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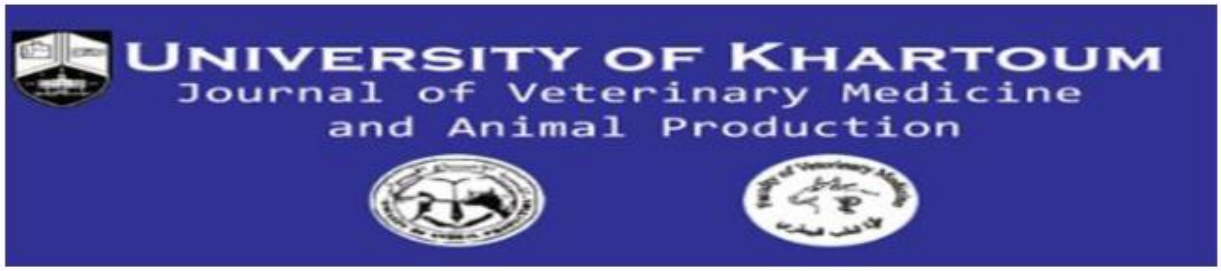

Production and Use of Veterinary Vaccines in Sudan

Review paper

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Abstract

This article aims at reviewing the production of veterinary vaccines used in the Sudan to control diseases of livestock and poultry. The paper covers the principals and a procedure employed in the production of live and inactivated viral and bacterial vaccines and specifically describes the production of ten viral vaccines used in Sudan including Rinderpest and Peste des Petite Ruminants (PPR), Rift Valley Fever, Lumpy Skin Disease, Sheep Pox, Blue Tongue, Camel Pox, African Horse (AHS), Newcastle Disease, Fowl Pox and Gumboro. It also describes the production of four bacterial vaccines; namely vaccines against Anthrax, Hemorrhagic Septicemia Black Quarter and Brucellosis, as well as vaccines against two Mycoplasma diseases, namely Contagious Bovine Pleuropneumonia and Contagious Caprine Pleuropneumonia.

The paper concludes that, Sudan with its many strong and diversified facilities is qualified for vaccine production, to cover the need for control of all diseases in the OIE list of socioeconomic significance for the country and the region. These facilities include an animal wealth estimated to be 100 million tropical animal units (TAU), and an excellent geostrategic position as far as marketing of animals and animal products are concerned; together with Veterinary services and research institutions established since the turn of the 20th Century, and research facilities together with available trained an well experienced personnel.

Modern and up-to-date research methodology is necessary for development of vaccine production techniques and generation of new and improved veterinary vaccines so that vaccine production laboratory in the Sudan becomes rated as a Regional Research Center and a Reference Laboratory for training of personnel and vaccine production in Africa and Arab countries. However, recent advances in bio-technology and information technology provide enormous promises and opportunities; however, political will, strategic planning adequate funding and proper training of the personnel are needed.

Keywords*: vaccines, production, diseases, veterinary.*

المستخلص

تهدف هذه الورقة لإستعراض اللقاحات الفيروسية والبكتيرية المنتجة فى السودان والمستخدمة لمكافحة ألأمراض الوبائية والسارية لحماية القطيع القومي والدواجن. تغطى الورقة أسس و طرق إنتاج اللقاحات الحية منها والميتة. تغطى الورقة أسس وطرق أنتاج عشرة لقاحات ضد الأمراض الفيروسية مثل لقاحات أمراض الطاعون البقري و طاعون المجترات الصغيرة , حمى الأخدود الإفريقي, مرض الجلد العقدى فى الأبقار ,جدرى الضان, مرض اللسان الأزرق, جدرى الإبل, مرض النجمة فى الخيول, مرض سمير فى الدواجن , جدرى الطيور ,القمبورو.كما أنتجت أربعة لقاحات بكتيرية ضد أمراض الجمرة الخبيثة, أبو زقالة, التسمم الدموى, والبروسلا, ولقاحين ضد أمراض المايكوبلازما هى أبو قنيت و أبو نينى.

تخلص الورقة إلى أن السودان ينعم بإمكانات كبيرة و متنوعة تؤهله للقيام بإنتاج وتطوير لقاحات ضد كل الأمراض المضمنة فى قوائم منظمة الأوبئة العالمية و تتمثل تلك الإمكانات في ثروته الحيوانية التي تقدر بمائة مليون وحدة حيوانية و الوضع الجيوإستراتيجى المميزلتسويقها. كما يتمتع ببنيات تحتية جيدة لإنتاج اللقاحات البيطرية متمثلة فى مرافق الخدمات ومراكز البحوث البيطرية التي أنشئت منذ مطلع القرن العشرينو توفر الكفاءات العلمية المقتدرة في مجالات البحث العلمي والتي ساهمت في أنتاج لقاحات بمواصفات عالمية. لا بد من إتباع مناهج البحث الحديثة لتطوير طرق إنتاج اللقاحات وصولا لأنجعها, مما سيمكن معامل البحوث البيطرية السودانية من أن تشكل حاضنة للبحث العلمى فى السودان وتكون فى مصاف المختبرات المرجعية لأنتاج اللقاحات فى محيطيها الأفريقى والعربى. ولن يكون ذلك مستحيلا لو توفرت الأرادة السياسية والتمويل الكافي والتدريب اللازم, خاصة وأن التطورات الهائلة فى مجالات التقانة الحيوية والمواصلات والأتصالات تتيح فرصا كبيرة لتحقيق ذلك.

كلمات مفتاحية: *لقاحات، إنتاج، أمراض، بيطرية.*

Introduction

Immunity to infection could be obtained in two ways: passive immunity by giving ready-made antibodies or gamma globulins in colostrum or serum; and active immunity, which could be obtained through natural infection or vaccination.

When exposed to real infection by a pathogen or vaccination, the immune system of the host recognizes antigenic determinants (usually proteins, carbohydrates or nucleic acids) which could be structural parts of the pathogen or soluble components of it. These antigenic determinants are highly variable among variants of the pathogen. The infected or vaccinated animal responds by production of antibodies by B-cells and/ or cytotoxic T cell (CTC) action that attacks the pathogen. Both the B-cells and the CT cells get help from the T helper lymphocytes.

