PREVALENCE, DIAGNOSIS AND ECONOMIC LOSSES OF BOVINE CYSTICERCOSIS IN PUNJAB

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2014
IN THE NAME OF ALLAH, THE MOST BENEFICIENT,
THE MOST MERCIFUL
To

The Controller of Examinations

University of Veterinary and Animal Sciences

Lahore.

We, the Supervisory Committee, certify that the contents and form of the thesis, submitted by Muhammad Saeed, Reg. No. 2009-VA-254, have been found satisfactory and recommend that it be processed for the evaluation by the External Examiner(s) for award of the Degree.

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WISDOM IS THE PART AND PARCEL

OF MY RELIGION,

KNOWLEDGE MY WEAPON,

PATIENCE MY DRESS,

FAITH MY DIET, AND SINCERITY

MY COMPANION

HADIS – E – NABVI
(PEACE BE UPON HIM)
Oh Lord,

Make me
An Instrument of Your Peace
Where, there is Hatred
Let me sow Love
Where, there is Injury, Pardon
Where, there is Doubt, Faith
Where, there is Despair, Hope
Where, there is Darkness, Light
And where, there is Sadness, Enjoy

DEDICATED TO

HOLY PROPHET (PBUH)
The Greatest Social Reformers

To
My
LATE
beloved MOTHER and affectionate LATE FATHER
Who taught me
The first word to speak
The first alphabet to write
And
The First step to take
And to
My intellectual Supervisor, Prof. Dr. Aneela
And to
Those who live in my mind
In my heart
Throughout the whole span of my life
And are
Nearest, Dearest and Deepest to me
Specially to my
Wife, Brothers, Sisters, sons and Daughter
And to
Prof. Dr. Sagar M. Goyal
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TABLE OF CONTENTS

Acknowledgements.........................................................................................i

Table of Contents...............................................................................................ii

List of Tables......................................................................................................iii

List of Figures.....................................................................................................v

Abbreviations...................................................................................................vii

