000) and Kojima *et al.* (1997) reported the different organisms, which were not recorded during the present study. The presence of the organisms in the local livestock vaccines was due to the several practical reasons. It could be due to use of poor

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

CONCLUSIONS

Some locally manufactured vaccines possess extraneous bacterial contaminants, while imported vaccines were free of bacterial contamination. Some local vaccine manufacturers use poor quality or less concentration of preservative (formalin) in dead vaccines, which results the presence of live contamination. Furthermore, anthrax (live spore) vaccines contain bacilli i.e., *B. cereus* and *B. subtilis* along with actual vaccinal organisms.

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