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Development and Dosage Determination of the "Muguga Cocktail Vaccine": An "Infection and Treatment Method" (ITM) for Managing East Coast Fever in Cattle in Pakistan

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Abstract:- "*Theileria parva*" (TP), an "apicomplexan parasite" that causes "East Coast Fever" (ECF) in cattle, in Pakistan. East coast fever (ECF) is a disease spread by ticks that is thought to cause at least $300 million in annual economic losses. The production procedure & other facets of the vaccine have not been sufficiently standardised, despite the fact that it is the only commercially available vaccination for this lethal cattle illness. Notably, the "Pakistan Veterinary Research Institute (PVRI)" produced its most recent production batch at the beginning of 2022. A pathogen-free tick colony and parasite-free cattle are used in the manufacturing of this vaccine, and both are closely watched during the entire procedure. This article describes the procedure used in the most current Infection and Treatment Method (ITM) vaccine production and introduces process improvements that are not present in earlier production versions. These upgrades include improved quality control measures. The report also describes how a three-phase in-vivo investigation with different vaccine stability dilutions are used to establish the ideal field dose. After post-production evaluations verified the vaccine's viability and safety, it is decided that a field dosage of one millilitre (ml) at a 1:100 weakening would be appropriate.

Keywords:- *Theileria parva; East Coast Fever; Pakistan Veterinary Research Institute; Vaccine.*

I. INTRODUCTION

Theileria parva (TP) is the cause of the severe sickness called as "East Coast fever" (ECF), which affects livestock. The "*Rhipicephalus appendiculatus* brown ear tick" is the trajectory for the spread of this parasite. According to Mc Leod and Kristjanson (Mc Leod and Kristjanson 1999), the disease affects 11 nations in Africa (central, eastern, and southern states) and has a substantial economic impact with $300 million in annual losses. The ITM, a vaccine strategy, is fortunately accessible to protect cattle from East Coast Fever (ECF). During this approach, live *T. parva*-sporozoites are inoculated into the animals by intramuscular together with a long-term effecting Procaine Penicillin G and Streptomycin therapy (Radley 1981). A homogenate of infected ticks that has been partially purified serves as the sporozoite preparation used in this procedure Patel, Mwaura et al. (2016).

This technology has evolved as a result of important discoveries. Ten adult "*R. appendiculatus* ticks" that are infested by *T. parva* are applied to calves, according to Theiler and du Toit's 1928 research (Theiler 1927). The ability of a suspension of homogenised ticks carrying *T. parva* to contaminate animals is also established. In a second study, (Neitz 1953) showed that Procaine Penicillin G and Streptomycin by a dosage of ten mg/kg might prevent early-stage infections in cattle from progressing to clinical ECF. Based on their work, (Wilde, Brown et al. 1968) demonstrated that cryopreserved sporozoite solutions may be delivered to animals while still maintaining their infectious qualities when kept in liquid nitrogen. Further investigation by (Radley, Brown et al. 1975) indicated that immunising animals through a range of the "parasite stabilates" resulted in larger shield than immunising them through only one stabilate, probably due to antigenic variation across *T. parva* strains. The "Muguga cocktail" (MC), which mixes several *T. parva* isolates, is created as a result of these findings and is now widely used as the primary method of animal vaccination against East Coast Fever (ECF) (Waladde, Young et al. 1993).

The "ITM Muguga-cocktail vaccine" is created with funding from the "Food and Agriculture Organisation (FAO) of the United Nations" and was initially created in the 1970s at the "Muguga laboratory Research Organisation" (Veterinary) of East Africa in Kenya (Clark, Smith et al. 2007). Several significant steps are made throughout the production process. The Muguga cocktail (MC), which consists of the 3 "*T. parva* stabilisers Mugaga, Serengeti-transformed, and Kiambu 5" is initially given to amature cattle (calves). The infected ticks are then pre-fed on calves before being delivered to the cattle as *R. appendiculatus* ticks, allowing the sporozoites to mature. To extract the required components, the infected ticks are homogenised at the end. In the ensuing years, laboratories in Kenya, Zimbabwe, and Zambia developed and produced alternate ITM formulations using single parasite stabilates.

Due to an increase in demand, the FAO approached the International Livestock Research institute (ILRI) in the middle of the 1990s to create a big batch of the ITM vaccine (Morzaria 1997). This led to the development of a comprehensive protocol that included in-vivo cross-immunity testing, measures to calculate and guarantee equivalent representation of every component in the ultimate vaccine stabilate, and thorough molecular characterization of

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each vaccine component using stock-specific markers (Bishop, Geysen et al. 2001). Two batches of "the vaccine—FAO 1 and FAO 2"—are produced. In 2021, PVRI once more developed the Muguga cocktail ITM vaccine under the name ITM ECF MC PVRI08 in response to a request from regional veterinary authorities. For each parasite component, three reference stabilates are also created using the identical tick batches.

