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Biochemical Properties of Bacterial Contaminants Isolated from Livestock Vaccines

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Abstract: In present study, 40 livestock vaccines were tested for bacterial contaminants. Four different bacterial species were identified from the vaccine samples. The species were *Escherichia coli, Pasteurella multocida, Bacillus cereus* and *Bacillus subtilis*. Of the 40 livestock vaccines studied, 1 Haemorrhagic septicaemia (H.S) and 2 Anthrax vaccines were found positive for bacterial contaminants, possessing batch numbers 057, 079 and 010 respectively, while 37 samples were observed without any bacterial growth. The percentage prevalence of positive vaccine samples was recorded as 7.5%. The pure contamination was recorded in 1 (33.33%) Anthrax vaccine sample with batch number 079, while 2 (66.67%) samples, 1 H.S and 1 Anthrax with batch numbers 057 and 010 respectively were recorded for mixed bacterial species. During investigating biochemical properties, it was observed that *Escherichia coli* show the positive reaction to catalase, and negative to oxidase, urease and indole. While *Pasteurella multocida*, *Bacillus cereus* and *Bacillus subtilis* were positive to catalase and oxidase, while negative to urease and methyl red.

Key words: Livestock vaccines, contamination, biochemical properties

INTRODUCTION

Vaccination or active immunization is the artificial introduction of antigens from a microbe into an individual in a controlled way, leading to the stimulation of the immune system without the symptoms of the full-blown disease. 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). There are several types of vaccines, which are used in veterinary practice like, Attenuated whole-agent vaccines (live vaccines), Inactivated whole-agent vaccines (dead vaccines), Toxoid vaccines, Subunit vaccines, Conjugated vaccines and Nucleic acid vaccines (Tortora *et al*., 2001).

During the vaccine manufacturing process, pathogens are cultivated on artificial or living media for to obtain their bulk amounts. For viral vaccines, viruses are grown in animals or chick embryo. The virulence of these microorganisms is reduced by passage through a series of animals other than the normal host species. Live viruses may be inactivated by phenol or ultraviolet rays, while bacteria usually by formalin (West, 1998).

The advantages of vaccines that contain dead organisms are that, they are safe with respect to residual virulence, since organisms are already dead. These are commonly available in liquid form along with formalin and have very little risk of 'alive' contamination. While live vaccines may posses residual virulence and these are usually available in freeze-dried form and always run the risk of contamination with unwanted organisms. Out breaks of reticuloendotheliosis in chickens in Japan and Australia has been traced to contaminated Marek's disease vaccine (Tizard, 1995). Samad (2001) reported extraneous contaminants in different manufactured anthrax vaccines. He dected *Bacillus megaterium*, *Bacillus cereus*, *Bacillus mycoids*, and *Bacillus subtilis* from anthrax live-spore vaccines through the cultivation of vaccine batches on Brain Heart Infusion Agar (BHIA). Feeling the gravity of the situation, it is therefore planed to carry out study of the bacterial contaminants of livestock vaccines and their reorganization on the basis of biochemical properties.

MATERIALS AND METHODS

Collection of vaccine samples: Forty livestock vaccines (both live and 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 Khalil (1992). The species of the organisms were recognized by checking their biochemical properties.

RESULTS

The prevalence of bacterial contaminants in livestock vaccines: The number and percentage prevalence of bacterial species as contaminants recognized from livestock vaccines are presented in Fig. 1. A total of 40 livestock vaccines were examined (Table 1), 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%) were exhibited no growth and recorded as negative for any bacterial contaminants.

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. 2 and 3. 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 *Pasteurella multocida* + *Escherichia coli* and Anthrax vaccine with batch number 010 were *Bacillus cereus* + *Bacillus subtilis* (Fig. 3).