<table>
<thead>
<tr>
<th>CHAPTER No.</th>
<th>TITLE</th>
<th>PAGE No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTRODUCTION.</td>
<td>01</td>
</tr>
<tr>
<td>2</td>
<td>REVIEW OF LITERATURE</td>
<td>07</td>
</tr>
<tr>
<td>3</td>
<td>MATERIALS AND METHODS</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td>RESULTS</td>
<td>62</td>
</tr>
<tr>
<td>5</td>
<td>DISCUSSION</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>SUMMARY</td>
<td>109</td>
</tr>
<tr>
<td>7</td>
<td>LITERATURE CITED</td>
<td>114</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE NO.</th>
<th>TITLE</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Overall prevalence of C. bovis in cattle and buffaloes in Punjab</td>
<td>65</td>
</tr>
<tr>
<td>4.2</td>
<td>Zone wise prevalence of C. bovis is cattle and buffaloes in Punjab</td>
<td>66</td>
</tr>
<tr>
<td>4.3</td>
<td>Abattoir wise prevalence of C. bovis is cattle and buffaloes in Punjab</td>
<td>67</td>
</tr>
<tr>
<td>4.4</td>
<td>Sex -wise prevalence of C. bovis is cattle and buffaloes in Punjab</td>
<td>68</td>
</tr>
<tr>
<td>4.5</td>
<td>Breed-wise prevalence of C. bovis in cattle and buffaloes in Punjab</td>
<td>69</td>
</tr>
<tr>
<td>4.6</td>
<td>Age-wise prevalence of C. bovis in cattle and buffaloes in Punjab</td>
<td>70</td>
</tr>
<tr>
<td>4.7</td>
<td>Body condition wise prevalence of C. bovis in cattle and buffaloes in Punjab</td>
<td>71</td>
</tr>
<tr>
<td>4.8</td>
<td>Tissue-wise prevalence of C. bovis is cattle and buffaloes in Punjab</td>
<td>72</td>
</tr>
<tr>
<td>4.9</td>
<td>Overall prevalence of C. bovis in cattle/ buffaloes in Punjab by ELISA</td>
<td>76</td>
</tr>
<tr>
<td>4.10</td>
<td>Zone-wise prevalence of C. bovis is cattle and buffaloes in Punjab by ELISA</td>
<td>76</td>
</tr>
<tr>
<td>4.11</td>
<td>Abattoir wise prevalence of C. bovis in cattle and buffaloes in Punjab by ELISA</td>
<td>77</td>
</tr>
<tr>
<td>4.12</td>
<td>Sex-wise prevalence of C. bovis in cattle and buffaloes in Punjab by ELISA</td>
<td>77</td>
</tr>
<tr>
<td>4.13</td>
<td>Breed-wise prevalence of C. bovis in cattle and buffaloes in Punjab by ELISA</td>
<td>78</td>
</tr>
<tr>
<td>4.14</td>
<td>Age-wise prevalence of C. bovis in cattle and buffaloes in Punjab by ELISA</td>
<td>78</td>
</tr>
<tr>
<td>4.15</td>
<td>Body condition wise prevalence of C. bovis in cattle/buffaloes in Punjab by ELISA</td>
<td>79</td>
</tr>
<tr>
<td>4.16</td>
<td>Blood values of healthy and infected cattle</td>
<td>84</td>
</tr>
<tr>
<td>4.17</td>
<td>Blood values of healthy and infected buffaloes</td>
<td>86</td>
</tr>
<tr>
<td>4.18</td>
<td>Blood chemistry of healthy and C. bovis infected cattle</td>
<td>88</td>
</tr>
<tr>
<td>4.19</td>
<td>Serum biochemistry of healthy and <em>C. bovis</em> infected buffaloes</td>
<td>90</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE NO.</th>
<th>TITLE</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Comparison of prevalence of <em>C. bovis</em> in cattle and buffaloes</td>
<td>65</td>
</tr>
<tr>
<td>4.2</td>
<td>Percentage of positive cattle and buffaloes in different area of Punjab province</td>
<td>66</td>
</tr>
<tr>
<td>4.3</td>
<td>Percentage of positive cattle and buffaloes in public and private sector abattoir</td>
<td>67</td>
</tr>
<tr>
<td>4.4</td>
<td>Percentage of positive animals among different sex in cattle and buffaloes</td>
<td>68</td>
</tr>
<tr>
<td>4.5</td>
<td>Breed-wise prevalence of <em>C. bovis</em> in cattle and buffaloes in Punjab</td>
<td>69</td>
</tr>
<tr>
<td>4.6</td>
<td>Age-wise prevalence of <em>C. bovis</em> in cattle and buffaloes in Punjab</td>
<td>70</td>
</tr>
<tr>
<td>4.7</td>
<td>Body condition-wise prevalence of <em>C. bovis</em> in cattle and buffaloes in Punjab</td>
<td>71</td>
</tr>
<tr>
<td>4.8</td>
<td>Tissue-wise prevalence of <em>C. bovis</em> is cattle and buffaloes in Punjab</td>
<td>73</td>
</tr>
<tr>
<td>4.9</td>
<td>Cysticerci in Cross section of Liver</td>
<td>80</td>
</tr>
<tr>
<td>4.10</td>
<td>Cysticerci on surface of Liver</td>
<td>80</td>
</tr>
<tr>
<td>4.11</td>
<td>Cysticerci in Cross section of Tongue (bovine)</td>
<td>81</td>
</tr>
<tr>
<td>4.12</td>
<td>Cysts in body muscles (bovine)</td>
<td>81</td>
</tr>
<tr>
<td>4.13</td>
<td>Cyst in Liver</td>
<td>82</td>
</tr>
<tr>
<td>4.14</td>
<td>Cysts in body muscles</td>
<td>82</td>
</tr>
<tr>
<td>4.15</td>
<td>Cysticerci in esophagus (larval form)</td>
<td>83</td>
</tr>
<tr>
<td>4.16</td>
<td>Cysts in liver of Buffaloes</td>
<td>83</td>
</tr>
<tr>
<td>4.17</td>
<td>Blood values of healthy and infected cattle</td>
<td>84</td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>4.18</td>
<td>Blood values of healthy and infected buffaloes</td>
<td>86</td>
</tr>
<tr>
<td>4.19 (1)</td>
<td>Comparison of serum biochemistry values of healthy and C. bovis infected cattle</td>
<td>89</td>
</tr>
<tr>
<td>4.19 (2)</td>
<td>Comparison of serum biochemistry values of healthy and C. bovis infected cattle</td>
<td>89</td>
</tr>
<tr>
<td>4.20 (1)</td>
<td>Comparison of serum biochemistry values of healthy and C. bovis infected buffaloes</td>
<td>91</td>
</tr>
<tr>
<td>4.20 (2)</td>
<td>Comparison of serum biochemistry values of healthy and C. bovis infected buffaloes</td>
<td>91</td>
</tr>
<tr>
<td>4.21</td>
<td>Section of the liver from buffalo showing histopathological changes</td>
<td>93</td>
</tr>
<tr>
<td>4.22</td>
<td>Section of the liver from cattle showing histopathological changes</td>
<td>94</td>
</tr>
<tr>
<td>4.23</td>
<td>Section of the tongue from buffalo showing histopathological changes</td>
<td>94</td>
</tr>
<tr>
<td>4.24</td>
<td>Section of the tongue from cattle showing histopathological changes</td>
<td>95</td>
</tr>
<tr>
<td>4.25</td>
<td>Sections of the esophagus from buffalo showing histopathological changes</td>
<td>96</td>
</tr>
<tr>
<td>4.26</td>
<td>Sections of the esophagus from buffalo showing histopathological changes</td>
<td>96</td>
</tr>
<tr>
<td>4.27</td>
<td>Sections of the tongue from buffalo showing histopathological changes</td>
<td>97</td>
</tr>
<tr>
<td>4.28</td>
<td>Sections of the tongue from buffalo showing histopathological changes</td>
<td>97</td>
</tr>
</tbody>
</table>
ABBREVIATIONS