This article goes into great detail about both the production of ITM, ECF, and MC PVRI08 and the evaluation of a harmless and effective field dosage. Additionally, it emphasises the process enhancements made that weren't applied to earlier ITM vaccination batches. The implementation of more delicate quality control techniques is among these advancements. The report also covers the benefits and drawbacks of performing experimental immunisation trials as well as their effectiveness in answering important queries about vaccination potency and field safety. Random clicks of a cattle and attached tick are shown in figure 1 and figure 2.

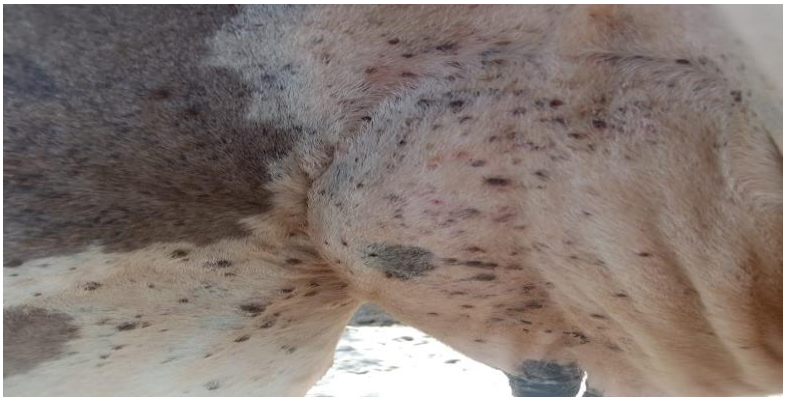

Fig. 1: Ticks prevalence on a cattle body

"The University of Edinburgh's Royal (Dick) School of Veterinary Studies" did research on the topic of livestock vaccination as a crucial strategy for managing numerous animal diseases (Allan and Peters 2021). "East Coast fever" (ECF), a sickness spread by ticks and caused by "*Theileria parva*", is now only preventable in cattle by a novel approach known as the "Muguga cocktail." In this procedure, long-acting Procaine Penicillin G and Streptomycin therapy is combined with the administration of live *T. parva* separately(Di Giulio, Lynen et al. 2009).

A thorough systematic evaluation is carried out using both meta-analyses and narrative summaries to assess the safety and effectiveness of the Muguga combo vaccination. Total 61 studies fulfilled the review's inclusion requirements. The bulk of these investigations proved or presented evidence of the "MS vaccine's safety & effectiveness". The efficiency of the vaccine is noticed to be impacted by the presence of buffalo, & "reports of vaccine component shed & transmission affected" the overall assessment of protection. The ability of policymakers and decision makers of livestock about the welfare of their livestock and their livelihoods depends on our ability to better comprehend the available control strategies for this deadly livestock disease.

Fig. 2: Ticks detached from the body of animal

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This paper has been organized into four section. The first section provides an introduction and literature review, presenting background information on the topic. The second section describes the materials and methods used, including animal selection and other relevant details. The third section presents the results obtained from the study. Finally, the last section engages in a comprehensive discussion, offering analysis and interpretation of the findings.

II. MATERIALS AND METHODS

A. Animal Selection & Ethical Approval

The "Institute Animal Care and Use Committee" (IACUC) regulations for PVRI's IACUC, are followed in all animal operations described in this work.

The PVRI Lahore farm's cattle (Bosindicus) are used to prepare the vaccine. All of these animals are sprayed with acaricides on a weekly basis as part of a tight acaricide control regime. Friesians, Friesian hybrids, or Ayrshires between the ages of 7 and 8.5 months are used in the dose determination trials. All animals are transported to the Institution of Lahore farmhouse and kept there for minimum 60 days in an environment permitted of ticks already being employed in a making and examing operations. The PVRI's small animal (calves) unit also raised and looked after, seven hundred white (calves) from Islamabad (Pakistan) and black from Indian imported calves in addition to experiment for the maintenance of the tick colony, this calve colony, which has been around for more than twenty years, acts as a closed population. In accordance with PVRI IACUC Protocol the ticks consumed the calves.

