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Immunological Studies for Using of Combined Inactivated Respiratory Virus Vaccine (Pneumo-3) and Sheep Pox Vaccine in Goats

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Abstract: This study was conducted for explaining the use of combined inactivated pneumo-3 vaccine (bovine viral diarrhea, bovine herpes virus -1 and parainfleunza-3 viruses) as a diluent for sheep pox virus vaccine to be used as a vaccine for goats in one shot. 16 balady adults' male goats were used which were seronegative against each virus used in this study. All goats were randomly divided into four groups each one consists of four goats. The first group was vaccinated against sheep pox using sheep pox vaccine (Veterinary Serum and Vaccine Research Institute, Cairo) subcutaneously (s/c). While the second group vaccinated with pneumo-3 vaccine (Veterinary Serum and Vaccine Research Institute, Cairo) in two doses with two weeks apart by 3ml s/c. The third group was vaccinated with the pneumo-3 and sheep pox vaccines as one mixture in one shot s/c. The fourth group was left as non-vaccinated control group. Serological investigation using SNT and ELISA revealed that there was no competition or interaction between each fraction of both vaccines but in contrast the results obtained revealed that the immune response induced by vaccination of the mixture prepared from both vaccines gave elevated and higher immunity than that of the second group. In conclusion, using of sheep pox and pneumo-3 mixture vaccines could be used safely as one shot vaccine and is recommended to be used in the field for protection of goats against such infections.

Key words: Pneumo-3 · Goat · Pox · SNT · ELISA

INTRODUCTION

Pneumonic enteritis complex syndrome is considered as typical models of a multifactorial cause disease as the ovine and caprine pneumonia are acute infectious disease of sheep and goats affecting all ages especially nursing and feedlot animals. Pneumonia is caused by a complex interaction of stress-producing environmental factors and a variety of micro-organisms especially viruses as IBR and Parainfluenza type 3 that synergistically work to damage the cell lining of respiratory tract and compensated host response [1, 2]. Sheep and goats pox (SGP) is highly contagious disease of small ruminants caused by Capri pox virus. They are endemic in most countries of Africa, Middle East and Asia [3-6].

Both diseases, either respiratory viruses infection and sheep and goat pox (SGP) were representing the most important and drastic upset among fattening sheep and goat. They are widely spread throughout the world. So, the obvious economic impact of these infections is not only from deaths but also due to substantial economic drain resulted from reduced feed gain efficiency in addition to general unthriftiness and reduced resistance to subsequent infections [7-9].

Vaccines are the most efficient and cost effective method for controlling such diseases in Egypt and at the same time the trend of using more than one vaccine for the same animals at same time is greatly simplify the prophylaxis control of diseases of livestock besides saving costs, efforts and time during vaccination.

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The aim of the present work is to use the sheep and goat pox virus vaccines as an immune-stimulant to the goats, currently elevate of the immune response triggered in animal body against Pneumo-3 vaccine. Also, at the same time using of Pneumo-3 vaccine as diluent for freeze dried disc of the sheep and goat pox vaccine to be incorporated to be used as one shot inoculation for inducing of maximum protection against such infections.

MATERIALS AND METHODS

Viruses

Bovine Viral Diarrhea Virus (BVD): Egyptian Reference virus strain (Iman Strain). Its titer is 10^7 $TCID_{50}$/ml. It was isolated and identified in Tahrir province by Baz [10].

Bovine Herpes Virus Type 1 (BHV-1): Egyptian reference virus strain of Infectious Bovine Rhinotracheitis virus (Abu Hammad), its titer is 10^8 $TCID_{50}$/ml. It was isolated and identified by Hafez *et al.* [11].

Parainfleunza Type 3 Virus (PI3): Egyptian reference strain (Strain 45). Its titer is 10^8 $TCID_{50}$/ml. It was isolated and identified by Singh and Baz [12].

