# CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgments</td>
<td>xxii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xiii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xv</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>xix</td>
</tr>
<tr>
<td><strong>ABSTRACT</strong></td>
<td>xxiii</td>
</tr>
<tr>
<td><strong>1 GENERAL INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td>1.1 Hypodermosis: Ectoparasitic Infestation</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Classification of Hypodermanidae</td>
<td>3</td>
</tr>
<tr>
<td>1.3 Morphological Characteristics</td>
<td>4</td>
</tr>
<tr>
<td>1.4 Lifecycle of <em>Hypoderma</em></td>
<td>4</td>
</tr>
<tr>
<td>1.5 Grub Feeding</td>
<td>5</td>
</tr>
<tr>
<td>1.6 <em>Hypoderma</em> Mating, Dispersal and Transmission</td>
<td>7</td>
</tr>
<tr>
<td>1.7 Economic Losses</td>
<td>8</td>
</tr>
</tbody>
</table>
1.8 Control and Eradication

1.9 Enzymatic secretions for Serodiagnosis

1.10 Purpose of Study

2 DEVELOPMENT OF INDIRECT ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) AND COMPARISON WITH CONVENTIONAL PROCEDURES

2.1 INTRODUCTION

2.2 REVIEW OF LITERATURE

  2.2.1 Diagnosis of Hypodermosis

  2.2.2 Conventional Procedure

  2.2.3 Serological Techniques

  2.2.4 Clinical Signs

  2.2.5 Conclusion

2.3 MATERIALS AND METHODS

  2.3.1 Study Area

  2.3.2 Study Plan

    2.3.2.1 Phase-I (Development and validation of ELISA)
2.3.2.2 Phase-II (Comparison with conventional procedure)  

2.3.3 ELISA Development  

2.3.3.1 Larvae collection and storage  

2.3.3.2 Isolation of antigen  

2.3.3.3 Purification of Hypodermin C from crude extract  

2.3.3.3.1 Dialysis  

2.3.3.3.2 Estimation of Protein concentration  

2.3.3.3.3 Obtaining and characterization of antigens of *Hypoderma*  

2.3.3.3.4 SDS PAGE  

2.3.4 Seroepidemiological Information  

2.3.5 Collection of Blood Samples  

2.3.5.1 Bovine sera  

2.3.6 Indirect ELISA (Purify HyC: *Hypoderma*)  

2.3.7 ELISA (Crude Extract: *Hypoderma*)  

2.3.8 Calculation of Cutoff values  

2.3.9 Palpation Method
2.4 RESULTS AND DISCUSSION

2.4.1 DEVELOPMENT OF INDIRECT ELISA AND COMPARISON WITH CONVENTIONAL PROCEDURES

2.4.1.1 Antigen Preparation

2.4.1.2 Determination of Protein Concentration

2.4.1.3 SDS-PAGE for Biochemical Analysis

   2.4.1.3.1 Crude extract

   2.4.1.3.2 Purified proteins

2.4.1.4 Lyophilization

2.4.1.5 ELISA Standardization

   2.4.1.5.1 Crude ELISA results

   2.4.1.5.2 Hypodermin C (HyC) ELISA results

2.4.1.6 ELISA Validation for Two Hundred Positive Samples

2.4.1.7 Comparison of ELISA and Conventional Procedures

3 SERO-EPIDEMILOGICAL INFORMATIONS ON THE BASIS OF ELISA ASSAY
3.1 INTRODUCTION

3.2 REVIEW OF LITERATURE

3.2.1 World Wide Prevalence of Hypodermosis

3.2.2 Prevalence of Hypodermosis in Pakistan

3.3 MATERIALS AND METHODS

3.3.1 Study Area

3.3.2 Seroepidemiological Information

3.3.2.1 Questionnaire

3.4 RESULTS AND DISCUSSION

3.4.1 Clinical Inspections through Palpation Method

3.4.1.1 District wise prevalence (based on topography)

3.4.1.2 Village wise prevalence in Northern Punjab, Pakistan

3.4.2 Seroepidemiological Prevalence based on the ELISA

3.4.2.1 District wise seroprevalence in Northern Punjab, Pakistan

3.4.2.2 Village wise seroprevalence in Northern Punjab, Pakistan

3.4.2.3 Topographical wise seroprevalence in Northern Punjab, Pakistan
3.4.2.4 Farm management wise seroprevalence in Northern Punjab, Pakistan

3.4.2.5 Water bodies based seroprevalence in Northern Punjab, Pakistan

3.4.2.6 Seroprevalence based on Grazing Pattern

3.4.2.7 Seroprevalence based on Animals Breed

3.4.2.8 Seroprevalence based on Animals Age

3.4.2.9 Seroprevalence based on Previous Exposure

3.4.2.10 Seroprevalence based on Previous Medication

3.4.2.11 Seroprevalence based on Sex

3.4.2.12 Seroprevalence based on Herd Size

3.4.2.12.1 District Rawalpindi

3.4.2.12.2 District Attock

3.4.2.12.3 District Jehlum

3.4.2.12.4 District Chakwal

3.4.2.13 Month wise prevalence of warbles
TO APPLY GEOGRAPHIC INFORMATION SYSTEM (GIS) FOR RISK MAPPING OF BOVINE HYPODERMOSIS TO DEVELOP EFFECTIVE CONTROL STRATEGIES

4.1 INTRODUCTION

4.2 REVIEW OF LITERATURE

4.3 MATERIALS AND METHODS

4.3.1 Risks Mapping of Bovine Hypodermosis

4.3.1.1 Descriptive statistics

4.3.1.2 Statistical analysis

4.4 RESULTS AND DISCUSSION

4.4.1 Risks Mapping and GIS Model

4.4.1.1 Descriptive statistics

4.4.1.2 Cluster analysis

4.4.2 Risk Factor Analysis

5 GENERAL DISCUSSION

SUMMARY

CONCLUSION

LITERATURE CITED
APPENDIX  169

PLATES  253