Vaccination is a deliberate administration of Veterinary medical product to an animal in order to induce active immunity that either prevents infection or reduces multiplication and shedding of the pathogen. A good effective vaccine should therefore, be able to stimulate as many of the body's defense mechanisms as possible and should be very similar to natural infection without causing clinical disease.

The outcome of vaccination depends on the ability of the vaccine strain to induce active immunity, on one hand, and host susceptibility. On the other hand; this in turn, depends on genetic, health, immune status of the host, and other environmental factors.

The Veterinary authorities in Sudan used vaccines besides other control measures to combat and contain the most important livestock diseases. Vaccine production in the country started as early as 1924 using classical and conventional means of processing (Babiker, 1996). With the progress of vaccine production the Sudanese veterinary authorities, supported technically and financially by international donors were able to introduce modern technologies replacing the conventional means. Besides a few imported vaccines 15 viral, bacterial and Mycoplasma, vaccines are currently produced and/or used in Sudan (Ibtisam *et al.*, 2018).

This paper will give a brief description of the Veterinary vaccines produced in Sudan with special emphasis on their production and use.

Principals of Vaccine Production

There are two principals of vaccine production:

- The first is the production of live vaccines.

Production of live vaccines depends on either selection of a naturally weak or weakened (modified or attenuated) strain of a pathogen or selection of temperature sensitive mutants and/or construction of modified live vaccines by deletion of genes responsible for virulence; or insertion of genes that code for certain immunizing antigens in the genome of a non-virulent vector using recombinant DNA (rDNA) technology.

All live vaccines should be able to efficiently multiply in the host to elicit protective immune response without causing clinical disease.

- second principal, which is the production of dead or killed vaccines, depends on three processes:

1. Obtaining a large amount of the pathogen then subjecting it to an inactivation process using chemical agents such as phenol, formaldehyde, and B-propiolactone; or physical conditions such as ultraviolet or Gamma rays.
2. Production of toxoids, when the immune system is directed against certain products or metabolites produced by the pathogen e.g. against the toxins of *Corynebacterium diphtheria or Clostridium tetani*. Here only the portion of the

toxin that is responsible for the neutralization of the toxin is used as a vaccine.

3. Production of unit or split-vaccines made of purified subunits of a pathogen: usually the virus coat or the capsular carbohydrates of bacteria.

4. A fourth generation vaccines are the recombinant proteins, synthetic peptides vaccines and Purified DNA vaccines

Thus, vaccines for active immunization can be divided into six groups: Live vaccines, inactivated vaccines, sub-unit vaccines, recombinant DNA (rDNA) proteins vaccines, synthetic proteins vaccines and purified DNA vaccines

Live vaccines

1.1. Live viral vaccines

Live viral vaccines are of four categories:

I. **Selection of a naturally weak strain.**

A good example to cite on selection of a naturally weak virus strain that can efficiently multiply in the host to elicit protective immune response without causing clinical disease is Newcastle disease virus (NDV), which is characterized by having great variation in the pathogenicity of its different strains. Thus, NDV strains are classified, from most to least virulent, as velogenic, mesogenic and lentogenic pathotypes.Velogenic pathotypes are further divided into velogenic vescerotropic pathotypes, which cause predominantly diarrhea and visceral hemorrhages. Velogenic neurotropic pathotypes, predominantly cause respiratory and nervous signs. Velogenic strains commonly cause acute disease in chickens with high mortality. Mesogenic strains of NDV may produce acute respiratory and nervous signs with less than 10% mortality. lentogenic strains are associated with subclinical infection marked by mild respiratory signs (OIE terrestrial manual, 2018).

Experimental classification of NDV strains is based on determination of the Median Death Time (MDT) upon allantoic inoculation of chick embryos. Mortality at less than 60 hours, 60-90 hours and more than 90 hours are considered velogenic, mesogenic and lentogenic, respectively. The Intracerebral Pathogenicity index (ICPI) which is based on clinical signs and death pattern in infected birds is also used in classifying NDV pathotypes.

Both mesogenic strains of NDV like Kamarove strain (K strain), Roaklin and Muktiwas and lentogenic strains likeHitchinersB1, F as well as Ulster and V4 have been extensively used as live vaccines against ND. However, only lentogenic vaccine strains with ICPI >0.4 are allowed as commercial vaccine (Oris *et al.*,2006).

II. **Selection of temperature sensitive mutants.**

Temperature sensitive mutants (TS) are viruses adapted to grow at suboptimal temperatures; generally, they have reduced virulence and can be used as vaccines. The virus is selected for 2 related properties e.g. replication at a lower temperature and inability to cause viremia. The Cold adapted influenza vaccine to be used intranasally exemplifies this (33 C in most mammalian species).

III. **Attenuated live vaccines.**

The principal of attenuation is based on selecting a wild strain of a pathogen (virus or bacteria) that lends itself to attenuation through serial passage in a non-natural host such as laboratory animals, avian embryos, culture media or cell cultures. Adaptation to growth in such a non-natural host is accompanied by progressive loss of virulence for the natural host, and is considered a process that selects for a vaccine strain with an ability to multiply in the host without causing clinical disease i.e. preparation of high immunogenicity and restricted pathogen city. Thus, the optimal attenuation level has to be determined, often at a passage level where the attenuated strain induces mild clinical signs in few of the recipient animals.

IV. **Recombinant vaccines.**

Recombinant vaccines are those utilizing bacteria and viruses as vectors for expression of viral antigens, or deletion of a virulence related gene from an organism.

The first concept involves inserting the gene coding for the antigen of a virus causing the disease of interest into the genome of an avirulent bacteria or a DNA virus or a plasmid. This modified avirulent bacteria, virus or plasmid is then administered as a live vector that replicates within the host expressing the viral epitopes on the surface of bacteria and / or viruses. Enteric bacteria that multiply naturally in the gut would be ideal for DNA viruses as vectors. Other examples are the human Hepatitis B surface antigen (HBs.Ag.) gene flanked by the non-essentialvaccinia gene for thymidine kinase and its promotor was inserted into a plasmid. Mammalian cells infected with vaccinia virus and then transfected with this chimeric plasmid produce

vaccinia virus carrying the HBs. Ag. by homologous recombination (Hilleman, 2003).
The recombinant virus is then used as a hepatitis B vaccine. Several RNA viruses such as picorna viruses and sindibis virus are also being developed as vectors.
The second concept involves construction of mutant viruses by deletion of genes essential for the virulence or viability of a pathogen within a host organism. e.g. Deletion of thymidine kinase gene from pox virus.(Shyambabu *et al.*, 2019).

