The State of Eritrea
Ministry of Agriculture Newsletter
A Monthly Newsletter Prepared by the Public Relations Division, Issue No 60, June 2023

A Glance at the Historical Journey and Recent Achievements of Vaccine production: 1903-2023

Recently, the Ministry of Agriculture (MoA) announced the commencement of production of two animal disease vaccines for Peste des Petitis Ruminants (PPR) and Newcastle Disease (NCD). The Public Relations Division of the MoA presents, Mr. Efrem Ghebremeskel, Director of the National Animal and Plant Health Laboratory (NAPHL), Mr. Amanuel Mebrahtom, Head of Vaccine Production Unit and Ms. Ghirmawit Habtom, Head of Quality Control Unit, to provide our readers with the necessary details on this significant accomplishment, preceded with substantial information regarding the process of the two vaccines in a question-and-answer (Q and A) form.

Mr. Efrem Gebremeskel

Question: Mr. Efrem Gebremeskel, could you give us brief information on the historical background of the NAPHL?

Mr. Efrem: This laboratory was one of the few pioneering laboratories in Africa. The active period of this laboratory's history extends from 1903 to 1972. At the beginning of this period, the Italian government used to purchase livestock, including cattle, sheep and goats from India to supply its soldiers with meat. However, the purchased animals were severely affected by Rinderpest and, therefore, the Italian government set up a study team in a bid to assess the situation and to find solution to control the outbreak. To this end, a team was setup; conducted its study; and came up with an idea that the army can rely on meat supply from Eritrea. Subsequently,

a new vaccine production laboratory with the name 'Istituto Siero-Vaccinogenito' was established in 1903, in Villagio, Asmara. The laboratory started active work like performing various laboratory activities and rendering services: Vaccine production, veterinary diagnosis, food safety and quality testing for products of animal origin, as well as diagnostic supporting research works and others.

Since its establishment to this date, the laboratory assumed different names: Istituto Siero-Vaccinogenito, Veterinary Research Institute, Central Veterinary Laboratory, and NAPHL, among others. This laboratory work was not a smooth sail. Its operational history was punctuated by events of progression, regression and shut down.

Question: Can you give us more details regarding the subsequent fates of the laboratory, with more emphasis on history of vaccine production?

Mr. Efrem: Documents reveal that, at the beginning, more than ten different types of veterinary vaccines and anti-venom sera were produced by this laboratory and utilized locally. It even satisfied vaccine demands from Ethiopia and other neighboring countries. Some of the then-prominently produced vaccine types include against diseases like Rinderpest, Fowl Fox, Fowl Pox Septicemia, Rabies, African Horse Sickness, Contagious Caprine Pleuro Pneumonia (CCPP), Black Leg and Anthrax. Apart from vaccine production, the institution was actively involved in animal disease treatment, field service activities and research studies. As a result, many scientific journals and documents were published on the basis of the findings that reflected status of various diseases among diverse animal species. Considerable types of laboratory animal species were also raised within its premises and used for vaccine production, diagnosis and research purposes.

The period 1972 to 1991 constituted a status of total regression and shutdown of vaccine production operation due to the destructive war with the Derg regime of Ethiopia. Especially with the intensification of the war in 1975, vaccine production came to an end and eventually the laboratory was totally abandoned. Part of the entire premises and the laboratory houses were even used as military camp by the Ethiopian regime.

After independence, a new dawn ushered and the laboratory operations showed a sign of come-back with a good momentum. The Government of Eritrea, through the MoA made all possible efforts to rehabilitate the laboratory through constructing various new laboratory infrastructures, equipping it with the required facilities and training laboratory personnel. Vaccine Production and Quality Control Laboratory Establishment Project was launched in 2005 with a fund from African Development Bank (ADB) under the National Livestock Development (NLDP) Project. Major infrastructures of the laboratory including the fence of the premises, laboratory building, laboratory animal housings and other subordinating houses were constructed through this project. Besides, various laboratory equipment and supplies were procured. In 2010, the MoA in collaboration with the International Fund for Agricultural Development (IFAD), focused on the

The 1903 built labaratory

The newly built laboratory

completion of the civil works, construction of water and drainage systems, procurement of the remaining essential equipment and supplies, recruitment of vaccine production and quality control experts (Sudanese and Cubans), training of laboratory personnel, as well as conducting trial activities. Apart from this, certain laboratory construction works were funded by the government and the civil work was mainly done by government parastatal construction companies namely Debwin and Segen construction companies. Some of the construction works have been active until 2019. However, as of 2017, the vaccine production training and trial paces were progressing well side by side.