ALP  Alkaline phosphatase
ALT  Alanine aminotransferase
AST  Aspartate transaminase
FAO  Food and Agriculture Organization
ESP  Economic Survey of Pakistan
GDP  Gross domestic product
OIE  Office International des Epizooties
IFA  Incomplete Freund’s adjuvant
CFIA  Canadian Food Inspection Agency
SPA  Scolex Protein Antigen
CP  Cysticercus bovis
PBS  phosphate-buffered saline
C.  Cysticercus
B.  Bovine
°C  Celsius
ASM  American Society for Microbiology
IMOA  Intramuscular oncosphere assay
ELISA  Enzyme linked immunosorbent assay
EITB  Enzyme-linked immunotransfer blot assay
SEM  Small-Ear-Miniature
L-- SEM  Landrace-Small-Ear-Miniature
T. solium  Taenia solium
T. saginata  Taenia saginata
CSF  Cerebrospinal Fluid Samples
AG  Antigen
AB  Antibody
AlPh  alkaline phosphatase
AcPh  acid phosphatase
SDH  Succini dehydrogenase
LDH  Lacto dehydrogenase
ATP  Adenocine triphosphate
DNA  Deoxyribonucleic Acid
EFSA  European food safety authority
E C  European Parliament and council
DTH  Delayed-type dermal hypersensitivity
DEAE  Diethylaminoethyle
DLE  dialyzable leukocyte extract
TF  Transfer factor
PCR  Polymerase Chain Reaction
O D  Optical Density
RFLP  Restriction fragment length polymorphism
ITS1  Internal transcribed spacer 1
ITS2  Internal transcribed spacer 2
CO1  Cytochrome c oxidase 1
IHC  Immunohistochemical assay
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCGF</td>
<td>T cell growth factor</td>
</tr>
<tr>
<td>PZQ</td>
<td>Praziquantel</td>
</tr>
<tr>
<td>MoAb</td>
<td>Monoclonal antibodies</td>
</tr>
<tr>
<td>MSO4</td>
<td>Magnesium sulphate</td>
</tr>
<tr>
<td>PAMCO</td>
<td>Punjab Agriculture and Meat Company</td>
</tr>
<tr>
<td>HACCP</td>
<td>Hazard analysis and critical control points</td>
</tr>
<tr>
<td>PCV</td>
<td>Packed cell volume</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean corpuscular volume</td>
</tr>
<tr>
<td>MCH</td>
<td>Mean corpuscular hemoglobin</td>
</tr>
<tr>
<td>MCHC</td>
<td>Mean corpuscular hemoglobin concentration</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td>W.H.O</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>GIS</td>
<td>Geographic Information Systems</td>
</tr>
<tr>
<td>ACVP</td>
<td>American College of Veterinary Pathologists</td>
</tr>
<tr>
<td>CYT-Ab</td>
<td>Cysticercosis Antybody</td>
</tr>
<tr>
<td>ETO</td>
<td>Echinococcus granulosus <em>Taenia Saginata</em> and <em>Taenia Ovis</em></td>
</tr>
<tr>
<td>ACVP</td>
<td>American College of Veterinary Pathologists</td>
</tr>
</tbody>
</table>
There are about 188.3 million buffalo and 1382.2 million cattle in the world (FAO, 2009). Pakistan is enriched with a variety of livestock genetic resources. In fact, Pakistan is a key center of animal domestication in this part of the world. The livestock population of Pakistan is well adapted to local environmental conditions. In Pakistan, there are 34.6 million buffaloes and 39.7 million cattle (Economic Survey of Pakistan 2013-14). Livestock is considered as a key indicator for the economics of any state. In Punjab province, there are 21.9 millions buffaloes, 19 million cattle, 24 millions sheep, 37 millions goats and 22 millions camels (Economic Survey of Pakistan 2013-14). Meat industry in Punjab is flourishing and many new abattoir setups are being constructed in addition to those already established, playing vital role in the economy of Pakistan.

During 2013-14 livestock business contributed 55.9 percent to the agriculture sector; and 11.8 percent of the total National GDP (Gross Domestic Product). Recent reports have shown saturation or decline in the growth of different industries, but livestock sector has been flourishing as of late. Livestock value estimates grew from Rs. 756.3 billion (2012-13) to Rs. 776.5 billion (2013-14), an increase of 2.7 percent. The rapid population growth of Pakistan places pressure on the domestic agronomy by accelerating demand for. Strong growth in the livestock sector is helping to satisfy this demand; additionally, livestock exports give Pakistan a considerable boost in the international trade ledgers. Milk production in Pakistan over the last
three years illustrates this growth: 16,741, 17,372, and 18,027 tons from cattle and 29,473, 30,350, and 31,252 tons from buffaloes during 2011-12, 2012-13, and 2013-14, respectively (Economic Survey of Pakistan 2013-14).

In Pakistan, export meat trade has shown robust growth. The export of Halal meat increased to US$ 230.2 million during the financial year 2013-14, up from US $ 211.1 million in similar period last year (9 %). This increase in meat exports indicated an increase of 13.9 percent per year. Producing disease free meat is one primary challenge facing Pakistan’s livestock agronomy; as such, controlling and containment of infectious animal diseases is crucial for both domestic and export sectors of the livestock industry. The animals used for milk can be subsequently slaughtered for human consumption. When a female has completed its milking life and becomes too expensive for milk production then that female can be used for human consumption as a beef animal. When males (calves and bulls) at dairy farms are useless for further use in breeding functions can also be used for beef purpose. Pakistan ranks at # 4 in the world for the export of Halal meat products (Mughal, 2013).