1.2. **Live bacterial vaccines**
Live attenuated bacteria such as the attenuated strain of tuberculosis, which is a bovine strain of *Mycobacterium tuberculosis* attenuated by several hundred serial passage in bile containing media, and known as Bacilli Calmette- Guerin (BCG) which is used to vaccinate humans against tuberculosis.
Other examples of attenuated bacterial vaccines are those which are used as vehicles to deliver antigens such as *S.typhi*. There are currently a number of vaccines being developed based on communal microorganisms such as vectors (Lactococcus, Streptococcus, Lactobacillus and Staphylococcus) or attenuated pathogenic organisms (Shigella, Bacillus, Yersinia,Vibrio, Corynebacteria, and Bordetella), all of which are being evaluated for their ability to induce protective immunity (OIE Terrestrial Manual, 2018).

2. Dead or inactivated vaccines.
Some organisms cannot be attenuated and they have to be inactivated to be used as vaccines. Inactivated vaccines can be produced from inactivated whole organism (virus or bacteria) or from a fraction of it. Pathogens are inactivated chemically by addition of materials that reduce infectivity but preserve antigenicity such as formalin, phenol, ethyleneimine or B- propyl-lactone. B- Propiolactone and ethyleneimide become completely hydrolyzed to non-toxic products in a few hours. Pathogens can also be inactivated by physical means e.g. heat or irradiation-(UV or Gamma rays).
However, the chemical or physical treatment used to reduce infectivity may damage immunogenicity and may result in an immune response that is shorter in duration, narrower in antigenic spectrum, weaker in cell mediated and mucosal immune responses, yet they are effective against extracellular organisms because they induce an adequate humoral immune response. For these reasons, the vaccination course comprises two or three injections and further booster doses. Adjuvants may also be needed to maintain immunity (Bergman *et al.*,1996).

2.1. Dead viral vaccines

I. Inactivated whole organism (virus or bacteria) vaccines.

The principal here depends on obtaining a large amount of the pathogen and then subjecting it to the inactivation process. The first step is the acquisition of an appropriate antigenically stable strain of a known causative agent of a disease of a particular animal species and establishment of a seed bank free from contaminants. It is also important to be sure that the growth requirements and conditions consistently yield a safe, stable and effective product, preferably following the guide lines of a recognized certified system of vaccine manufacture such as that of the European Pharmacopeia (European Pharmacopeia 7.0 -2012) or that of the OIE(OIE Technical Standards for manufacture and quality control of veterinary vaccines, 2019).

Subunit vaccines.

The concept that the entire organism is not needed to elicit protective immunity came about when Hepatitis B vaccine was prepared as purified hepatitis B surface antigen (HBs.Ag.) from human blood of chronically infected carriers of the virus. (Hilleman, 2003).Those observations brought about methods to produce subunit or split vaccines that have been extracted from cultures or rDNA and have been shown to be safe because they contain only the essential antigens and not all the other molecules that make up the microbe,a matter that reduces the chances for adverse reactions to the vaccine.

Two approaches are used to obtain split vaccines:
- Lipid solvents such as sodium deoxycolate are used in the case of enveloped viruses to solubilize virions and release the components including the glycoprotein spikes of the envelope.
-Fractionation of viruses and use of suitable purified fractions such as the capsids or coats. Differential centrifugation is used to semi purify these components which are used as split vaccines. Examples of split vaccines are those of herpes

viruses, corona virus and influenza virus (Young *et al.*, 2014).

II. Recombinant DNA (rDNA) proteins.

Formation of recombinant DNA is generally made by finding the particular gene, cut it out and insert it into the genome of another organism. Generally the steps for engineering rDNA vaccine protein are:

1) Identification of the immunogenic proteins which is made by dissection of the microbe into subunits and separation of those subunits, usually by polyacrylamide gel electrophoresis and testing their biological activity.

2) Identification of the genes coding for these proteins is done by expressing these genes in a suitable expressing system (vectors) in vitro and precipitating the products with neutralizing antibodies.

3) Several vector systems are available for its expression. Two very commonly used vectors are plasmids and bacteriophage. Plasmids are none essential extra chromosomal circular DNA molecules present in many bacteria. Because plasmid DNA is different from bacterial DNA they can be separated by density gradient centrifugation.

To achieve this, the DNA segment has to be covalently linked to the vectors DNA which is able to multiply within the bacteria. The inserted DNA segment replicates as the bacteria multiplies and expresses the foreign gene product in form of a protein, which can be purified and be used as an antigen (vaccine).

Bacteriophage Lambda who's DNA contains two *E coli* RI sites is extracted and treated with E ColiRI and then inserted into the bacterial genome.

If, however the gene is a part of an RNA virus genome, it must first be transcribed into DNA before it can be inserted into the vector DNA- (using reverse transcriptase).

Recombinant proteins can be manufactured in great quantities and have the advantages of freedom from fragments responsible for side effects. However, they are difficult to remove from cells in which they were produced.

III. Synthetic peptides vaccines.

Synthetic peptides containing only the epitope of a protective antigen can also be used as vaccines.

IV. Purified DNA, Plasmid, or Gene Vaccines.

It was observed earlier in the 1960s that cutaneous inoculation of phenol extracts of DNA from Shope Papilloma virus induced papilloma in rabbit skin at the site of inoculation.

It was also observed in the early 1990s, that a plasmid construct that included the B- galactosidase gene, when inoculated into mouse skeletal muscle, expressed the enzyme for up to 60 days post-inoculation.

It was also observed that, for many viruses, completely viral DNA, RNA or cDNA of viral RNA when transfected into cells could undergo full cycle viral replication. It was then, deduced that viral DNA can be used as a vaccine. In principal, the technique cuts out the step of constructing an infectious viral or bacterial vector.