In 2019, professional communication was established between the NAPHL and the African-Union-Pan African Veterinary Vaccine Center (AU-PAN-VAC) laboratory, which is based in Ethiopia. The two leading directors of the AU-PANVAC visited the NAPHL in 2019 and expressed positive promises to technically support the vaccine production laboratory with the required vaccine seeds, cell line and some reagents. Lack of vaccine seeds and cell lines was a critical bottleneck in the previous years. Then they sent us Peste des Petitis Ruminants (PPR) and Newcastle Disease (NCD) vaccine seeds in 2020. They further sent one expert to train our laboratory staff on PPR and NCD vaccine production and quality control techniques for two weeks. This was in 2022 and the laboratory has been carrying out several scientific internal trial activities.

Finally, it was in a position to produce the actually required two vaccines with full confidence. As a result, three batches of NCD vaccine (327,000 doses in total) and one batch of PPR vaccine (30,000 doses in total) were produced in 2023. Samples from the vaccines were tested through internal quality measures and well-passed the recommended test parameters. Then, for more confirmation and to meet international standard requirements, representative samples were sent to the AU-PAN-VAC laboratory pursuant to the procedure given. Ultimately, the AU-PAN-VAC laboratory tested the vaccine and confirmed their good quality and granted certificates to this end. The certificates for PPR and NCD vaccines were issued on the 3rd of April and 13th of May this year respectively.

The progress so far indicates that the NAPHL has developed and is equipped with the required capacity in terms of laboratory setup and human resource (knowledge and skill) to produce these. Moreover, it implies that we can embark upon production of other related vaccines with the help of some technical adjustment and adoptions mechanisms.

Question: Why were the PPR and NCD vaccines given a priority?

Mr. Efrem: PPR and NCD are very infectious diseases with a long period of prevalence in Eritrea. Depending on the situation and degree of infection, PPR and NCD can cause mortality rate up to 100% in small ruminants (mainly in kids) and poultry flocks respectively. For this very reason, the Ministry of Agriculture, almost yearly, has been importing vaccines for these diseases and others in big quantities. On the other hand, these vaccines relatively do not require a complicated technology, unlike some of the very expensive vaccines. These two vaccines were selected for production in the first phase, taking these facts into account. It is also worth mentioning that the NCD is further linked with the strategic plan of the MoA for small-holder farmers where every household in the village is encouraged to keep at least a small poultry flock. This gives NCD vaccine production a priority as chicken need to be immunized four times with NCD vaccine to maintain a good immunity level.

Question: What kind of advantage can Eritrea secure from PPR and NCD vaccine production?

Mr. Efrem: Comparatively, notable advantages and benefits can be obtained from these vaccines when they are produced locally. Based on the ministry's report in the last 10 years' vaccine imports, it was roughly estimated that, every year, about 3.9 million doses of PPR vaccine and 3.3 million doses of NCD vaccines were imported at costs of about 383,000 and 30,000 dollars respectively. The cost of production for the same amounts of dose, i.e. considering the expenses used for processing the two vaccines locally (excluding the lab facilities and other assets) stands at around 31,000 and 20,000 dollars for PPR and NCD correspondingly. This implies that we can save up to 353,000 and 10,000 dollars respectively.

Procurement and import of vaccines require hard currency. Moreover, vaccines are not ready for sale at the shelf. Most of the time, veterinary vaccines are produced on demand basis. Procurement process also has its own complications. Consequently, such delays and complications can cause further/ extensive spread of a disease to susceptible population.

However, the well-equipped laboratory that has been established and the nurturing of knowledgeable, with well-trained and highly motivated young laboratory staff constitute a great asset for our country. The laboratory facility and its staff are versatile and can serve in many laboratory settings. Other vaccines can be produced through the knowledge and skills that have been acquired and the available laboratory facilities, i.e. with some modification, adoption and training, depending on the future required products. The laboratory also provides groundwork for furthering disease diagnostic techniques and research works.

Question: Mr. Amanuel Mebrahtom, let's come to you, will you shed light on the critical process and journey the NAPHL went through with a view of justifying standard production capacity?

Mr. Amanuel: A good-quality vaccine always comes as a result of integrating trained capacity with all features of the installed system. To attain such a good result, the laboratory building, rooms and all materials within need to be designed in such a way that it ensures safety and quality of the vaccine. The entire process should not be compromised at any cost. All the procedures, guidance, personnel training manual etc. should be aimed at and designed with major consideration to safety and quality of the vaccine. All the stated undertakings are performed in a proper way, then vaccine quality can be on the safe side. Therefore, the same and satisfactory procedure was followed in our case.

Once the vaccine processing environment and facilities were ready for the production of standardized vaccine, the process proceeded and recommended tests were done at every stage, from the beginning to the end. The Vaccine Quality Control unit took the required samples from the finished products and carried out all the recommended tests. Meanwhile, the Regulatory Services Department (RSD) of the MoA conducted inspection activities on all steps and areas of the

Mr. Amanuel Mebrahtom

laboratory and ensured compliance of the entire process, in addition to issuing the permit thereof. Then, representative samples from each batch of newly produced vaccines were collected and sent to the African Union – Pan-Africa Veterinary Center (AU-PAN-VAC) to be further tested and validated for quality in the recommended international laboratory. Eventually, all the samples perfectly met the requirements for standard qualification and we received certificates confirming good result for each sample.