B. R. Appendiculatus tick colony

The PVRI Tick Unit has been caring for Muguga stock (MS) for *R. appendiculatus* impulses, which are utilized in the study, as a locked colony for over thirty years. This colony underwent thorough testing for viruses including the Bunya and Bovine Viral Diarrhoea viruses three decades ago, which proved it was devoid of tick-borne hemo-parasites. This colony has been utilised effectively by the PVRI Tick Unit on numerous occasions to make stabilates that were safely administered to cattle inoculations (Mutugi, Njuguna et al. 1997).

C. Screening through examination

Before being carried to the PVRI farms, livestock are examined aimed at the prevalence of parasites' tick-borne & other illnesses. As will be discussed in more detail below, the screening methods used are polymerase chain reaction (PCR), blood smears, and serology. Weekly screenings of the animals are done at the facility as a precaution.

D. Blood smear & serology

An ELISA[1] is carried out on the samples to evaluate whether cow serum had previously been exposed to *T. parva*, *Anaplasma marginale*, *Babesia bigemina*, and *Theileria mutans* (Katende, Goddeeris et al. 1990, Morzaria 1997, Katende, Morzaria et al. 1998, Tebele, Skilton et al. 2000). To find antibodies against the Bovine Leukosis Virus, a further serology test is performed utilising the "IDEXX Leukosis Serum x2 Ab Test." The presence of the aforementioned parasites as well as Ehrlichia species are determined by carefully examining Giemsa-stained blood smears.

E. PCR Analysis

A nested approach of PCR is utilised to identify *TP* in livestock using specific primers & PCR situations formed to intensify "p104 gene" (Odongo, Sunter et al. 2010). For1 and Rev1 are the initial set of primers that are intended to flank the gene. For1 primer sequence: "5'-ATTTAAGGAACCTGACGTGACTGC-3". Rev1 primer sequence: "5'-TAAGATGCCGACTATTAATGACACG-3". For2 and Rev2 are internal primers that are created to amplify a smaller portion of the gene. The For2 and Rev2 primer sequences are "5'-GGCCAAGGTCTCCTTCAGAATACG-3" and "5'-TGGGTGTGTTTCCTCGTCATCTGC-3" respectively. This approach can identify as few as one parasite per microliter of blood, according to previous sensitivity testing.

F. Screening for Extraneous Pathogens

Prior to the creation of the new ITM vaccination batches, thorough examine for bacterial, viral, and fungal contaminants is performed on all animals and seed stabilates. Biological examining is done at Laboratory of the Pakistan veterinary and research institute Islamabad MRU[2] Located in Pakistan. The 2-step opposite transcriptase method is used to target the bacteria and viruses. These viruses are chosen due to their propensity to infect cattle and the fact that they are found in the *R. appendiculatus* ticks that are found in the hilly areas near to iran (Sang, Onyango et al. 2006). Viruses include the Kupe, Dhori, Thogoto, Dugbe, Bhanja, and Kadam as well as the Crimean Congo Haemorrhagic Fever virus are tested. At the "Aga Khan University Hospital" in Pakistan's Karachi city, tests for bacteria and fungi are conducted using several antibiotic-free agars medium.

G. Immunization of cattle by Parasite Stabilates

The parasite stabilates are rapidly defrosted in a water soak at thirty-seven-degree Celsius and then put on ice. Each straw held 0.5 ml of them. Injecting 1 ml of the weak stabilate solution subcutaneously beneath and in forward-facing of the right "parotid lymph" gland required diluting the stabilate as necessary. After vaccination, animals are carefully observed for 20 minutes to look for any indications of anaphylactic shock caused on by too much tick material in the vaccine. Though no animals showed signs of anaphylactic shock throughout the observation period, the authorised procedure called for giving the animal 2-4 ml of adrenaline and withdrawing it from the research in the event that such a reaction happened. Along with the stabilising agents, the animals also received a concurrent dosage of thirty mgof Procaine Penicillin G and Streptomycin made about gluteal muscle throughout the immunisation procedure. Cattles are observed routinely to measure their parasitological and clinical responses. Temperature, the existence of the schizonts in local lymph nodes, and the existence of piroplasms in the marginal blood are among the significant factors that are noted.

[1]Indirect enzyme-linked immunosorbent assay

[2]Medical Research Unit

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According to (Anonymous 1989), the clinical reactions that are seen may be divided into four categories: non-response/reactors (NR), mild reactions, moderate reactions, and severe reactions. In non-reactors, there are no clinical symptoms or parasites found, yet there is an apparent clinical reaction. Mild reactors showed a small number of schizonts and up to a four-day fever. A persistent fever and schizonts are present in moderate reactors for more than four but less than nine days. The schizont count is high and the fever persisted for at least eight days in severe reactors. During the monitoring period, the severity and length of the animal reactions are evaluated using these classifications.