Recombinant plasmids are constructed to contain genes capable of expressing viral antigens. The DNA inserted in the plasmid, on injection transfects cells. The expressed protein elicits an immune response that stimulates, for the particular antigen of interest, the response elicited by the viral infection. Both humoral and cell mediated responses are detectable and are protective (David Weiner and Kennedy, 1999)

2.2. Dead bacterial vaccines.

Dead or inactivated bacterial vaccines are divided into:

1). Killed bacteria such as the *Vibrio cholera* vaccine and *Bacillus anthracis* vaccine.

2). Capsular polysaccharide vaccines such as those derived from many serotypes of *Streptococcus pneumonae*, *Niseriameningitis*, and *Hemophilus influenzae*.

3). Inactivated exotoxins or toxoids, such as those prepared from *Corynebacterium diphtherae* and *Clostridium tetani* and *Clostridium chouvaei* (OIE Terrestrial Manual, 2018).

Vaccine Production

In all cases of vaccine production, two factors are of paramount importance; these are the establishment of a seed bank and the system of vaccine production.

The first step in the establishment of a seed bank is to acquire an antigenically consistent and stable strain of a known causative agent of a disease of a particular animal species e.g., Brucella strain 19 and ensure that it is free of contaminants. The

second step is to ensure that the growth requirements and conditions consistently yield a safe effective and stable product.

Aliquots of the seed should be kept under cold conditions (-70 C or in liquid nitrogen or lyophilized), for long periods of time, in such a way that production could be started without subjecting the strain to serial passages each time, thus maintaining the appropriate passage level in case of attenuated vaccines.(OIE Terrestrial Manual, 2018).

Microbial fermenters are often used in mass vaccine production plants to speed up strain selection, increase production and optimize manufacture conditions. In fact, the introduction of the Goettingen-IBT bioreactor technology for vaccine production to the Sudan in 1985 resulted in significant improvements in bacterial vaccine production both quantitatively and qualitatively. In addition to other benefits, the running cost of production was reduced by at least 80% (Babiker, 1996).

It is also important that vaccines should be manufactured in accordance with recognized certified systems such as the European Pharmacopeia European and OIE guidelines; that include specifications regarding premises, equipment, qualified personnel, and strict quality assurance systems including establishment of a seed bank, safety, potency and purity of the vaccines (OIE Terrestrial Manual, 2019).

Vaccines Produced and Used in Sudan

In reviewing the production of veterinary vaccines used in the control of livestock diseases in Sudan, it would be more informative and more beneficial to cover the topics of how vaccines were made in the past, including the origin and characteristics of the vaccine strains, production procedures and quality control measures employed to ensure safety, efficiency and purity of the vaccines according to the OIE technical standards for manufacture and quality control of veterinary vaccines (OIE, 2019),and also cover how vaccines were improved to provide the vaccines in use today, and some future prospects as major advances in biology and biotechnology are in progress.

Most of the important animal diseases have been diagnosed in Sudan e.g. Rinderpest, Pestes des Petite Ruminants(PPR), Foot and Mouth Disease, Rift Valley Fever, Contagious Bovine Pleuropneunia, Anthrax, Hemorrhagic Septicemia, Black Quarter, Brucellosis, Bovine Tuberculosis, Bovine Farcy, Lumpy Skin Disease (LSD), Sheep Pox, Caprine Pleuropneumonia, Heart Water,. African Horse (AHS), New Castle Disease, Fowl Pox, Fowl Cholera and many parasitic infestations.

These diseases, collectively and individually, cause high casualties within livestock in Sudan, estimated to be 100 million tropical animal unit (TAU) including wildlife, and consequently have a negative impact on the socio-economic buildup of the Sudanese community.

For decades, the veterinary authorities in the country used vaccines besides other control measures to combat and contain various disease incidences. Vaccine production started as early as 1924 using classical and conventional means of processing (Babiker, 1996). With the progress of vaccine production the Sudanese Veterinary authorities, supported technically and financially by international donors were able to introduce modern technologies replacing the conventional means. Besides a few imported vaccines 15 viral, bacterial and Mycoplasma vaccines are currently produced and/or used in Sudan (Ibtisam *et al.,* 2018).

Vaccine production is mainly conducted in the Central Veterinary Research Laboratory (CVRL) in Khartoum (Soba) with limited activities in two regional laboratories in Nyala and Al Obeid. Production and application of the vaccines strictly abides with the World Organization of Animal Health (OIE) standards and recommendations.

The following is a brief description of the veterinary vaccines produced in Sudan with special emphasis on their production and use.

Vaccination is based on one or more of the following policies and/or strategies:

1. Normal disease control polices, in case of endemic diseases i.e. border vaccination where vaccination is conducted along the border of an infected area or zone to prevent spread of infection from or into a neighboring country or zone; or ring vaccination of all animals around an infected focus.
2. Eradication polices (Elimination of a pathogen from a country or zone e.g. Rinderpest, where annual massive or blanket vaccination is conducted for several years. An immune status study is usually done.
3. Stamping out polices: Killing of all animals which are affected and those suspected or are in contact (vaccinated or unvaccinated) and their carcasses destroyed by burning or burial, followed

by cleaning and disinfection, and then introduction of vaccinated animals.

4. Export and import policies, where target vaccination is conducted; quarantine and certification is required.

1. Viral disease vaccines

1.1. Rinderpest Virus Vaccine.

Rinderpest or cattle plague, the most important disease of cattle in Sudan, is caused by a morbilli virus that belongs to the Family Paramyxo viridae.

Prior to the use of vaccines, serum against Rinderpest was ordered from South Africa and Egypt. Then an anti Rinderpest serum production station was established in Malakal in 1927(Gillispie, 1966).

This was followed by the introduction of the first animal virus vaccine (The Rinderpest spleen tissue vaccine) produced in Sudan in 1935 (Bennet, 1935).This tissue vaccine was used to supplement serum treatment, a practice that effectively reduced the incidence of Rinderpest in the country at that time (Sudan Vet. Authorities Report 1933-1936), a fact that encouraged the veterinary authorities to establish a laboratory in Nyala for the production of an inactivated glycerinated, lyophilized Rinderpest spleen tissue vaccine in August 1947(Wilson, 1979).