Beef is the major source of protein domestically. The biochemical composition of bovine meat closely resembles that of human skeletal muscle tissue and constitutes an excellent source of protein. Meat contains of all amino acids essentials for human health. Beef is also an essential source of vitamin B, D and E. It is also a most important source of selenium, Iron and zinc. Carbohydrates and mainly fibers, vitamin C and K are not provided by the beef in diet. Lean beef has protein, fat and energy.

In spite of all above benefits the improper handling and cooking of beef may cause many diseases (Waris, 2010). These diseases may result from consuming meat contaminated with harmful agents. These agents are organic or inorganic chemical substances and animal parasites
as well as pathogenic bacteria. Among the most important parasites is *Taenia saginata* whose intermediate stage is *Cysticercus bovis*. This is a causative agent for human cysticercosis.

*Cysticercus bovis* is an infection of cattle muscles which by the larval stage of *T. saginata*. *Taenia saginata* is a cestode which is found in human intestine. The distribution of this parasite is global (Minozzo et al. 2002) but is very common in certain regions (Doyle et al. 1997). The adult Taenia infection in human is known as taeniasis and the condition which is due to the larval stage is called as cysticercosis (Hancock et al. 1989). The occurrence of *Taenia saginata* is higher in developing countries where hygienic measures are poor and where the residents habitually consume rare or inadequately cooked or sun-cured meat (Frolova, 1982; Symth, 1994; Minozzo et al.2002). The disease is also a problem in developed countries where considerable undercooked beefsteak (a beef dish) is eaten as meal. It is important to note that parasitic eggs can survive all stages of sewage treatment. It is significant; too, that even the high levels of monitoring in abattoirs of developed countries have not succeeded in eliminating this parasite (Frolova, 1982; Symth, 1994).

The occurrence of bovine cysticercosis is less in dairy farms than conventional and feedlot farming systems of animals. The factors for *T. saginata* spreading may be due to the presence of causal workers and human to animal contact in feedlot farming (Dorny et al. 2002). Bovine cysticercosis is most prevalent in countries where poor hygiene practices are followed on farms. In Pakistan prevalence of cysticercosis is due to open lavatory practices and unhygienic eating habits like under-cooked beef steak, burgers, tikka, kababs, and other fast foods at local food streets. Another major factor is failure to observe precautionary measures during slaughtering in abattoirs. There is little proper meat inspection in domestic abattoirs. Illegal slaughtering is common. Lack of strict implementation of legislation relating to meat inspection is also responsible for poor and unhygienic meat in the market. The consumption of
semi cooked food prepared from beef causes cysticercosis. Due to health oriented nature, this problem must be addressed at national level with same pace as International efforts.

This parasite (*Taenia saginata*) is present globally both in developing and developed countries as well (Gracey and Collins, 1992; Cabaret et al. 2002). According to World Health Organization data, 50 million cases of this infestation occurred in the world and death toll is 50,000 annually (WHO, 1996). In Ethiopia, the occurrence of *T. saginata* (bovine cysticercosis) has been reported by a number of individuals. Florova in 1982 reported a prevalence of 100%, the highest in the world. In some parts of Ethiopia, due to the practice of eating inadequately cooked beef dishes like kourt and kitffo (that are served in raw or undercooked form) are the source of *T. saginata* infection in man (Teka, 1997). Another report from Ethiopia indicates infection rates up to 89.41% for the same reason (Tembo, 2001).

Cysticercosis in man is known as taeniasis which is associated with symptoms of restlessness, vomiting, gastroenteritis and abdominal pain, excessive or lack of hunger, weakness, loss of weight and intestinal obstruction (Neva and Brown 1994). Rarely, there is a serious disorder due to mobile gravid segments like appendix and biliary tract infection. In cattle the condition is called bovine cysticercosis. There are no symptoms in live cattle having *C. bovis*; however there may be myocarditis or heart failure due to heavy infestation by the larvae (Gracey and Collins 1992).

Life cycle of *Taenia saginata* (bovine cysticercosis) is completed in cattle/ buffaloes and human beings. Animal usually become infected by grazing on pasture contaminated with human feces (which can come from sewage water or direct contamination), then, the cycle is man-feces-cattle-man. Bovine cysticercosis is not transmitted directly from cattle-to-cattle nor is transmitted directly from person-to-person. There is no treatment in infected animals, only strict post-mortem examination in abattoirs and cooking of beef at high temperatures can reduce the
chances of cysticercosis. When this infected and improperly cooked (<60°C) beef is consumed by humans or direct contact by butchers during slaughtering / handling beef then the parasite completes its life cycle in human beings and becomes adult tapeworm. By adopting proper hygienic measures, washing hands with soap after defecation, sufficient meat inspection, abstinence from eating raw or inadequately cooked beef (thorough cooking of meat at a temperature of 60°C or above and freezing the infected carcass at -10 °C for 10 days prevent human infection. Treatment in humans decreases the spread of eggs and disease in cattle (Solusby, 1982).

In developing countries inappropriate education about health and non availability of taenicides are the main causes for the spread of the infection (Pawlowski, 1996). The variations in epidemiological patterns of taeniasis/cysticercosis throughout these countries are a reflection of the numbers and distribution of human and cattle populations (Harrison et al. 1996). In East African countries 30 to 80% prevalence rates have been documented (Tembo, 2001). In majority of these countries this infection constitutes a major public health challenge (Minozzo et al. 2002).