H. Stabilates of the Seed

The stabilisers seed that are engaged from the crops are Serengeti-transformed sixty-nine, Muguga seventy-three, and Kiambu 5 sixty-eight have been ordered and import from Africa. Due to the fact that they had been in storage for above twelve years following their initial creation, the sporozoites' practicability is evaluated. 2-animals (calves) each received subcutaneous injections of 0.5 / 0.7 ml of each stabilised substance, and the cattle are then watched to see how they responded.

➢ *Origin of Seed stabilates*

"The table 1 provides details on the origins of various seed stabilates used in research or production processes. These stabilates are associated with different dates and locations. For instance, Muguga Serengeti-transformed Kiambu 5 stabilate, with an unknown date, originated from EAVRO in Muguga, Kenya. Muguga 10 ST 72 K5 E542c stabilate, collected on April 22, 1988, also originated from EAVRO in Muguga, Kenya. Similarly, other stabilates, such as Muguga 147 ST 47 K5 32 and Muguga 57 ST 161e K5 102, had different dates and origins from institutions like CVL in Lilongwe, Malawi, and VPC in Lilongwe, Malawi, respectively. These stabilates play a crucial role in vaccine research and production, allowing for the evaluation of their effectiveness."

I. Generation of diseased ticks for the creation of vaccines

Each of the three seed stabilisers are administered to three groups of cattle in a different way (SC and IM). After injecting the vaccine for ten days, the cattle are relocated to the Lahore Tick Unit. The twelfth day saw the placement of unproductive *R. appendiculatus* on cotton fabric covers, that are then adhered to the cattle's spines for four days straight with contact glue. Each cow received a total of 36,000 nymphs, and after 4-6 days of self-indulgence, the nymphs split from the cows. Nymphal tick drops from each day are collected and stockpiled as a same batch. Microscopy is used to assess the parasite loads, and the nymphs are housed at 24 °C for six weeks to encourage moulting into adults.

J. Assessing the ticks' infection rate

The infection rate is determined by feeding 100 impulses from per batch on bunnies for 96 hours to permit the sporozoites. Then, thirty male & thirty female ticks per batch (representing the nerve-gathered from a 1 animal on a given time) are divided using the method outlined by (Büscher and Tangus 1986) to get their salivary glands. The diseased glands are blemished through BDH Chemicals (Schiff reagent), & a compound microscope is used to determine the approximate number of infested animals The contagion rates are determined using the average numeral of infested acini per tick, which is estimated from four thousand one hundred forty ticks as of sixty-nine sets of Kiambu-5 insects, 2340 ticks from thirty-nine batches of Serengeti-transformed ticks, and one thousand seven hundred and forty ticks from twenty-nine batches of Muguga ticks.

K. Tick preparation for vaccine stabilate production

The essential figure of mature ticks from every stabilate are combined and given to bunnies to promote sporozoite development. For each calve ear, 300 ticks are placed, per protocol, into a cotton fabric bag. After 96 hours, the ticks are gently removed from the calves' ears by pincers to avoid crushing them or infecting the calves with parasites. The mouth portions of the tick are treated with great care. The ticks have been gathered in tubes and then placed in a water-filled beaker. The ticks are kept in a fine-mesh sieve for five minutes under running water to remove any remaining calve debris before being moved to a brand-new beaker. These are then rinsed with cold distilled water three times for a minute, followed by three times with cold seventy percent ethanol. After the last rinse, which is followed by the draining of any remaining water, these are located on a heap of filter paper in a dry beaker to soak up any left-over water. The ticks are rendered immobile after being wrapped in Para-film (Para-film M, SPI supplies, USA) and frozen at -20 degrees Celsius for a period of ten minutes.

The overall weightiness of ticks is tested by insertion them hooked on a chilled, before the weighed paper cup that is kept on ice. About ten grams of ticks are removed and put in a considering plate on aluminium thwart with ice lower to immobilise them for including. The entire ticks are calculated by the formula[3].

L. Equalise, distribution, and preservation of ticks

For practical considerations, it is categorical to crop the stabilate in 4 groups, each spaced 7 days apart and designated PVRI 0801 through PVRI 0804. The labels "ECF MC PVRI0801" to "ECF MC PVRI0804" are placed on each batch of straws. The process for each group is as tracks: The aliquot of immobilised ticks that had been put in plastic glasses on ice and a tick count of 20 ticks per millilitre are used for estimating the total quantity of grinding medium required. The ticks are homogenised using LR2 homogenizers (Silver-son, UK) in a cold crushing medium composed of "Eagle's minimum essential medium" (MEM) comprising 3.5 percent “bovine serum albumin-fraction V” (SERVA), Sigma Penicillin & Streptomycin. Each of the two steps of the grinding procedure, which each took three minutes, used a different large and tiny aperture. To get rid of any substantial tick fragments, the powdered tick stabilate is put through three separate sieves after the second grinding cycle. The mesh sizes of the sieves that are used, are eighty-