The attenuated Rinderpest goat virus vaccine, originally adapted to goats in East Africa (Doubney, 1949) was introduced into Sudan in September 1950. Two years later a lapenizedRinderpest vaccine (The Nakamura strain) was also used. Both vaccines were imported from Kenya and continued to be used until 1959 when Adlan and Evans (1961), started the production of the attenuated Rinderpest goat virus vaccine in Sudan.

The cell culture attenuated Rinderpest vaccine production was introduced to Sudan by Ali in 1970. (Ali, 1971) The seed virus was the Kabete O Rinderpest virus at its 98th passage level in calf kidney cells. The virus strain was originally adapted to tissue culture by Plowright and Ferris in 1959 (Plowright and Ferris, 1962). This vaccine was used in the Inter-African Campaign against Rinderpest (JP15) that was started in West Africa in 1962, continued through six stages, and ended in 1976. During this campaign 120 million animals were vaccinated in Africa (about 22 million dosed were used in Sudan between 1969 and 1976).The successes of JP15 were evident at the end of the campaign where only two countries in Africa reported the disease while it was prevalent in 17 countries at the beginning. In Sudan 176 reports were registered in 1969, at the beginning of the campaign but only one report was registered at the end in 1976 (Hassan, and Hajer, 1989). The vaccine is still in use in Sudan.

Because the nucleotide sequence of the full PPR virus genome was found to be very similar to that of the Rinderpest virus, the two genomes were considered homologous (Dalan *et al.*,2005). Accordingly, in Sudan, vaccination of sheep against Peste des Petits Ruminants using the Rinderpest Kabete O tissue culture attenuated vaccine strain was established since 2002, but no organized vaccination campaigns were practiced (Intisar *et al.*, 2009).

1.2. Rift Valley Fever Vaccine.

The causative agent of Rift valley fever is the insect transmitted Rift Valley Fever virus (RVFV) that belongs to the family Bunyaviridae, genus Phlebovirus. There are now three licensed commercially produced vaccines, the Smithburn vaccine and the Formalin-Inactivated vaccine, and Clone13. These vaccines are produced by three different laboratories, The Onderstepoort Biological Products limited (OBP) in South Africa, Kenya Veterinary Vaccine Producing Institute (KEVEVAPI), and Egypt's Veterinary Serum and Vaccine Research Institute (EVSVRI), respectively. The two vaccines currently used for the control of RVF in Sudan, Saudi Arabia and Egypt are a live attenuated vaccine and an inactivated vaccine. The live attenuated vaccine is based on the Smithburn Vaccine Strain, which was derived from the virulent Entebbe strain, isolated from mosquitoes in Western Uganda in 1944 and passaged 79-85 times by intracerebral inoculation of mice (this resulted in loss of hepatotropism, acquisition of neurotropism and the capacity to immunize sheep safely when administered parentally (Smithburn, 1949). The 103 and 106 mouse brain passage levels of the virus are used to produce the vaccine in cell culture in South Africa and Kenya respectively, using BHK cells.

Because live vaccines of Rift Valley fever based on the Smithburn virus have induced abortions and teratogenesis in the fetuses of vaccinated dams an inactivated RVF vaccine was produced to vaccinate cows that can then confer cloistral immunity to their off springs. Given the poor immunogenicity of this vaccine in cattle, it requires a booster three to

six months after initial vaccination, followed by annual inoculations (Barnard, 1979); for those reasons, Clone13, which is a naturally attenuated isolate of RVF virus with a large deletion in the S segment, was introduced. It was cloned by plaque purification of non-fatal human case isolate (74HB59 strain), obtained during 1974 RVF outbreak in Central African Republic, and proved to be highly immunogenic leading to long-term immunity (Bird *et al.*,2008).

1.3. Lumpy Skin Disease (LSD) Vaccine.

In Sudan, LSD is controlled by vaccination. Two vaccines are currently available: an attenuated live virus vaccine (Neethling) passed 60X in lamb kidney cells followed by 20 passages in embryonated chicken eggs, from South Africa, and from Kenya a low passage strain of sheep/ goat pox propagated in tissue culture. The latter has very recently been produced and used in Sudan. (Ibtisam *et al.,* 2018).

1.4. Sheep pox

Attenuated live virus and inactivated vaccines are available. Recombinant vaccines based on the use of Capri Pox Viruses as vectors are in development. In Sudan, the attenuated live vaccine is produced in Lamb Testicle cell (LT) monolayers. The harvest is mixed with an equal volume of chilled 5% lacto albumen hydrolysate and 1% sucrose and kept in lyophilized form. (Abbas *et al.,* 2007)

1.5. Camel Poxvaccine

Camel pox is caused by Orthopox virus camelivirus, which belongs to the genus orthopox virus that belongs to the family Poxviridae. Based on nucleotide sequencing Camel Poxvirus is considered very closely related to variola virus, the etiological agent of Small Pox. Attempts to attenuate camel poxvirus in Vero cells had successful results. There are intentions to produce a live attenuated vaccine in CVRL (Ibtisam *et al.,* 2018).

1.6. Foot and Mouth Disease vaccine

Foot and mouth disease (FMD) is caused by a virus of the genus *Aphthovirus*, family *Picornaviridae*. It was the first animal virus to be identified by Loeffler and Froschin (1898) as a non filterable agent. It was also the first virus shown to have antigenic plurality as shown by Vallare and Curre in 1922. (There are seven serotypes of FMD virus namely; O, A, C, SAT 1, SAT 2, SAT 3, and Asia 1, that infect cloven-hoofed animals). Infection with any one serotype does not confer immunity against another.

The importance of FMD is that it causes drop in productivity, and has a negative impact on trade in animals and animal products. The epidemiology of FMD in endemic regions of sub-Saharan Africa has unique features that render the control of the disease extremely complex: due to the prevalence of six of the seven FMD serotypes and the reservoir role played by wildlife.