Biochemical properties of bacterial contaminants isolated from livestock vaccines: After isolation bacterial organisms were checked for their biochemical properties (Table 2). During investigating biochemical properties, it was observed that *Escherichia coli* show the positive reaction to catalase and negative to oxidase, urease and indole. While *Pasteurella multocida*, *Bacillus cereus* and *Bacillus subtilis* were reacted positively to enzymes catalase and oxidase, while negative to urease and methyl red. For Voges proskauer *Escherichia coli* and *Pasteurella multocida* were negative, while *Bacillus cereus* and *Bacillus subtilis* were positive. For gelatin liquefaction and citrate utilization *Escherichia coli* was negative, while *Bacillus subtilis* was positive.

DISCUSSION

During present investigation, a total of 40 livestock vaccine samples were examined, out of which 3 (7.5%) were found positive for various bacterial contaminants possessing batch numbers 057, 079 and 010, while 37(92.5%) were found free from bacterial species (Fig. 1 and 3). The bacterial contaminants identified from livestock vaccines during this study are given in Figure 3. A total of 40 samples of livestock vaccines were examined, 1 (33.33%) and 2 (66.67%) were determined having pure and combined bacterial species respectively (Fig. 2). The pure bacterial contaminant was *Bacillus cereus* from Anthrax Vaccine, while the mixed bacterial contaminants were in H.S vaccine and Anthrax vaccine samples.

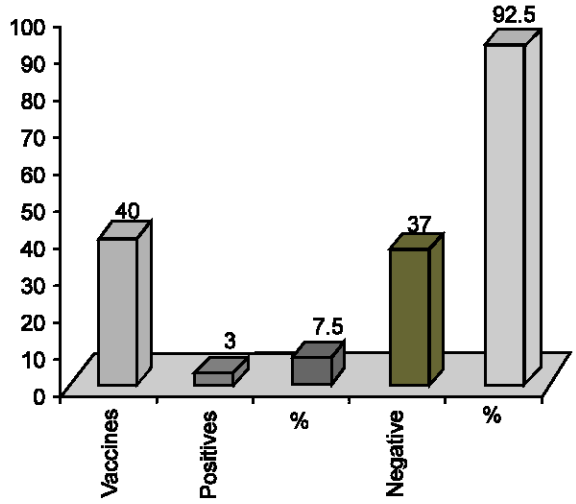

Fig 1: Number and percentage prevalence of bacterial contaminants in livestock vaccines

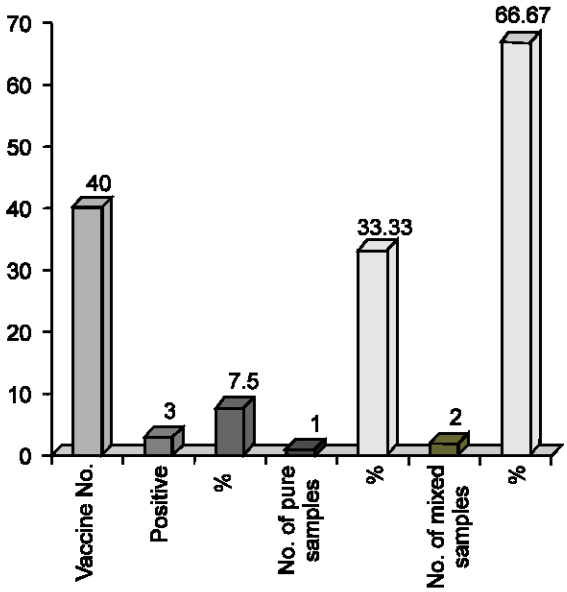

Fig 2: Number and percentage prevalence of pure and mixed bacterial contaminants in livestock vaccines

Table 1: Prevalence of bacterial contaminants in livestock vaccines

Sr#	Vaccines	No. of samples examined	No. of positive samples
1	H.S (Hemorrhagic septicemia)	10	01
2	E.T.V (Enterotoxaemia Vaccine)	04	00
3	B.Q (Black quarter)	03	00
4	Anthrax	05	02
5	FMD (Foot and mouth disease)	07	00
6	C.C.P.P (Contagious Caprine Pleuropneumonia)	03	00
7	Rabies	08	00