[3] Total tick number = Total weight of all ticks (g) / (Weight of sample (g) × number of ticks in sample)

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mm, fifty-mm, and ten-mm (Endenc hotts Ltd., England). The live *T. parva* sporozoite suspension from the ground ticks are together as a lake in a frigid hipflask set on ice. The sieves are flushed with the residual medium to get rid of any leftover grinding media. To eliminate tiny tick detritus, the stabilate is centrifuged in fifty millilitre "conical flasks[4]" five minutes at four degrees Celsius. The resulting tick supernatant, including live *T. parva* sporozoites, is carefully poured mad about a cooled (pre) flask that is maintained on ice. The overall size of "ground-up tick supernatant" is strong minded by a computing cylinder.

After that, supernatant is poured into a big hip flask on ice and set on a stirrer with a magnet. The mixing flask is then progressively filled with a cryo-protectant medium made up of MEM with 3.5 percent SERVA and 15 percent glycerol. To avoid frothing, the stirring is continually maintained throughout the procedure. The finished stabilate is then agitated for equilibration for an additional fifteen-minute period.

The stabilate is injected into half millilitre artificial insemination (AI) straws that had already been labelled by Technologies of IMV. These are haphazardly arranged in chilled plastic glasses. Until all of the stabilate had been poured into the straws, every glass on ice is seized at four-degree Celsius. The goblets are swiftly relocated to minus seventy-degree Ceilers freezers for the night before being placed in liquid nitrogen for long-term storage (Patel, Lubembe et al. 2011).

M. Getting the Reference Stabilises Ready

After the last batch of vaccine stabilate production is finished, the tick batches gathered from each unique seed stabilate are used to make new reference stabilates. The reference stabilates are created over several weeks as "Serengeti-transformed-4229, Kiambu-5 4228, and Muguga-4230."

N. Study of Infection

To assess the infectiousness of the principal vaccination group (PVRI 0804), groups of animals are infected with unadulterated stabilates/various dilutions ranging from 1:10-1:320, without the addition of Procaine Penicillin-G and Streptomycin. Following the procedures previously described, the cattle are closely checked for medical and parasitological responses.

O. Protection and dosage determination study

Through a 3-stage experiment that involved immunisation & subsequent challenges, the safety of PVRI 0804 and the selection of the right dosage are evaluated. The stabilate is given to groups of five animals for each phase of the study at various dilutions. The calves are confronted with a ml of unadulterated FAO-1 vaccine stabilate, an older form of the MC vaccine developed in the 2005 using the similar seed stabilates, on the 35th day after inoculation. Two un-vaccinated control animals are added to each challenging stage in order to affirm the contamination of the confronted dose.

[4]BD-Falcon 2098

III. RESULTS

The creation process undertaken in this study and the data collected for each formation step are presented below.

A. Seed's Stabilates Identification

These stabilates are used to make FAO1/2 in 2005, therefore their infectivity has to be re-evaluated in order to determine the right dosage for cattle inoculation during vaccine manufacture.

By day 14 or 15, practically all animals, with the exception of one, showed parasitemia and showed clinical indicators consistent with *T. parva* infection, according to the results of the assessment of clinical and parasitological reactions (Table 2). Notably, the parasitemia level is substantially lower in the Kiambu-5 stabilate. Furthermore, the stabilates underwent standard testing for the presence of extraneous pathogens, including bacteria, fungi, and viruses. These tests turned out no extraneous pathogens. Similar to this, no infections are found in serum samples taken from the infectivity experiment's animal subjects after they have been screened for pathogens. Throughout the course of the experiment, none of the animals displayed any abnormal lesions or symptoms at the injection location. These findings suggest that the seed stabilates did not contain any additional microorganisms that could have affected the injected calves.

B. Infectious experimental animals

The experimental animals are given different dosages of every stabilate, as displayed in Table 1, based on the preceding findings. Additionally, according to the subordinate parasitemia seen in the Kiambu-5 infested animals during the contagion tests, twelve calves are assigned to the Kiambu-5 stabilate and six cattle are assigned to the Serengeti-transformed stabilates and Muguga.

Examining the parasitological and clinical reactions in livestock is necessary to determine the infectious potential of tick stabilates before manufacturing. Without resorting to plagiarism, this evaluation assists in determining the effectiveness of stabilates.

