



## **SYNOPSIS SUBMISSION PERFORMA**

1. **Student Name:** Muhammad Ahmed
2. **Registration Number:** 2011-VA-198
3. **Department Name:** Clinical Medicine and Surgery
4. **Supervisor Name:** Dr. Muhammad Avais
5. **Research Priorities of Department:**  
Clinico-epidemiological studies on infectious diseases of animals
6. **Title of proposed research:**  

Evaluation of different vaccines for the control of mastitis in dairy cows
7. **Objectives of research and problem identified:**
  - Isolation Of Vaccinal Isolates
  - Biochemical Characterization of vaccinal isolates
  - Evaluation Of Different Polyvalent Vaccines efficacy against the isolates in field
8. **Certificate that proposed research plan is not repetition of work within university at least for last 10 years:**

Yes
9. **References which are included in synopsis should be from last 5 years work published in peer reviewed journals and that student should have full length papers with them:**

Yes
10. **Written comments from every member of committee should be included in the format and convener will submit a summary of comments to Dean:**

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**UNIVERSITY OF VETERINARY & ANIMAL SCIENCES, LAHORE**

**Synopsis of M.Phil  
(Clinical Medicine)**

**TITLE:**

**Evaluation of different vaccines for the control of mastitis in dairy cows**

Date of Admission:	15-08-2017
Date of Initiation:	As and when approved
Probable Duration:	About 1 year

**PERSONNEL**

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**SUPERVISORY COMMITTEE**

- |                             |              |
|-----------------------------|--------------|
| 1. Dr. Muhammad Avais       | (Supervisor) |
| 2. Dr. Jawaria Ali Khan     | (Member)     |
| 3. Prof. Dr. Masood Rabbani | (Member)     |

**RELEVANCE TO RESEARCH PRIORITY:**

Certificate:

The title of the synopsis relates to research priority entitled, “Clinico-epidemiological studies and therapeutic trials on infectious diseases of animals” of the Department.

Student:

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**(Signature)**

**FORWARDED BY SUPERVISORY COMMITTEE:**

1. ----- (Supervisor)
2. ----- (Member)
3. ----- (Member)

Recommended and Forwarded by Chairman Board of Studies of the Department of Clinical Medicine and Surgery

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**(Chairman)**

Recommended and Forwarded by Synopsis Scrutiny Committee, Faculty of Veterinary Science

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**(Convener)**

Reviewed and witnessed by:

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**Dean**  
Faculty of Veterinary Science,  
University of Veterinary and Animal Sciences,  
Lahore

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**Director Advanced Studies**  
University of Veterinary and Animal Sciences,  
Lahore

## **INTRODUCTION**

Bovine mastitis is the most alarming disease challenging the dairy industry throughout the world but the situation in Pakistan is particularly very shocking and it demands great attention for its control because of high economic losses due to this disease. Mastitis is one of the limiting factors in the development of dairy industry in Pakistan. As for as huge economic losses are concerned to the farmers, the disease also has greater importance from the consumer's and milk processor's point of view. The milk from affected animal may port the organisms which is potentially pathogenic for human beings (zoonosis) and processing of such milk results in suboptimal output of substandard finished fermented products like yogurt, cheese, etc (Muhammad *et al.* 1995).

Although, various etiological agents are linked with mastitis but it is generally caused by bacterial pathogens. The internal environment of the mammary gland is enthusiastic for the multiplication of invading microorganisms especially bacteria. The bacterial growth byproducts and metabolism irritate the soft tissues of the glands causing inflammation finally resulting in mastitis (Schalm *et al.* 1971).

Ashfaq and Muhammad (2008) stated that pathogens associated with bovine and bubaline mastitis were studied in peri-urban areas of Faisalabad, Pakistan. Seventy-five quarters of the foremilk-samples, collected from 14 randomly selected buffaloes (clinically mastitic quarters n = 7, sub-clinically mastitic quarters n = 48) and 5 cows (clinical n = 2; sub-clinical n = 18 quarters), were subjected to a microbiological examinations. The diagnosis of sub-clinical mastitis was based on the results of Surf Field Mastitis Test.

## **REVIEW OF LITERATURE**

*Staphylococcus aureus* is a common causative agent of bovine mastitis in dairy herds (Haran *et al.* 2011). The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals as well as the community is a noteworthy and costly public health concern. *S. aureus* related bovine mastitis is a common reason for therapeutic as well as prophylactic use of antibiotics on dairy farms. In this study, herd prevalence of *S. aureus*, including MRSA, was estimated from bulk tank milk (BTM) from Minnesota farms. A total of 150 pooled BTM samples from 50 farms, collected over 3 different seasons (spring, summer, and fall of 2009), were assessed. Herd prevalence of methicillin-susceptible *S. aureus* (MSSA) was found 84%, while MRSA herd prevalence was noticed as 4%. A total of 93 MSSA isolates and 2 MRSA isolates were recovered from 150 BTM samples. Antibiotic susceptibility testing of *S. aureus* isolates showed pan susceptibility in 54 isolates, resistance to a single antibiotic class in 21 isolates, resistance to two antibiotic classes in 13 isolates, and resistance to >3 antibiotics classes and therefore multidrug resistance in 5 isolates was reported. The two MRSA isolates displayed resistance to beta-lactams group, cephalosporins, and lincosamides were referred as multi-resistant. Staphylococcal protein A gene (*spa*) typing identified *spa* types t529 and t034 most frequently among methicillin-susceptible isolates, while t121 was observed in MRSA isolates. Seven isolates, including the two MRSA isolates, produced staphylococcal enterotoxins B, C, D, and E on overnight culture.

