# CONTENTS

List of Abbreviations xiii
List of Tables xvi
List of Figures xix
List of Plates xxvii
Acknowledgements xxviii

## ABSTRACT xxxi

1 GENERAL INTRODUCTION 1

1.1 FASCIOLIASIS 2

1.2 ETIOLOGY 3

1.3 INTERMEDIATE SNAIL HOSTS 4

1.4 LIFE CYCLE 5

1.5 CLIMATE AND ENVIRONMENT 6

1.6 GEOGRAPHIC DISTRIBUTION 7

1.6.1 Human Fascioliasis 7

1.6.2 Animal Fascioliasis 9

1.7 PATHOLOGY AND SYMPTOMATOLOGY 9

1.8 FASCIOLIASIS RESISTANCE 10

1.9 ECONOMIC SIGNIFICANCE 11

1.10 DIAGNOSIS 12

1.10.1 Humans 12

1.10.2 Animals 13

1.11 TREATMENT 13
1.11.1 Humans                           13
1.11.2 Animals                14
1.12 CONTROL                       14
1.13 STUDY RATIONALE             15

2 PHENOTYPIC CHARACTERIZATION OF FASCIOLID
ADULTS/EGGS FROM THE BUFFALO OF CENTRAL
PUNJAB PAKISTAN BY APPLYING COMPUTER IMAGE
ANALYSIS SYSTEM (CIAS)

2.1 INTRODUCTION                   18
2.2 REVIEW OF LITERATURE          21
  2.2.1 Geographical Distribution of Fasciolids  21
     2.2.1.1 Fasciola hepatica                   21
     2.2.1.2 Fasciola gigantica                  22
  2.2.2 Fasciolid Adults Phenotyping     22
  2.2.3 Fasciolid Eggs Phenotyping        25
  2.2.4 Present Study                   26
2.3 MATERIALS AND METHODS          27
  2.3.1 Study Area                    27
  2.3.2 Morphometry                   27
     2.3.2.1 Liver flukes collection         28
     2.3.2.2 Staining                      28
     2.3.2.3 Egg collection from adult liver fluke uteri  28
  2.3.3 Morphometrical Measurements    30
     2.3.3.1 Morphological characteristics of adult fasciolids  30
     2.3.3.2 Eggs characteristics            31
     2.3.3.3 Shape of adult liver flukes and eggs  32
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3.3.4</td>
<td>Grouping criteria</td>
<td>32</td>
</tr>
<tr>
<td>2.3.4</td>
<td>Statistical Techniques</td>
<td>32</td>
</tr>
<tr>
<td>2.4</td>
<td>RESULTS</td>
<td>36</td>
</tr>
<tr>
<td>2.5</td>
<td>DISCUSSION</td>
<td>49</td>
</tr>
<tr>
<td>2.5.1</td>
<td>Conclusions</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>DEVELOPMENT AND ANALYSIS OF RISK MAPPING</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>BY USING CLIMATIC DATA AND FORECAST INDICES TO MITIGATE THE IMPACT OF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FASCIOLIOSIS IN SUB-TROPICAL PUNJAB, PAKISTAN</td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>INTRODUCTION</td>
<td>56</td>
</tr>
<tr>
<td>3.2</td>
<td>REVIEW OF LITERATURE</td>
<td>59</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Effect of Climate Change</td>
<td>62</td>
</tr>
<tr>
<td>3.2.1.1</td>
<td>Influence of temperature</td>
<td>64</td>
</tr>
<tr>
<td>3.2.1.1.1</td>
<td>Eggs</td>
<td>64</td>
</tr>
<tr>
<td>3.2.1.1.2</td>
<td>Miracidium</td>
<td>64</td>
</tr>
<tr>
<td>3.2.1.1.3</td>
<td>Intermediate host (Snail)</td>
<td>65</td>
</tr>
<tr>
<td>3.2.1.1.4</td>
<td>Intramolluscan cercarial production</td>
<td>65</td>
</tr>
<tr>
<td>3.2.1.1.5</td>
<td>Metacercariae</td>
<td>66</td>
</tr>
<tr>
<td>3.2.1.2</td>
<td>Influence of moisture/rainfall</td>
<td>66</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Environment and Transmission of Liver Fluke</td>
<td>67</td>
</tr>
<tr>
<td>3.2.2.1</td>
<td>Seasonality</td>
<td>67</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Climate and Fascioliosis Forecast Indices</td>
<td>68</td>
</tr>
<tr>
<td>3.2.4</td>
<td>Remote Sensing</td>
<td>70</td>
</tr>
<tr>
<td>3.2.4</td>
<td>Present Study</td>
<td>71</td>
</tr>
<tr>
<td>3.3</td>
<td>MATERIALS AND METHODS</td>
<td>71</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Study Area</td>
<td>71</td>
</tr>
</tbody>
</table>
3.3.1.1 The Punjab plain  
3.3.1.2 Main climatic characteristics  
3.3.1.3 The Indus Bassin River irrigation system  
3.3.2 Climatic Data  
3.3.3 Mt Forecast Index  
3.3.4 Water-Budget-Based System (Wb-bs) Forecast Index  
3.3.5 Seasonal and Geographical Assessment of the Disease Transmission Risk  
3.3.6 Remote Sensing Data  
3.3.7 Animal and Human Prevalence Data  
3.3.8 Statistical Validation of Index Information  
3.4 RESULTS  
3.4.1 Analysis of Climatic Data  
3.4.2 Rainfall Surface Water Availability Analysis  
3.4.3 General Fascioliasis Risk by the Mt Index  
3.4.3.1 Monthly evolution of Mt  
3.4.3.2 Yearly evolution of Mt  
3.4.4 Discriminating F. hepatica and F. gigantica infection risks by the Wb-bs index  
3.4.4.1 Monthly evolution of Wb-bs  
3.4.4.2 Yearly evolution of Wb-bs  
3.4.5 Assessment of Fascioliasis Distribution Heterogeneity by the NDVI Index  
3.4.5.1 Geographical distribution of NDVI
3.4.5.2 Monthly evolution of NDVI
3.4.6 Index/Prevalence Correlation Results