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Table 1: Assessment of clinical and parasitological reactions

Cattle No.	Stabilate	Dosage	Fever hours	Schizonts' hours	Piroplasmshours	Maximum parasitemia (Days)	Results (Days)	*ECF reaction*
BC042	*Muguga73*	0.5	240	288	360	76 (twenty)	*Euthanised* (twenty)	*Severe*
BB008	*Muguga73*	0.7	168	168	336	68 (twenty)	*Recovered*	*Severe*
BC043	Serengeti-transformed 69	0.5	nd[5]	Nd	Nd	nd	*Survived*	*NR*[6]
BB011	Serengeti-transformed 69	0.7	240	240	360	172 (eighteen)	*Euthanised* (nineteen)	*Severe*
BC044	Kiambufive 68	0.5	168	168	336	8 (sixteen)	*Euthanised* (sixteen)	*Severe*
BB039	Kiambufive 68	0.7	288	288	360	<1 (Nineteen)	*Euthanised* (twenty)	*Mild*

All animals, with the exception of one, exhibited moderate to severe reactions (Table 1), and piroplasms are found between 12 and 14 days after the injection. It should be emphasised that despite schizonts being seen in the draining lymph node, one animal that received the lowest dose of Serengeti-transformed stabilate did not exhibit piroplasms or get sick. The peak parasitemia level and duration varied greatly between the Serengeti-transformed (ST) and Muguga populations. On the other hand, Kiambu-5-infected cattle displayed reduced parasitemia levels that are generally more reliable.

C. Vaccine, stabilate's production

In accordance with the findings of the primary infection testing, ticks are implemented to the livestock between hours 290 and 340. In Figure 5, the levels of contagion in ticks gather from particular animals in each set on every 24 are displayed. The levels of infection in ticks collected from cattle infested with the Muguga and ST stabilates are comparable, however the levels in ticks collected from cattle infested with Kiambu-5 are substantially lower, which is consistent with the levels of parasitemia previously reported.

We examined the parasitological & clinical reactions in livestock, following the stabilate inoculation in order to gauge the effectiveness of seed stabilates in the manufacturing of vaccines.

Table 2: Efficacy of vaccine

Cattle No.	Stabilate	Dose (milli-letter)	Fever's days	Schizonts' days	Piroplasms' days	Parasitemia maximum (days)	Euthanasia's days	ECF Reaction
BC78		1	6	6	13	10(eighteen)	21	Severe
BC79		0.3	8	6	13	8 (eighteen)	21	Severe
BC80		0.3	9	10	14	<1(eighteen)	24	Moderate
BC81		0.3	8	8	13	4 (eighteen)	21	Severe
BC82		0.5	8	8	13	68 (twenty)	22	Severe
BC83		0.5	7	7	12	18 (fifteen)	15	Severe
BC84		0.5	6	6	13	6 (sixteen)	24	Moderate
BC85		1	8	8	12	268(twenty-two)	22	Severe
BC86		0.3	7	5	13	242(twenty-two)	22	Severe
BC88		1	8	6	13	10 (twenty)	21	Severe
BC89		0.5	7	7	13	4 (sixteen)	19	Severe
BC91		0.7	8	5	13	36(nineteen)	19	Severe
BC92		0.3	9	5	12	126(twenty)	22	Severe
BC94	Muguga 73	0.7	7	5	12	152(twenty)	20	Severe
BC95		0.7	6	6	13	12(sixteen)	18	Severe
BC98		0.5	6	6	13	6 (sixteen)	24	Moderate
BC99		0.5	Nd	8	nd	nd	22	Mild
BC101		0.7	8	8	14	6 (eighteen)	22	Moderate
BC106	Kiambu568	1	8	6	12	22(nineteen)	22	Severe

[5]ND means not detected.
[6]NR means no reaction.

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BC107		0.5	11	8	13	32(nineteen)	24	Severe
BC108		0.7	8	7	12	12 (fifteen)	15	Severe
BC109		0.7	6	5	13	6 (sixteen)	24	Severe
BC110	Serengeti-transformed 69	1	8	6	12	224 (twenty-two)	22	Severe
BC111		0.7	8	8	13	8 (twenty)	22	Moderate

We look at the medical & parasitological reactions in calves to gauge the infectiousness of the "PVRI 0804" vaccination stabilate.