Question: Please explain to us the major steps in NCD vaccine production process.

Mr. Amanuel: In the first place, it is important to note that the eggs used for NCD vaccine production must come from specific pathogen-free (SPF) flocks or from healthy flocks that are free from vertically transmitted agents such as Salmonella Pullorum, Mycoplasma Gallisepticum, Mycoplasma Synovae, etc. The eggs and embryos are checked using candling technique for viability and fertility. The external part of the egg shell is properly cleaned and disinfected before inoculation and incubation to avoid contamination. Fertile eggs are incubated from 9-11 days in a humidified incubator for the growth of the embryo and eggs with enlarged blood vessels are inoculated with vaccine seed through the allantoic route. After 96 hours of incubation, the allantoic fluid is harvested and samples are sent to Quality Control Laboratory for sterility, while it is kept under bulk titration process. Hence, through titration, concentration of the virus present in the solution, as well as the amount of virus that can trigger antibody production on the target animals can be measured. Once titration is successfully accomplished, Lactalbumin Hydrolysate and Sucrose – on free dry medium– are added to the vaccine solution to maintain its stability and increase shelf life.

Question: How about the major steps in PPR vaccine production process.

Mr. Amanuel: In the virus seed used for PPR vaccine it is inoculated into Vero cells that are prepared from African monkey kidney. The Vero cells are inoculated with passaged working seed. The Vero cells are cultured and made to grow on different growth media such as GMEM, MEM while frequently being checked for sterility and level of cytopathic effect (CPE) i.e. shown in the media. Normally, first harvest starts when it shows 40-50% CPE on the 6th day, and we continue harvesting and passaging until it reaches 70-80% CPE. Titration is carried out in 96 well micro plates with 10-fold serial dilution and result reading,

from day-7 to day-14, using inverted microscope. The solution of the vaccine in vials is placed in free drier machine at least for 48 hours to remove its moisture content through sublimation.

Finally, the vaccine vials are packed, labeled and stored in cold room until quality control certificate are obtained from the Quality Control Unit. In our earlier case, representative samples from each batch of production were sent to the AU-PAN-VAC laboratory for quality control test and certification at this stage.

Question: Ms. Ghirmawit Habtom, please tell us how the Quality Control Unit dealt with the process of PPR and NCD vaccine development?

Ms. Ghirmawit: Any vaccine produced should be tested and pass the minimum requirement of quality control test parameters including purity, safety, stability, potency and identity before it is released for use. Consistent with this precondition, the Quality Control Unit performed tests on starting raw materials, in-process quality factors and the final product quality. Before the start of vaccine production process, the required raw materials; including eggs, media and Phosphate Buffer Solution (PBS); equipment and master seed were collectively assessed to ensure sterility avoiding further contamination. In-vivo (in embryonated eggs) and in vitro (in cell-culture) potency test was performed during the process as well as the final vaccine products to gauge the titer of the virus. In-vivo testing was further conducted through challenging target animals (chicks) to determine the immune response of their bodies, and thus gauging the titer using serological tests such as Haem-Agglutination (HA), Haem-Agglutination Inhibition (HI) and Enzyme-Linked-Immunosorbent assay (ELISA). Afterwards, identity test is done using Polymerase Chain Reaction (PCR) technique to ensure that the product contains only the required vaccine strain. Safety test was also done on immune response of the target animals, making sure that there is no adverse effect due to the vaccine on them. Safety test can be done both on target animals and lab animals. Likewise, Stability test was executed using vacuum and moisture test approaches on the vaccine to determine the product's stability rate during storage period.

Ms. Ghirmawit Habtom

Moreover, the Regulatory Services Department of the MoA, worked side by side with us, starting from controlling the quality of buildings, ways and standards of the vaccine production.

Question: What can we expect from NAPHL's future plans vis-à-vis vaccine production?

Mr. Efrem: I feel that there are three short-term goals worth-mentioning now. First, the case of these two vaccines is just a beginning. Only limited amounts are being produced. So, we are looking forward to producing the required amount of vaccines pursuing the demand and request from the Agricultural Extension Department (AED) of the MoA. Secondly, based on the list of prioritized diseases, two more vaccines mainly Infectious Bronchitis (IB) among poultry and Sheep/ Goat Pox among ruminants will be produced within same laboratory facility. Lastly, depending on circumstances, further development of the laboratory and roadmap of the MoA etc, the laboratory will put up efforts to accommodate these recommended tasks accordingly.

Mr. Efrem Gebremeskel, Director of NAPHL, Ms. Ghirmawit Habtom, Head of Quality Control Unit, and Mr. Amanuel Mebrahtom, Head of Vaccine Production Unit, thank you for being willing to take this interview.

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