All viruses were kindly supplied from Rinderpest like diseases department, Veterinary Serum and Vaccine Research Institute, Cairo. All viruses were propagated and adapted then titrated on Madin Darby Bovine Kidney cell (MDBK) cell line which was proved to be free from any adventitious agents especially non cytopathic strain of BVD virus. Viruses were harvested and inactivated by binary ethyleneamine (BEI) of 0.1M and used in preparation of pneumo-3 vaccine.

Sheep Pox Antigen: Sheep pox antigen was prepared according to El-Bana [13]. This antigen was tested against specific positive serum and was used in ELISA testing.

Vaccines

Pneumo-3 Vaccine: Pneumo-3 vaccine is combined inactivated vaccine contains BVD, BHV-1 and PI3 viruses adjuvaneted with aluminum hydroxide absorbable gel. It is already registered and routinely produced in Rinderpest like diseases department, Veterinary serum and vaccine research institute-Abbassia, Cairo. It was used in dose of 3ml for sheep and goats by two doses with two weeks apart.

Sheep Pox Vaccine (Kenvan Strain): It was prepared in Pox research department, Veterinary serum and vaccine research institute-Abbassia, Cairo. The virus had a titer of $10^{5.2}$ $TCID_{50}$/ml and of vaccinal dose of 10^3 $TCID_{50}$/ml.

Preparation of Tested Mixture of Sheep Pox Vaccine and Pneumo-3 Vaccine: Sheep pox virus vaccine in form of freeze dried disc vial containing 100 vaccinal dose to be dissolved in 5 ml of sterile physiological saline solution, 2.5 of this diluent was pulled by sterile syringe to be added to pneumo-3 vaccine bottle (containing 50 vaccinal doses) and this mixture is ready to be used to vaccinate goats with 2ml inoculated s/c in first dose of vaccination and boostering after 2 weeks by 2ml s/c of pneumo-3 vaccine alone.

Cell Culture: Monolayer Maiden Darby Bovine Kidney (MDBK) cell culture was tested to be free from the non cytopathic BVDV and used for propagation and titration of viruses.

Immunization of Goats: Sixteen apparently healthy balady male goats about 2 years old housed in an isolation facility in a private farm at Giza Governorate, all animals were proved to be seronegative for the all viruses included in the work. Goats were randomly assigned into 4 groups with 4 balady male goats in each group, goats were schemed for vaccination and illustrated by numbering them as follow:

***Group I*:** Each animal was vaccinated with Pneumo-3 vaccine using 2 ml s/c with 2 doses, 2 weeks apart.

***Group II*:** Each animal was vaccinated s/c with sheep pox vaccine (1 dose 0.5 ml containing 10^3 $TCID_{50}$/ml). The vaccine vial was dissolved in 50ml of sterile saline solution and 0.5ml used as vaccinal dose.

***Group III*:** Each one was immunized in the first dose of vaccination by the prepared mixture of sheep pox and Pneumo-3 vaccines (2 ml s/c). Then, animals were followed by booster dose after 2 weeks by Pneumo-3 vaccine only (2 ml s/c).

***Group VI*:** Male goats were kept as non-vaccinated control group.

All animals were kept under clinically observation, body temperature daily recorded and monitored for any adverse post vaccinal reaction either locally or systemically during the whole experimentation period.

Serological Investigations

Serum Neutralization Test (*SNT*)

Snt for Pneumo-3 Vaccine: It was performed using a microtiteration plate assay according to Ahmed [14] and Obando *et al*. [15].

SNT for Sheep Pox Vaccine: It was applied as described by Martin *et al* [16] to all sera samples collected from goats.

***Enzyme linked immune sorbent assay* (*ELISA*)**

***ELISA for Pneumo-3 Vaccine*:** It was carried out according to Voller *et al*. [17] and Durham and Hassard [18].

ELISA for Sheep Pox Vaccine: Indirect ELISA was conducted according to Babiuk *et al*. [19].