Control of this parasite currently relies on visual detection of suspected parasites or lesions in infected animals at slaughtering and laboratory confirmation of the etiological agent. Detection of infected animals is by the routine inspection of various traditional or “predilection” sites in the carcass, and laboratory diagnosis is dependent on visual, stereomicroscopic and histological examination of specimens. The routine procedure of inspection for screening of carcasses is well recognized to have low sensitivity of detection of animals with light infections (Saini et al. 1997), even when suspected lesions are detected. Lesions are often so degenerated that definite diagnosis by current methods is rather difficult.
It is therefore important that sufficient emphasis be given to this problem so as to improve health, quality and quantity of beef in order to satisfy the domestic requirements and qualify the minimum standards set by international market. Thus the development of suitable tests that are capable of identifying infected animals before slaughter is helpful for diagnosis. These tests can help to estimate the prevalence of the disease and used to identify parasite free areas animals/body parts. Such tests would greatly assist in identifying the sources and allow for improvements in efforts aimed at controlling this disease. Development of serological tests is necessary for diagnosis of the disease in live animals. The tests may be used as alternate for the diagnosis of *bovine cysticercosis* within the local setting and to qualify animals for international export with an acceptable degree of confidence (Wrights, 1998).
2.1. **History**

Infections of this parasite have been observed in history from 1500 B.C. and have been known as one of the earliest human parasites. The infection of *Taenia solium* has been known since biblical times and the life cycle of parasite has been identified by the 1850s (Garcia, 2001). *Taenia saginata* was distinguished at first time by its family member *Taenia solium* in 1782. At the first time *T. saginata* was discovered in 1700 by Audry, but it was very difficult to distinguish the proglottids (segments) of the two parasites. The worm was described by Goeze and an experimental study was conducted by Leuckart and Goeze in which proglottids of *T. saginata* were fed to calves and were developed into *cysticerci* (larval form of tapeworm) muscular tissues of the calves in 1863. Finally, Oliver discovered that when humans eat *Taenia saginata*, they developed adult *T. saginata*. In general, *T. saginata* is such type of tapeworm which "leeches" nutrients off of their hosts. This type of parasite resides in badly cooked beef and is a parasite of both cattle and humans. These worms can also be found in such type of places where there is poor hygiene and human feces are improperly disposed of such as bathrooms (Lightowlers, 2005; Jeon et al. 2006).

*Cysticercus bovis* is found throughout the world but is especially prevalent in the undeveloped and developing areas due to poor hygienic environment, bad managerial practices for cattle and inadequate meat inspection (Carlos et al. 2003). Livestock has an
important role in the societal structures and economy of the most of human population in African and Asian countries. Moreover, bovine Cysticercosis is rising public health hazard in the rural societies as well as in the urban areas where infected cattle are unofficially transported slaughtered and consumed (Carlos et al. 2003).

2.2: **Description of the parasite**

2.2.1: **Taxonomy of the Taenia**

*Taenia saginata* and its metacestode *Cysticercus bovis*, the unarmed beef tapeworm, belong to the class Cestoda order Cyclophyllidea Family Taeniidae and Genus Taenia (Soulsby, 1982; Symth, 1994; Urquhart et al. 1996).

Kingdom, phylum, class, order, family, genus and species are animalia, platyhelminthes, cestoda, cyclophyllidea, taeniidae, Taenia and *Taenia saginata* repectively. Genus Taenia has two species-*Taenia saginata* and *Taenia solium*.

Taenia tapeworms belong to the family Taeniidae, order Cyclophyllidea, and class Cestoda. The scientific nomenclature for Taenia species is somewhat confusing, as within some species, the adult and larval stages have been assigned different scientific names. This is the result of an historical assumption that the larvae and adults, found in intermediate and definitive hosts, respectively, were different species in different genera. Thus the larvae of *Taenia saginata* and *T. Solium* were named *Bovine cysticercosis* and *Cysticercus cellulosae*, respectively. It was not until the mid-nineteenth century that scientists such as Kutchenmeister and Leuckart demonstrated the link between larval and adult stages within species (Grove, 1990).

However, the dual nomenclature persists to this day. The more recently described *T.saginata asiatica* has been correctly assigned one scientific name for all life stages. Infection of intermediate hosts with the larval stage of each of these species is most appropriately referred to
as *Taenia saginata* cysticercosis or bovine cysticercosis, *T. Solium* cysticercosis or porcine cysticercosis, or *T. saginata asiatica* cysticercosis.
2.2.2: Morphology of Taenia

The length of adult tapeworm can be several metres and consists of an anterior scolex (≤2mm in diameter) and a dorso-ventrally flattened series of progressively maturing hermaphroditic reproductive segments, which comprises the strobila. The scolex has four muscular suckers and, depending on species, may have an apical rostellum armed with two rows of hooks. The larval cysticercus is oval in shape, normally achieving a maximum dimension of approximately 2-10 mm when fully developed, depending on species. It consists of a fluid-filled bladder with an invaginated scolex-anlage, contained within a connective tissue membrane or capsule of host origin at the interface with the host tissue matrix. The bladder is presumed to protect the developing cysticercus from the pressure of the surrounding host tissue (Slais, 1970).