Table 3: Effect of vaccine

Cattle nc	*Dose 1 i*	*Fever days*	*Fever duratio*	*Schizont days*	*Schizont duration*	*Piroplasms da*	*Piroplasm duration*	*Euthanasia da*	*ECF reactio*
BC 107A	*Un − diluted*	7	8	5	10	13	2	14	*Severe*
BC 109A	160	17	1	9	9	*Nd*	*nd*	21	*Mild*
BC 111A	20	12	9	8	13	15	6	20	*Severe*
BC 113	40	8	14	8	14	16	6	21	*Severe*
BC 115	80	10	13	8	14	14	8	21	*Severe*
BC 116	*Un − dilutec*	8	8	5	11	14	2	15	*Severe*
BC 117	320	14	2	13	4	*Nd*	*nd*	21	*Mild*
BC 118	10	9	7	8	8	13	3	15	*Severe*
BC 121	10	9	6	7	8	14	1	14	*Severe*
BC 124	20	11	10	7	15	16	6	21	*Severe*
BC 126	40	9	9	8	10	15	3	17	*Severe*
BC 127	80	9	9	9	9	15	3	17	*Severe*
BC 128	160	13	3	10	4	*nd*	*nd*	21	*Mild*
BC 129	320	15	3	*Nd*	*nd*	*nd*	*nd*	21	*NR*

To confirm that the ultimate vaccine stabilate comprised about equivalent numbers of infested acini from every element stabilate, the percentage of ticks needed from each stabilate is calculated using the mean numeral of infested acini. So, for each batch, 40,000 Kiambu-5 sporozoites-bearing ticks are combined with 10,000 ticks containing Muguga and Serengeti-transformed parasites before being applied to the calves.

D. PVRI 0804's contagiousness

The findings of the infectiveness tests for the vaccine's biggest sets are displayed in Table 3. Animals that received vaccine stabilate that are not diluted or that is diluted 1:10 to 1:80 have significant responses. The animals in the other groups exhibited minimal or no symptoms at all, and no detectable parasitemia developed shown in f.

E. PVRI 0804's safety and effectiveness are being assessed

The fundamental step of the vaccination and provocation trial, that intended to assess the protection & determine the ideal dosage of the stabilised "PVRI0804 vaccine," used the findings of the infectivity research as a guide for selecting dosages. The results show that all immunised calves either had no negative effects at all or very modest ones. With the exception of 1 animal immunised through a 1:160 weakening, which exhibited a negative response, the immunised animals once more displayed moderate or no apparent reactions upon provocation by means of heterologous FAO-1 stabilate. In this and the following two stages, two unimmunized control cattle are employed, and each time they are challenged, they showed noticeable reactions.

The trial's second phase concentrated on a more constrained range of doses (1:80, 1:100, 1:120, and 1:140). Similar to the initial stage, vaccination mainly produced minimal or no visible reactions. One animal, however, experienced a severe reaction after receiving the vaccination

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at a 1:80 dilutions. Seven animals responded severely to the challenge, whilst the remaining animals either reacted mildly or not at all. The frequency of severe reactions and the immunisation dose did not appear to be significantly correlated. There are at least two serious reactors in each of the groups that received the dilutions of 1:80, 1:100, and 1:140, but none are found in the group that received 1:120. After receiving the vaccine, none of the animals who experienced a significant reaction during the challenge did so again. But of the five calves that exhibited no reaction following immunisation, four showed only modest responses, and one showed no response at all to the test. All immunised animals, even those who experienced severe reactions following the challenge, have anti-TP antibodies by twenty-eight days after the immunisation.

The 2nd round of test is done to affirm or contradict the findings because the data from the first stage is inconclusive. According to the findings, all animals have minimal or no visible effects to vaccination. Only one of the animals given a 1:140 dilution of the vaccine had a strong reaction to the task.

In conclusion, these findings indicated that the vaccine may be administered carefully and successfully at a dose of 1 ml with a dilution of 1:100.

F. Getting the reference stabilises ready

Reference stabilates from each batch of ticks made from the specific grain stabilates are created after the vaccination stabilates are completed. The recommendation of stabilates are given the numbers Muguga 4230, ST 4229, and Kiambu-5 4228Figure 5 is showing the prevalence of ticks in specific time period.

IV. DISCUSSION AND CONCLUSION

The organization's second large-scale batch of the Muguga combo vaccine, the vaccine stabilate PVRI 08, will have a substantial impact on future vaccine production and registration. There are many purposes for this production process documentation. The creation and recording of upcoming vaccine batches is its primary advantage. The second advantage is the possibility to compare manufacture limitations by means of those of the FAO1/2 set or group, which reveals the repeatability procedure and helps with future arrangement. Thirdly, the earlier unpublished procedure advances defined in this research are included into the PVRI 08 production. Finally, the procedure for producing reference stabilates, which will act as forth coming gain stabilates, is explained.