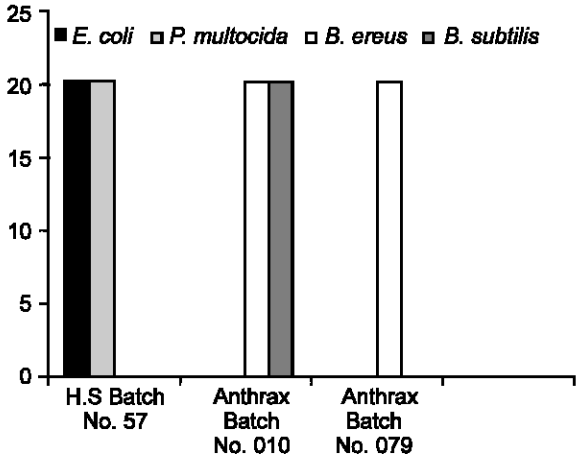

Fig 3: The incidence of individual bacterial species in livestock vaccines

Table 2: Biochemical properties of bacterial species recognized from livestock vaccines

Biochemical tests	*E. coli*	*P. multocida*	*B. cereus*	*B. subtilis*
Catalase	+v	+v	+v	+v
Oxidase	-ve	+ve	+ve	+ve
Coagulase	-	-	-	+ve
Indole	-ve	+ve	-ve	-ve
TSI	A/A	A/A	K/A	A/A
H2S	+v	-ve	-	+v
Urease	-ve	-ve	-ve	-ve
M R	+v	-ve	-ve	-ve
VP	-ve	-ve	+v	+v
GL	-ve	+v	-	+v
Citrate	-ve	-ve	+v	+v
NR	+v	+v	-	+v
Aesculin	-	-	+v	-

Whereas:
-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

The bacterial species isolated from anthrax live-spore vaccines by Samad (2001) as mixed contaminants were *Bacillus megaterium*, *Bacillus cereus*, *Bacillus mycoids*, and *Bacillus subtilis*. 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 *Mycoplasma synoviae* and *Mycoplasma gallisepticum* were predominant. Mbulu *et al.* (2004) reported *Mycoplasma 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 observed 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 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.

The findings of biochemical properties of isolated organisms are presented in Table 2. According to these cells of *Escherichia coli* were found catalase positive but oxidase negative. It exhibited A/A but did not produce H2S in TSI agar. It interacted positively with methyl red and nitrate reduction but negatively to indole, urea, Voges-Proskauer, gelatin and citrate. While, *Pasteurella multocida* was found positive to catalase, oxidase, indole and nitrate reduction, but negative to urea, methyl red and citrate. It exhibited A/A without production of H2S in TSI agar. Khalil and Gabbar (1992); Nizamani (1999); Devrajani (2005) and Dewani (2000) who recorded similar biochemical properties of *Escherichia coli* as demonstrated in the present investigation. Similarly, Khan and Rind (2001) and Fazlani (2005) also recorded similar biochemical properties of *Pasteurella multocida* as recorded in the present study. The findings about biochemical properties of *Bacillus cereus* observed in

this survey are also in line to the other workers (Fazlani, 2005; Dewani, 2000 and Devrajani, 2005). While results about *Bacillus subtilis* were in close agreement as observed by Samad (2001), Merchant and Packer (1999).Therefore one should say that these are the same species identified in their investigations, also recognized in the present study.

Conclusion: From the present study, it is concluded that some livestock vaccines possess extraneous bacterial contaminants. It is further observed that anthrax (live spore) vaccines contain extraneous bacilli along with actual vaccinal organisms. The bacterial organisms isolated from vaccines are same in biochemical properties as they have originally.

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