Morphological differences in the scolex and proglottid anatomy can be used for the diagnosis of tapeworm species, host species and scolex morphology are used to identify cysticerci (Proctor, 1972; Soulsby, 1982). The eggs having thick wall are about 30-45 μm in size released from “gravid” proglottids and morphologically identical among species and genera within the Taeniidae family.

2.3: Taenia saginata (Cysticercus bovis)

The infection of bovine cysticercosis is caused by C. bovis, which is the larval stage of T. saginata, a tapeworm consisting largely of hermaphroditic segments, with a total length from 4 to 12 m in length, whose definitive hosts are human beings. It contains an anterior part or scolex with four suckers followed by a short unsegmented section, called the neck or germinative area, and the rest of the body or strobile made up of proglottids.

Mature Taenia saginata is 4-8 m in length. The scolex has no rostellum or hooks, and the strobila consists of approximately 2000 proglottids each with 14-32 uterine branches and measuring up to 14 mm in breadth at maturity (Pawlowski and Murrel 2001; Flisser et al. 2004;
Cysticerci are oval, approximately 0.5 - 1 x 0.5 cm in dimension and have a scolex with no rostellum or hooks (Pawlowski and Murrel 2001; OIE, 2004c).

2.4: *Taenia saginata asiatica*

*Taenia saginata asiatica* has resemblance and is closely related to, but genetically and morphologically distinct from, *T. saginata* (Zarlenga et al. 1991). The adult tapeworm has a scolex with a hookless sunken rostellum, and proglottids with posterior protuberances having 11-32 uterine branches. The cysticercus is relatively small (approximately 2 mm) with a rostellum usually bearing two rows of rudimentary hooklets (Fan et al. 1995; Flisser et al. 2004; OIE, 2004c).

2.5: Zoonotic importance

Human being is the only final host where the adult *Taenia saginata* resides in the small intestine. The size reached by the adult worm is closely related to the number of worms present (Maeda et al. 1996). Multiple infections up to 20 tapeworms in one host are often occurring in developing countries (Mann, 1984). The effect on human health is generally slight and symptoms may be vague or absent. *Taenia* has a debilitating effect on people who already have live of protein deficient diets suffer from iron deficiency and infected by hookworm etc. (Mann, 1984). The most noticeable symptom is the spontaneous discharge of one or several proglottids, which often show individual muscular activity. These may creep out of the anus onto the perianal skin and even migrate over clothes of the distraught host or on the ground, shedding eggs as they go (Reinecke, 1983).

*Taeniasis* causes many symptoms, which probably depend so much on the physical and psychological characteristics of the host. Some patients lose their appetite and thus lose weight while others tolerate the infection (Florova, 1982). Sometimes there is obstruction and inflammation of the affected organs due to migration of gravid proglottids of *Taenia saginata* to
different organs appendix, pancreatic duct, nasopharyngeal pathways and bile ducts (Florova, 1982). Tapeworms can also cause intestinal obstruction (Doyle et al. 1997).

*Taenia saginata* in the small intestine of human being absorbs digested food. From the day the Cysticercus is ingested it may take 2-3 months for the parasite to produce ripe segments. As long as the scolices are attached to the intestinal mucosa of the victim new segments will continually grow to replace those that detach from the main body (Teka, 1997).

The zoonotic tapeworms *Taenia saginata, Taenia saginata asiatica, and Taenia solium* infect humans as the normal definitive (primary) host and domestic cattle or swine as intermediate hosts. Human infection with the adult tapeworm is referred to as taeniosis, while infection of Intermediate hosts with the larval stage, or cysticercus, is referred to as cysticercosis. Human Infection with the adult tapeworm of these species, while unpleasant, is easily treated with anthelmintics. Cysticercosis in naturally infected cattle does not generally cause clinical disease, although swine may occasionally manifest clinical signs. Humans also serve as intermediate hosts for *T. Solium*, but human cysticercosis can result in severe, sometimes fatal, neurological diseases (neurocysticercosis). An emerging global disease is neurocysticercosis with an estimated 50 million people affected, causes 50,000 deaths annually (Aubry et al. 1995).

Due to the public health implication of taeniosis and neurocysticercosis, and the negative aesthetic impact of infected meat, cysticercosis causes significant economic loss through condemnation of infected meat and offal, and trade restrictions for endemic regions. These diseases are viewed as threats to international food safety and fall under the mandate of the World Organization for Animal Health. There are no published reports of porcine cysticercosis in Canada, but bovine cysticercosis occurs sporadically and is a federally reportable disease necessitating immediate implementation of specific control measures (OIE, 2004).
2.6: Life Cycle of the zoonotic Taenia

These tapeworms have a life cycle which indirect and are comparatively host specific for both larval and adult stages. Human beings are the only usual definitive hosts of the mature tapeworm. The adult tapeworm is fully developed and reproductively mature as early as 10-12 weeks (depending on species) after infection of the host (Lloyd, 1998a). Once mature, the tapeworm regularly sheds its most posterior segments, called gravid proglottids, which are discharged from infected humans spontaneously or with defecation. These proglottids contain thousands of immediately infective eggs that can remain in the proglottid or be expelled free into the surrounding fecal matrix or environment (Kyvsgaard et al. 1988). On average, a single *T. saginata* tapeworm releases six to nine proglottids daily (Pawlowski and Murrel, 2001).