RESULTS AND DISCUSSION

Pneumo-enteritis disease complex syndrome and goat pox are highly contagious disease in large and small ruminants causing highly economic losses [20]. Till now, the vaccination programs are the main method for controlling both diseases in Egypt.

The successful trials of vaccination of animals with more than one vaccine at the same time were reported either between bacterial and viral vaccines as Rinderpest (RP) and contagious bovine pleuropneumonia (CBPP) vaccines [21], Anthrax and Foot and Mouth Disease (FMD) vaccines [22], Peste de Petites Ruminant (PPR) and Clostridia vaccines [23], live attenuated *Brucella melitensis* (Rev-1) and PPR vaccine [24]. Combined vaccination between viral and viral vaccines were conducted as Rift Valley Fever (RVF) and sheep pox virus (SPV) vaccines [25], PPR and RVF [26], PPR and SPV vaccines [27] and recently PPR and goat pox vaccines [28], all the aforementioned statements for simultaneous and compound vaccination programs were a way to save costs, time and efforts.

The participated prepared vaccine of Pneumo-3 and Sheep pox vaccine were used in this study for immunization of goats. Sixteen balady male goats were used in this study, randomly divided into 4 groups with 4 goats each. Group I was inoculated with Pneumo-3 vaccine s/c, while group II was inoculated with sheep pox vaccine. Group III was inoculated with the prepared participated compound vaccine, the Pneumo-3 was used as a diluent for sheep pox vaccine and Group VI kept as non-vaccinated control group. All animals were kept under observation and all goats had no local or systemic post vaccinal reaction which indicates that there is no competition or interaction between each component of both vaccines [29]. All serum samples reacted positively in the BHV-1, BVD and PI3 indirect ELISA and VNT indicating that all animals responded to vaccination properly with high individual Protection Level.

Results were illustrated in Figures (1 and 2) to compare the immune status against Pneumo-3 vaccine between animals of group I and III using SNT and ELISA respectively; it showed an exaggeration and elevation in the mean sera neutralizing antibodies in group III, indicated the immunostimulant effect of use of SPV in combination with other inactivated vaccine that results agrees with that obtained by Ghaly *et al*. [30].

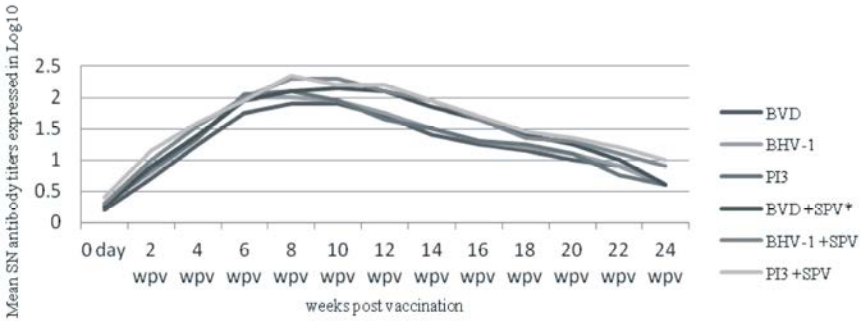

Fig. 1: Mean SNT titers against BVD, BHV-1 and PI-3 of vaccinated goats with Pneumo-3 vaccines alone and prepared mixture of Pneumo-3 and sheep pox virusvaccine