3.5 DISCUSSION

3.5.1 Geographical Distribution of Fascioliasis Risk
3.5.2 Critical Values of Risk Indices
3.5.3 Definitive Host Aspects Influencing Prevalences
3.5.4 Seasonality of Disease Transmission Risk
3.5.5 Global Change Impact
  3.5.5.1 Rainfall monoseasonality versus rainfall
  -irrigation seasonality and their relationships
to *Radix auricularia* population dynamics
3.5.6 Effects of climate change on fascioliasis in Punjab province
3.5.7 Time-Lags in Infection Risk Analyses
3.5.8 Human Infection Risk in Punjab Province
3.5.9 Conclusions

4 DETECTION OF ANTI-*Fasciola* IgG ANTIBODIES BY USING MONOCLONAL ANTIBODIES (Mab) BASED MM3 SERO-ELISA IN CATTLE AND BUFFALO OF SUB-TROPICAL PUNJAB, PAKISTAN

4.1 INTRODUCTION
4.2 REVIEW OF LITERATURE
  4.2.1 World Fascioliasis Occurrence
  4.2.2 Fascioliasis in Pakistan
    4.2.2.1 *Fasciola* species
    4.2.2.2 *Fasciola hepatica*
4.2.3.3 Fasciola gigantica 163
4.2.3 Serodiagnosis of Fascioliasis 164
4.2.4 Present Study 167

4.3 MATERIALS AND METHODS 167

4.3.1 Study Area 167

4.3.1.1 Pothwar region 167

4.3.1.1.1 Rawalpindi 168
4.3.1.1.2 Islamabad 169
4.3.1.1.3 Chakwal 169
4.3.1.1.4 Attock 169
4.3.1.1.5 Jhelum 169

4.3.1.2 Central Punjab 170

4.3.1.2.1 Lahore 170
4.3.1.2.2 Sargodha 170
4.3.1.2.3 Faisalabad 171

4.3.2 Animals 171

4.3.2.1 Management practices 171
4.3.2.2 Animal age and grouping criteria 172
4.3.2.3 Animal data statistics 172

4.3.3 Serology 173

4.3.3.1 Collection and preparation of sample 173
4.3.3.2 Diagnostic techniques 173

4.3.3.2.1 MM3-Sero ELISA procedure 173

4.3.4 Statistical Analysis 174
4.4 RESULTS

4.4.1 Level of *Fasciola*-antibodies in Cattle and Buffalo 178

4.4.2 Level of *Fasciola*-antibodies in Age Groups of Cattle and Buffalo 179

4.4.3 Level of *Fasciola*-antibodies in Studied Areas of Cattle and Buffalo 179

4.4.4 Level of *Fasciola*-antibodies according to Sex of Cattle and Buffalo 180

4.4.5 Relationship between Prevalence and Altitude 181

4.5 DISCUSSION 193

4.5.1 Conclusion 198

5 GENERAL DISCUSSION 199

5.1 RECOMMENDATIONS 213

SUMMARY 217

LITERATURE CITED 220

APPENDICES 270