Eggs can remain infective for several months under cool and moist environmental conditions, and can be disseminated by water and other fomites. Upon ingestion of contaminated feed or water by a bovine intermediate host, a hexacanth embryo, or oncosphere, hatches from the egg and penetrates the intestinal mucosa within a few hours to enter the cardiovascular or lymphatic system. Once it reaches a suitable muscle or other tissue site it develops into a cysticercus and becomes infective for a human host after about 10-12 weeks. In cattle, *cysticerci* are found predominantly in cardiac and skeletal musculature, and occasionally in other sites including liver, lungs, kidneys and lymph nodes. *Cysticerci* remain infective for several months to a year or more (WHO/FAO/OIE, 2005; OIE, 2012).

Although multiple and mixed species infections can occur, most taeniosis infections involve a single tapeworm. Upon ingestion by a suitable intermediate host, a hexacanth embryo, or oncosphere, hatches from the egg and uses its six hooks to penetrate the intestinal mucosa within a few hours to enter the circulatory or lymphatic system. It eventually reaches the tissue
site (such as the lymphatic space in skeletal muscle) where it eventually develops into a
cysticercus which is infective to a human final host after about 10-12 weeks (McIntosh and
Miller, 1960); or as early as 4 weeks for *T. saginata asiatica* (Fan et al. 1990). Domestic cattle
and swine are the intermediate hosts for *T. saginata* and *T. saginata asiatica cysticerci*,
respectively. A suitable intermediate hosts for *Bovine cysticercosis* is Reindeer (*Rangifer
tarandus tarandus*) which have been proven a good host (Kirichek, 1985).

In cattle, *cysticerci* are found predominantly in cardiac and skeletal musculature, and
occasionally in other sites including liver, lung, kidneys and lymph nodes (Ginsberg and Grieve,
1959; Mitchell, 1973; Schillhorn van Veen, 1979). There is evidence that pre-natal infection of
calves can occur (McManus, 1960; 1963). *Taenia saginata asiatica* cysticerci localize primarily
on the serosal surface and within the parenchyma of the liver of pigs (Fan, 1988; Fan et al. 1989;
Fan et al. 1992), and occasionally in extra hepatic peritoneal sites (Chung et al. 1996).
Experimental infections have also been reported for cattle, goats and monkeys (Lloyd, 1998a).
Domestic swine is the normal intermediate host of *T. Solium*, even though a range of other
species, including human beings and dogs, can serve as intermediate hosts (Lloyd, 1998a). In
pigs, *cysticerci* localize in the area of central nervous system, skeletal muscle, tongue, and
subcutaneous tissue. The cycle is completed when human consumes contaminated pork or beef.
When humans consume the larval stage of *Taenia solium*, *Taenia solium cysticercosis* develops
into to an adult cestode. *Cysticerci* (larvae) from pork muscle incubated in bovine bile undergo
evagination – this is the first step of the development pathway that eventually leads to a fully
mature tapeworm (Rabiela et al. 2000).
2.7: Clinical manifestations

2.7.1: In man

The clinical manifestations in humans include abdominal pain, nausea, debility, weight loss, flatulence, diarrhea or constipation. A patient may have one or several of these symptoms and some patients experience gastric hypo secretion. Individual reactions to the infection differ and may be influenced by psychogenic factors, since patients often notice symptoms only after they see proglottids (Symth, 1994). Signs like those of epigastric discomfort, hunger sensations and irritability were also observed in infested individuals (Harrison and Sewell, 1991).

*Taenia saginata* is the most widespread in sub-Saharan Africa, Latin America, Asia, and some Mediterranean countries. Tens of millions of persons are likely infected with *T. saginata* taeniosis worldwide, but reliable estimates are lacking due to the low pathogenicity and under-reporting of this infection. For many otherwise healthy humans infected with *T. saginata*, the symptoms are mild and unrecognized for many years until the parasite dies or are eliminated. The most common manifestation is mild non-specific gastrointestinal illness with symptoms such as pruritus ani, nausea, weight loss, abdominal pain, diarrhea, and anorexia, although more serious complications such as appendicitis have been reported. Cattle with cysticercosis typically do not exhibit any clinical signs. Safe and effective chemotherapy of human taeniasis is available in the form of a single oral dose of praziquantel or niclosamide (Craig, 2007).

Globalization of trade has allowed for the spread cysticercosis and taeniosis from endemic areas. Since humans as the definitive host are key to maintaining the parasite life cycle, a more complete epidemiological picture of *T. saginata* taeniosis is needed; this can be acquired by effective surveillance and mandatory reporting by public health agencies. Practical and effective control programs are also needed; these include thorough cooking of meat, education
regarding the life cycle of worm, implementing measures such as proper hygiene to prevent exposure of cattle to human feces, and taeniacidal treatment (Gajadhar et al. 2006).