Vaccines currently used against FMD throughout the world are multivalent to provide protection against the different serotypes likely to be encountered in a given region. For vaccine production, the virus is grown in BHK cell culture and the resulting preparation is clarified, inactivated using aziridine compounds (usually binary ethyleneimine, concentrated and blended with adjuvant (either saponin/aluminum hydroxide gel or various oil emulsions) to potentiate the immune response of the host. (Doel, 2005; Lubroth *et al.,* 2007).

Different forms of vaccination programmes are implemented in different regions of the developed world. The duration of immunity induced is short and booster inoculations need to be administered at 4 to 6 monthly intervals in most animals. In Sudan, the vaccine is imported and vaccination is conducted only on requirement from import counties.

1.7. Blue tongue Vaccine.

Bluetongue is an arthropod-borne viral disease of sheep and cattle, caused by a virus that belongs to the genus *Orbivirus of the* family *Reoviridae*.The virus has 24 known serotypes transmitted by *Culicoides* spp. vectors.

Eradication of BTV is second to impossible because of the multiplicity of serotypes; the ability of the virus to overwinter in the absence of adult vectors, and its persistence in healthy reservoir hosts such as cattle and some wild ruminants,

besides the widely distributed *Culicoides* spp. midge vectors.
Control of BTV in endemic regions of Africa including Sudan is done through vaccination.
The initial BT vaccine was developed more than 50 years ago in South Africa and has been improved over time to include 15 of the 24 serotypes known to occur in Southern Africa (Verwoerd. and Erasmus, 1995).
The vaccine currently used in Sudan, isa live attenuated pentavalent, cell-adapted, plaque-purified viruses obtained from South Africa. The teratogenicity of attenuated BT vaccine strains resulting in brain defects in the fetus when administered during the first half of gestation, the risk of reassortment and recombination between attenuated and virulent strains in the field and differences in the genetics of sheep and their susceptibility to BTV have lead some countries to opt for the inactivated or other forms of non-replicating vaccines. Inactivated BT serotype 2 and 4 vaccines have been developed produced and used (Boutrand *et al.*,2003, Di Emilio *et al.*, 2004).

1.8. African Horse Sickness vaccine

African horse sickness virus (AHSV) along with bluetongue virus belongs to the genus *Orbivirus* of the family *Reoviridae*. The virus is transmitted between equine hosts by Culicoid spp. (Anthony *et al.*,2009). To date, nine antigenically distinct serotypes have been identified. Of the nine serotypes, types 1 to 8 are found only in restricted areas of sub-Saharan Africa while type 9 is more or less widespread and has been responsible for virtually all epidemics outside Africa,
The earliest live attenuated vaccine was produced in South Africa by serial sub-passaging the virus some 100 times in embryonated eggs and intracerebral passage in adult mice brains. However, problems were encountered, with some of the mice brain attenuated strains causing neurological problems in vaccinated horses. Changes were made to the attenuation process, by reducing the number of egg passages or sub passaging using suckling mice brains or cell culture.A safer polyvalent vaccine adapted to Vero cells was developed by Erasmus in the Onderstepoort Veterinary Research Institute. (Erasmus, 1976) This led to the decision that all strains should be cell culture attenuated.
Inactivated recombinant polyvalent AHS vaccines were produced in the early 1990s (House, 1998). With the VP2, VP5 and VP7 baculovirus-expressed combination that was shown to induce neutralizing antibodies and protection (Martinez-Torrecuadrada *et al.*, 1996).
In Sudan a trivalent vaccine containing type 3, type 6 and type 9 of the neurotropic African Horse Sickness Virus strains after having been passaged 7 times in VERO cell cultures is produced. The seed bank was established from a local low passage isolate (not exceeding 6 passages) (Ali and Hajer, 1988).

1.9. Newcastle Disease virus (NDV) **vaccines**

.Newcastle Disease **(**ND) is caused by (NDV), a member of the genus rubulavirus of the family paramyxoviridae. ND is included in the list of communicable animal diseases of the Office International des Epizooties (OIE). (NDV) is characterized by having great variation in the pathogenicity of its different strains, which are classified, from most to least virulent, as Velogenic, mesogenic and lentogenic pathotypes.
Both mesogenic strains of NDV like Kamarove strain (K strain), Roaklin and Muktiwas and lentogenic strains like HitchinersB1, F as well as Ulster and V4 have been extensively used as live vaccines against N D. However. Only lentogenic vaccine strains with ICPI >0.4 are allowed as commercial vaccine (Oris *et al.*, 2006).
. The Kamarove (K) strain of NDV was first introduced to Sudan from Lebanon and has been recognized by Sudan Veterinary authorities since 1958 as the appropriate live vaccine and was propagated in chick embryos (Karar and Mustafa, 1964).
With the introduction of different breeds of poultry into Sudan milder lentogenic NDV vaccine strains such as Lasota, Hitchiner F and AVINEW(VG/GA strain) were obtained from commercial sources.
Depending on poultry husbandry conditions NDV vaccines are administered through nasal droppings, aerosol or in drinking water according to manufacture instructions.
Currently a temperature stable NDV vaccine strain is under trial in the Central Veterinary Research Laboratory in Khartoum (Ibtisam *et al.*, 2018).
The problem that could be faced in the future is the fact that some temperature sensitive mutants have the tendency to revert to virulence during replication in vaccinated animals.

1.10. Fowl pox Vaccine

Fowl pox is an important serious disease of poultry worldwide. There are two forms of the disease, the cuteness form and the diphtheritic form. Effective vaccines have reduced the great losses caused by the disease.

In Sudan, the Baudette vaccine strain of fowl poxvirus, of known history is used as a seed virus. The vaccine bulk final harvest is subjected to standard quality control tests including safety, potency and immunogenicity tests and issued in lyophilized form (Ali and Hajer, 1988).

1.11. Infectious bursal disease vaccine.

Infectious bursal disease (IBD), known as Gumboro,is an acute highly contagiousviral disease of young chickenscharacterized by severe bursal legions and immune suppression. The infectious bursal disease virus (IBDV), is a member of the avibirna virus genus that belongs to the family Birnaviridae. (Wu *et al.,* 2007).Two serotypes of IBDV, designated as type 1 and 2 are so far recognized.