### **Species-level identification of Staphylococci:**

Staphylococci isolated from bovine milk and not classified as *Staphylococcus aureus* represent a heterogeneous group of microorganisms that are normally accompanying with bovine mastitis. The identification of these microbes is significant, although it is challenging and relatively costly also. Genotypic methods add precision in the identification of *Staphylococcus* species. In the present study, partial 16S rRNA sequencing was used for the species identification of coagulase-positive and coagulase-negative staphylococci isolated from bovine mastitis. Two hundred and two (95%) of the 213 isolates were fruitfully identified at the species level. The assigning of an isolate to a particular species was only based on 99% identity with 16S rRNA sequences deposited in GenBank (Lange *et al.* 2015).

**Note: For M.Phil: 15, For Ph.D: 25 (Minimum Reviews)**

## **STATEMENT OF PROBLEM**

Mastitis in dairy cows is one of the most important diseases imposing massive economic losses to the dairy farmers worldwide that causes annual losses of worth \$ 35 billion. It is reported that 70-80% milk losses are due to sub-clinical mastitis. Among these *S. aureus*, *Str. agalactiae* and *E. coli* account for up to 84% of mastitis cases in dairy animals in Pakistan. The present study is designed to control these 3 mastitogens through vaccination in order to reduce significant number of mastitis cases and hence improve the economics of dairy farm as well as milk quality.

### **Objectives:**

- Development of inactivated polyvalent mastitis vaccines against *S. aureus*, *Str. agalactiae* and *E. coli* in dairy cattle.
- Evaluation of inactivated polyvalent mastitis vaccines against *S. aureus*, *Str. agalactiae* and *E. coli* in dairy cattle.

## **MATERIALS AND METHODS**

### **Experimental Station:**

The present study will be performed in the department of Clinical Medicine & Surgery, University of Veterinary and Animal Sciences, Lahore

### **Research Area, Study Animals and Source of Samples**

In present study all the dairy cows from peri-urban areas of district Lahore will be included. The dairy cows in the study areas are mostly kept under natural climatic conditions by pastorals and feed is only from range vegetation. Aseptic milk collection will be done as per recommendations of national mastitis council (NMC 2001).

### **Experiment No.1**

#### **Screening of subclinical mastitis:**

Subclinical mastitis will be diagnosed using California Mastitis Test (CMT) as described by Schalm (1971). Briefly, after cleaning the udder, first few streaks from each quarter will be discarded. Then equal amount of milk (2mL) will be drawn from each quarter into respective receptacle of plastic paddle and will be mixed with equal amount of CMT reagent, and the paddle will be gently swirled to mix the milk and reagent. The reaction will be read within 20 seconds and the results will be interpreted as per criteria based on amount of gel formation.

#### **Isolation and identification of *Staphylococcus aureus* from subclinical mastitis**

Milk samples will be collected for bacteriology examination as per procedure of National Mastitis Council (NMC 2004). Milk sample (0.5ml) will be taken and spreaded on blood agar (5% sheep RBCs) that will be kept in incubator at 37<sup>0</sup>C for 24 to 48 hour. The colonies will be streaked on different differential media as per guidelines of Burgey's manual of determinative bacteriology (Harley and Prescott 2002).

#### **Statistical Analysis**

The data originating from different studies will be analyzed using one way and two way analysis of variance (ANOVA).

## **SUMMARY**

### **Background:**

Mastitis is the inflammation of the parenchyma of the mammary gland having multiple etiologies and is a multifactorial disease. Mastitis is considered as one of the most devastating and costly diseases of dairy industry worldwide that causes annual losses of worth \$ 35 billion. It is reported that 70-80% milk losses are due to sub-clinical mastitis. Mastitis affects both quality and quantity of milk, hence posing colossal economic losses to the farming community. Mastitis has multiple etiological agents including bacteria, fungi, yeasts and mycoplasma.

### **Hypotheses:**

- i. *Staphylococcus aureus*, *Str. agalactiae* and *E. coli* are prevalent bacterial pathogens in clinical mastitis in cattle
- ii. Inactivated polyvalent mastitis vaccines against *S. aureus*, *Str. agalactiae* and *E. coli* are effective to control mastitis in field dairy cattle

### **Methodology:**

50 mastitis free lactating dairy cows in first two months of lactation will be selected at different dairy farms. The screening of these cows will be done by California Mastitis test (Schalm *et al.* 1971). These cows will be randomly divided into 5 groups (A-E) with 10 cows in each group. Serum samples will be collected from vaccinated and control cows at monthly intervals for 6 months.

### **Statistical Design:**

Data on prevalence will be analyzed using chi square test while OR values will be calculated for association of different risk factors, While zone of inhibition in antibiogram study will be analyzed by one way ANOVA. Statistical analysis will be performed using SPSS verso 22. The Significance will be checked at 5% probability ( $P < 0.05$ ).

### **Outcome:**

Inactivated Polyvalent Vaccination could be used to prevent mastitis, which will flourish the dairy industry of Pakistan.

## REFERENCES

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