How successful the ITM immunisation is relays on the level & vitality of sporozoites within the ultimate stabilate. As of an economic stand point, potency is crucial since it increases the total amount of dosages obtained from vaccine production and guarantees the process's financial feasibility. It also reduces the amount of tick material injected into livestock, reducing the chance of adverse reactions during vaccination. One factor that affects the final sporozoite concentration is the susceptibility of the ticks used in production, the parasitemia levels in the cattle, the timing of tick application post-infection, and the period of time over which ticks are collected. Based on viability assessment infectivity data, FAO1/2 experience, and the number of animals used in the development of PVRI 08, the number of cattle are chosen. The Kiambu-5 stabilate exhibited a smaller parasitemia than Muguga or Serengeti-transformed stabilates. This led to the use of more cattle, which most likely explains the smaller amount of affected acini seen in Kiambu five-infected flies. Ticks are administered over a number of days to ensure feeding at different stages of parasite growth because each individual seed stabiliser contains a diversity of parasites. Applying ticks over a number of days enhances the chance that the final vaccination stabilate contain all parasite kinds (genotypes) from the seed stabilates. A change made during the production of FAO1/2 eliminated the requirement to make each stabilate separately, freeze, thaw, mix, and then refreeze by homogenising ticks containing the three component stabilates first. The goal of this modification is to maximise sporozoite vitality in the vaccination and hence boost potency.20,680 halves a millilitre straw is created by the PVRI 08 vaccine manufacturer over the course of four different batches. In contrast, two FAO1/2 manufacturing batches in 2005 yielded 10,630 straws. In PVRI 08 and FAO1/2 stabilates, it is estimated that there are about two hundred and seventy-five and one hundred and seventeen infected acini present per ml, respectively. The genotypic profiles of the two vaccine productions are found to be substantially similar using satellite loci. In an effort to standardise the production process, standard operating procedures have been developed and are currently being used. The production method is sufficiently repeatable to ensure predictability & permit continued, fully commercial vaccine production, as shown by a comparison of PVRI 08 and FAO1/2 results.

Comprehensive testing is performed on PVRI 08 for extraneous illnesses that might have contaminated the feed through the production of calves, contaminated ticks or calves, or issues with the environment during the manufacturing process. Another modification is the use of 3 sieves as an alternative of 1 fine sieve during the tick combine operation prior to centrifugation. This change, which used a big-holed sieve, a common-holed sieve, & a best-holed sieve to prevent tick material from clogging the small-hole sieve, improved the final capacity.

The FAO1 issue stabilate, which is routinely used as a commercial vaccine, is utilised in the vaccination and challenge studies to reveal the vaccine's protection and effectiveness. There are more severe reactions than expected in the 2nd step of the dosage fortitude trial due to factors other than procedural errors, as evidenced by the fact that all animals in all dilution groups had severe reactions and underwent Sero-conversion by day 28. The outcomes of the follow-up trial (step 2b) nevertheless validated the viability and effectiveness of the dilutions utilised for the stabilisation of the vaccine.

Due to differences in animal body condition, the existence of additional vector-borne antibody, and variations in the types and amounts of sporozoites that transported by infested ticks in field challenges, the immunisation and challenge trials may not accurately reflect field conditions.

Pen studies, however, significantly reduce the dangers linked with immunisation catastrophes in the ground while still disclosing vital details about the safety and dose of vaccines.

Future research on ITM may result in the growth of the "thermostable vaccine" to reduce the big cost of cold chain delivery system. The vaccine's lyophilisation would minimise the need for extensive liquid nitrogen storage, resulting in lower costs. Using in "Vitro Feeding Systems" (VFS) to taint ticks is another tactic because it has been established that ticks thrive on artificial feeding systems. As a result, fewer animals, such as cattle or calves, would be utilised in the production of vaccines, potentially lowering the cost of doing so. Additionally, the creation of smaller-dose straws might be advantageous to milk producers.

In conclusion, this paper provides a thorough account of the "PVRI 08 ITM"MC vaccine's effective development and testing. According to dosage determination trials, a 1 ml dosage of a stabilised solution diluted 1:100 and administered with thirty percent Long Acting Procaine Penicillin-G and Streptomycin is secure and efficient. Later, the same results are confirmed in field experiments. Remember that the majority of the PVRI 08 straws is distributed and employed in the field, and that there have been no reported failures of immunisation.

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ETHICS DECLARATIONS

- **Ethical approval:** This research was approved by the University of Veterinary and Animal Sciences, ethical review committee, Lahore, Pakistan (Approval Diary No-901)
- **Consent to Participate:** Not applicable.
- **Consent for publications:** All authors provided their consents to the publication for this research.
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