Although the two serotypes infect chickens and turkey, vaccines are produced against type 1 only (Silva *et al.,* 2016).

Together with strict hygiene vaccination with live attenuated strains is often used to control Gumboro. Because live attenuated vaccines cause bursal atrophy and consequently low immune response to other vaccines, inactivated, subunit and viral vector vaccines have also been used. (Arnold *et al.,* 2012).

In Sudan, the disease was first reported by Shuaib *et al.,* (1982) and is considered to be endemic since then.

Commercial live vaccines such as Gumboro D/78 strain originally isolated in Delaw are , USA in 1962 and the CEVAC8 IBDL vaccine containing the Winterfeild 2.512 strain of IBDV(CEVA company-Budapest Hungary) are used to vaccinate young chickens. However, experimental trials are underway in the Central Veterinary Research Laboratory to produce a live attenuated IBDV vaccine by attenuation of amild local field isolate of IBDV via serial passage in embryonated eggs , or in tissue culture.(Ibtisam *et al.,* 2018).

Table 1.Summarizes veterinary viral vaccines produced or used in Sudan

S/No.	Viral Vaccine	Description
1.	Rinderpest& PPR	Live attenuated tissue culture vaccine
2.	Rift Valley fever	1. Smithburn based live attenuated and grown in BHK cells. 2. Also a killed vaccine.
3.	LSD	1. Live attenuated vaccine containing Capripox strain. 2. Neethling strain passed 60X in lamb kidney cells and followed by 20 passages in embryonated chicken eggs.
4	FMD vaccine	Multivalent vaccine grown in BHK and inactivated, used for export purposes.
5.	Bluetongue	Live attenuated pentavalent cell adapted and plaque purified vaccine.
6.	Sheep and goat pox vaccine	Live attenuated Capripox virus cultured in lamb testis cells
7.	AHS	Trivalent inactivated vaccine-containing type 3, 6 and 9 and passed 7X in VERO cells.
8.	NDV vaccine	1. Mesogenic Kamarove strain. 2. Lentogenic strains: Lasota; Hetchiner B and F;AVINEW(VG.GA).
9.	Fowl pox	The Baudettee attenuated live vaccine.
10	IBDV	Commercial live.

2. Bacterial vaccines produced in Sudan

Currently the Central Veterinary Research Laboratory (CVRL) and the regional laboratories in Sudan produce six bacterial vaccines, which are used locally against the following diseases:

2.1. Anthrax:

The disease was first recognized in Sudan in 1900-1901 (Smith, 1902;ElNasry, 1966).It became enzootic and was annually reported

nation-wide affecting many domestic animals and wildlife in the whole country (Musa *et al.,* 1993).
Before1946, the disease was controlled through quarantines. In that year Sudan Veterinary Authorities decided to vaccinate all export animals. Mass vaccination of animals in the field started in 1951.
The anthrax vaccine strain used in the Sudan is based on a seed lot from *B.anthracis* Sterne strain 34 F2developed by attenuating a toxigenic, rough variant of a virulent *Bacillus anthracis* from culture onto Serum Agar in an elevated carbon dioxide atmosphere. The variant Sterne 34F2 was incapable of forming a capsule (Mohammed Ahmed *et al.,* 2007).
For vaccine production, the master seed is prepared from Sterne 34F2 strain preserved as live spores and stored in lyophilized form and propagated to sporulate onto solid agar medium. When used as a vaccine the proportions of sporulated cells is adjusted to be at least 70%.. The spore culture is washed in normal saline and the vegetative cells are killed by heating on a water bath at 65C, then twice the volume of sterile pure glycerol is added. Saponin is added to make a concentration of 0.1%. Fermenters are often used to prepare vaccines in liquid media with an incubation period of 30-45 hours.
This type of vaccine is effective but repeated vaccinations are required for long- term protection; a single dose will only provide immunity for about a year. The regular vaccination practice with the live spore anthrax vaccine remarkably reduced the prevalence rate of anthrax in animals and till now no complaint about an adverse effect attributable to this vaccine has been reported.

2.2. Hemorrhagic Septicaemia (HS)

Hemorrhagic Septicaemia (HS) is a fatal disease of cattle and buffaloes, caused by *Pasteurella multocida.* Classification of *Pasteurella multocida*is based on the identification of its somatic and capsular antigens. Accordingly, *P. multocida* can be separated into 16 serotypes based on the characteristics of their lipopolysaccharide (LPS) antigens (Heddleston *et al.,*1972). It can also be separated into 5 serogroups based on the antigenicity of their capsule designated: A, B, D, E and F(Carter and lwis, 1989). On these basis, two serotypes designated B:6 and E:6 have been found to cause HS in cattle , buffaloes, fowl cholera in birds and atrophic rhinitis in pigs.
Since *Pasteurella* is a weak immunogenic a whole cell bacteria vaccine is preferred and live attenuated vaccines can protect against heterologous serotypes (Adler *et al.,*1999).
Three preparations are used; dense bacterins combined with either alum adjuvant or oil adjuvant, and formalin-inactivated bacterins. The oil adjuvant bacterins is thought to provide protection for up to one year and the alum bacterins for 4–6 months.
In Sudan a formalized whole culture bivalent vaccine is prepared from *Pasteurella multocida* type B:2 and type E:2.which are endemic and predominant in Sudan and other African countries (Shigidi and Mustafa, 1979)., Although this vaccine was used to efficiently control an outbreak that occurred in Zambia, an Aluminum hydroxide (AlOH) and a Zinc sulphate ($ZnSO_4$) treated formalized vaccine showed superiority over the plane vaccine (Mona et al. 1995)

2.3. Black quarter (BQ) Vaccine.

Blackleg is a worldwide acute, febrile, fatal disease of cattle and sheep caused by *Clostridium chauvoei* and characterized by emphysematous swelling, commonly affecting heavy muscles (Clostridial myositis). The vaccine currently produced in CVRL and used in Sudan to protect cattle and sheep contains killed cells and formal toxoids of *Clostridium chauvoei*, as It is prepared from *Clostridium chauvoei* grown in anaerobic medium and killed by formaldehyde solution.