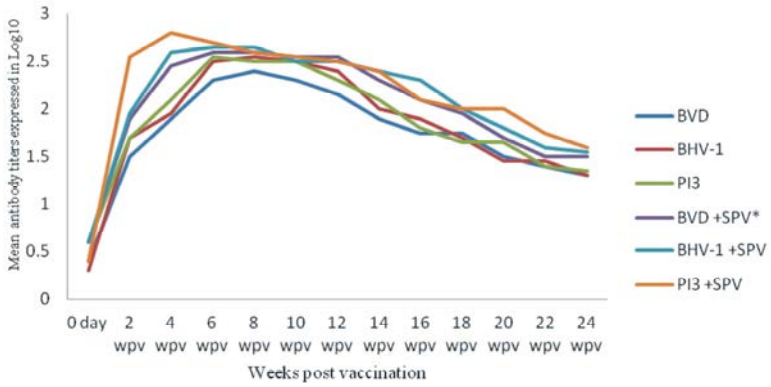

Fig. 2: Mean ILISA antibody titersagainst BVD, BHV-1 and PI-3 of vaccinated goats with Pneumo-3 vaccines alone and prepared mixture of Pneimo-3 and sheep pox virusvaccine

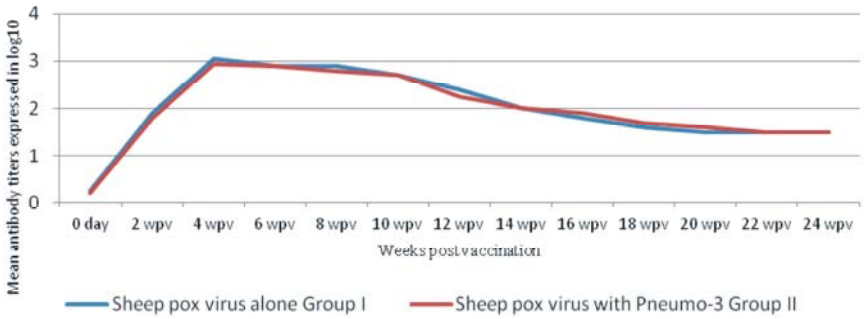

Fig. 3: Mean serum neutralization antibody titers of goats vaccinated with sheep pox virus vaccine alone and with Pneimo-3 vaccine

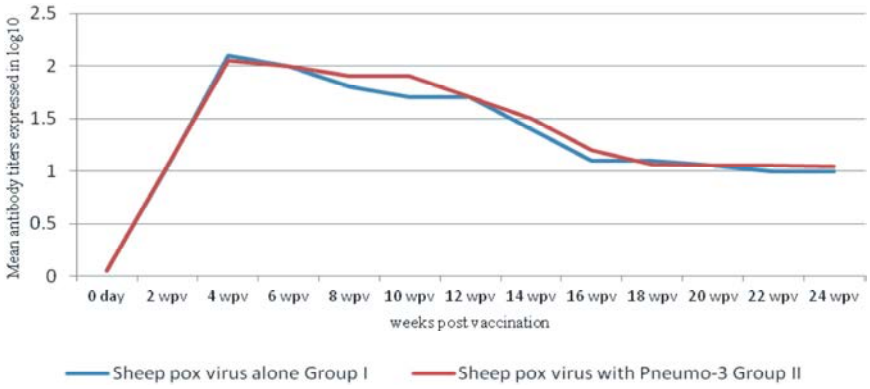

Fig. 4: Mean ELISA titers of goats vaccinated with sheep pox virusvaccine alone and with Pneumo-3 vaccine

Results presented in Figures (3 and 4) revealed that, the greatest mean titers of SPV was recorded at 4 weeks post vaccination in groups II & III reaching of 3.05 and 2.95 to SNT and 2.10 and 2.05 to ELISA respectively. On the other hand, when the humeral immune response was compared between goats vaccinated with Pneumo-3 vaccine or SPV alone with those vaccinated with mixed vaccines, there was clear elevation of antibody titer in group III with mixed vaccines.

In conclusion, the successful use of participation or forming mixture between sheep pox and Pneumo-3 vaccines in goats induced safety vaccination and provided a good protective level of antibody titers against diseases. In the same time, it will save time, cost and effort and recommended to be used in the vaccination program for goats. ELISA involved simple technique and produced results at least equivalent to the standard SNT with considerable savings in time and labor.

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