2.4. Brucellosis Vaccine:

Brucellosis is a worldwide, recognized bacterial zoonotic disease caused by bacteria of the
Genus *Brucella*, Gram-negative non-spore-forming non-encapsulated coccobacilli or short rods with rounded ends. Brucellosis is primarily caused by *B. abortus*, *B. melitensis and B. suis.*
The first report on the presence of brucellosis in Sudan dates back to 1908, (Amira 2015). Since then a series of studies have provided serological evidence for infection in cattle, goat, sheep and camels in different parts of Sudan (Hellmann *et al.,* 1984).

Currently three types of vaccines are used to protect cattle against *Brucella abortus:*

1. *Brucella abortus* strain 19 was isolated in 1923 from milk of a Jersey cow by Dr. John Buck (Buck, 1930). The original virulent strain was accidentally left at room temperature for one year. It was found to have lost some of its virulence when tested in guinea pigs and thus was used as a vaccine for calves. It is a smooth attenuated *B. abortus* (Mc Donald. 1957).
2. *Brucella abortus*RB51 vaccine strain is a rough rifampicin resistant strain developed in 1952. It was derived from a virulent smooth *Brucella abortusbiovar 1* strain 2308, which was a natural mutant adapted by serial passage on media containing sub-inhibitory concentrations of rifampicin and penicillin.
3. Killed *Brucella abortus* vaccines are also available.

Because immunity does not last for more than a year, the recommended vaccination procedure is first vaccination of calves above four months of age, and then a booster vaccination after 10 days and then annual vaccination

3. Mycoplasmal Vaccines..

3.1. Contagious Bovine Pleuropneumonia (CBPP) Vaccine.

The disease was first observed in 1875 in Darfur Province and later spread to Khartoum Province where it resulted in great losses among cattle. The disease disappeared during the Mahdia and it reappeared again in Kordofan Province in 1912 and became enzootic in the whole country (Shareef and Shareef, 2009).

Control of CBPP mainly includes restriction of animal movement, segregation, quarantine of infected herds and annual vaccination.

The early attempts to vaccinate against CBPP in Sudan was the nose vaccine which essentially involved the infection of a susceptible animal by inserting a slip of an infected lung from an animal suffering from CBPP into a cut made in the nose, and then cauterizing the site with a hot iron. (Wilson.1979). Because this method caused very severe swellings another method of injecting infected pleural exudates from a sick animal under the skin of the tail tip was practiced (Thiaucourt *et al.*,2003).

A safer and more effective tissue vaccine was introduced in 1929 and later on an attenuated culture and an egg adapted vaccine were introduced in 1952- 1953. Both vaccines are known to sometimes cause local reactions and tend to lose their potency in a short time.

In Africa, three strains are widely used as vaccines: the T1-44, the T1-SR and the KH1.The T1-44 is a naturally mild strain isolated in Tanzania in 1951.The 44 passage level is sufficiently attenuated and is being used as a vaccine.

The KH1(Khartoum1 or F strain was isolated in Sudan in 1944 from pulmonary exudates of a naturally infected case of a field strain of *M. mycoides* sub sp.*mycoides*.A seed from this strain was adapted by sub culturing in glycerin broth and then passaging in embryonated eggs and used as a vaccine in its 88th passage. (OIE Terrestrial Manual, 2018).The recommended dose was 1 ml.

During the 1970s, the FAO funded a technical cooperation programme in Sudan (TCP/ SUD/3001 A)) for CBPP surveillance. The TCP programme envisaged zoning of the country into infected, surveillance, buffer, and disease free areas, with specified disease control activities for each zone.

The successful implementation of this TCP resulted in increased knowledge of the status of CBPP in the country and upgrading of the technical capacities of field personnel and provision of adequate supplies for vaccine production that resulted in production ofa lyophilized form of CCPP vaccine facilitating its application and usage.

3.2. Contagious caprine pleuropneumonia (CCPP) Vaccine.

Contagious Caprine Pleuropneumonia (CCPP) is one of the most serious diseases of goats in Africa and Asia. Accordingly, it is included in B list of communicable animal diseases of the Office International des Epizooties (OIE).

In Sudan, the disease is widely distributed with higher incidences in winter and rainy months. It represents a real threat to goat population in the country. Although it is difficult to make a precise estimation of the economic impact of CCPP, it is obvious that losses due to the disease are considerable. Thousands of animals die annually in different states of Sudan due to CCPP outbreaks. In addition, restrictions in exportation

of goats due to CCPP affect the contribution of livestock in the national income. This situation prompted the veterinary authorities to fund trials for CCPP vaccine production. (Ghoraish *et al.*, 2018).

Table2. Summarizes veterinary bacterial and Mycoplasma vaccines produced or used in Sudan.

S/N	Vaccine	Description
1	Anthrax	Strain 34F2 sporulated in liquid media with glycerol and saponin added as adjuvants..
2	H.S.	Formalized whole culture bivalent vaccine, prepared from *Pasteurella multocida* types B-2 and E-2.
3	BQ	Formalized whole culture grown in anaerobic liquid medium.
4	Brucella	*Brucella abortus* strain 19 to be used for export purposes.
5	CBPP	*Mycoplasma mycoides* adapted by sub culturing in glycerine broth and then passaged in embryonated eggs and in its 88th passage level.
6	CCPP	Attempts are being made to produce a vaccine against CCPP in CVRL.

Conclusions

In a country like Sudan , with an animal wealth estimated to be 100 million tropical animal units (TAU), and an excellent geostrategic position as far as marketing of animals and animal products are concerned; together with veterinary services and research institutions established since the turn of the 20thCentury, and research facilities and trained personnel available at CVRL, much more emphasis should have been placed on vaccine production: so much as to cover the need for control of all diseases in the OIE list of socioeconomic significance for the country and the region.

Research and development should form the basis for the modernization of vaccine production techniques and generation of new and improved veterinary vaccines so that CVRL becomes rated as a research incubator and a reference vaccine production laboratory in Africa and Arab region.

Recent advances in bio-technology and information technology provide enormous promises and opportunities; however, political will, strategic planning adequate funding and proper training of qualified staff are needed.

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