2.7.2: In animals

Light or moderate cysticercosis in cattle is not usually associated with any defined clinical picture. Heavy infections, experimentally determined to be in the 200,000 to 1,000,000 T. saginata egg range, may give rise to fever, weakness, profuse salivation, anorexia, increase heart and respiratory rate and a dose of one million or more eggs may cause death between 14 to 16 days due to degenerative myocarditis (Oryan et al.1998).

2.8: Epidemiological study

In Zambia studies were conducted to estimate the prevalence cysticercosis in cattle. Prevalence was estimated using sandwich ELISA. A total 628 serum samples were tested. Only 38 samples were detected positive. In that group prevalence was found 6.1%. Bovine cysticercosis was significantly less common in dairy farms than in feedlots and in traditional farming systems. It is recommended that the contact between man and animals careless employees in feedlots may be the cause of spreading Taenia. Saginata (Dorny et al. 2002).

Experimental study was carried out to estimate the infection by Taenia saginata metacestode. Cysticercus bovis is source of infection in human population if infected; semi-cooked beef is consumed by humans. The controls for infected individuals rather than all bovine animals in a herd T. saginata presentations should help reduce costs and alleviate the current constraints in the management of an epidemic. To this end, we have developed a reliable diagnostic test for use in live animals to veterinary authorities would concentrate control strategies against the disease. The performance characteristics of the ELISA test suggest that it will be sufficient for the application in the field in the homes of bovine cysticercosis (Ogunremi and Benjamin 2010).
2.9: Prevalence of *C. bovis*

Taeniasis / B. cysticercosis is a severe public health issue in developing countries (Minozzo et al. 2002). In Ethiopia taeniasis is common in humans due to the frequent consumption of raw or semi-cooked beef and food dishes like kourt kitffo (Gebro-Emanuel Teka, 1997). In Ethiopia the high prevalence up to 89.41% due to custom of consuming semi-cooked beef in different agro-climatically zones of the country (Tembo, 2001). Taenicides are not widely available and the use of herbal medicines does not eradicate the human cestode (Ashwani Kumar et al. 2011).

Cysticercosis/taeniosis is one of the most prevalent parasitic infections transmitted from foodstuff throughout the world. An experiment was conducted at Assiut Governorate, Upper Egypt. The purpose of this study was to estimate the prevalence of *C. bovis* in animals and its effect on humans. The prevalence of bovine cysticercosis by postmortem was 0.8% in buffaloes and 1.6% in cattle. Prevalence was higher in females. It was 2.7% in female cattle and 1.3% in female buffaloes and was 1.4% and 0.5% in male cattle and buffaloes. When age wise prevalence was observed, it was found that it was higher in buffaloes and cattle which were more than two years (0.9% and 2.7%). It was found 1.1% in cattle and 0.6% in buffaloes. Usual meat inspection showed 1.4% and comprehensive examination showed 1.6% prevalence. This study revealed that bovine cysticercosis was common amongst buffaloes and cattle. Comprehensive meat inspection is suggested than usual meat examination (Basem et al. 2009).

*Cysticercus bovis* is the primary life stage acquired by humans through the consumption of incompletely cooked infected beef. Ideally, control strategies aimed at containing this parasite should focus on individual animals rather than an entire herd would help reduce costs and alleviate the current constraints in the management of an epidemic. To this end, we have developed a reliable diagnostic test for use in live animals to allow immediate discrimination
between infected and uninfected individuals. Removing infected animals exclusively, while keeping those uninfected will help bovine farmers avoid the cost of eliminating an entire herd. The performance characteristic of the ELISA test suggest that it will be sufficient for the application in the field to detect bovine cysticercosis (Ogunremi and Benjamin 2010).

Cysticercosis is a primary public health issue in many countries, and is particularly acute in the developing world. It also has an effect on agricultural development in endemic areas. For this purpose the incidence of Cysticercus bovis in cattle and buffaloes was determined in certain areas of Pakistan, 3731 cattle and 1105 buffaloes were examined from April to October, 2009 in an export slaughter house in Lahore by extended meat inspection at the time of postmortem (masseter and pterygoid muscles, freed tongue, heart, diaphragm, esophagus, M. triceps brachii). The proportion of carcasses found to be infected with cysts varied greatly with species (cattle: 2.1 %, buffalo: 4.7 %; P = 0.002), gender (higher in females; P < 0.001) and age (higher in animals > 2.5 years of age; P < 0.001). In detail, the percentages were in cattle and buffaloes 2.6 % and 6.0 %, respectively; for females (of all species) incidence was 1.9 % and 4.1 % for males. Incidence also varied with age when animal populations of a given gender were further subdivided into > 2.5 yr. and < 2.5 yr. groups (Nauman et al. 2013).

2.9.1: Prevalence of *C. bovis* in some African countries

The prevalence of *C. bovis* in many African countries has been studied extensively over the past few decades. Prevalence of *C. bovis* in Zambia was 6.1 % (Dorny, 2002), Namibia had 6.2% in communal and 2.3% in commercial cattle (Kumba, 2001), Ethiopia 3.2% (Tembo, 2001), Egypt 0.23% in native cattle and 7.25% in imported cattle (Haridy, 1999), Kenya 33.02% (Onyango-Abuje, 1996), Nigeria 10.2% (Frolova, 1982), Chad 6.67% (Frolova, 1982) and in Zaire 22.3 % (Frolova, 1982).
The first published data about the prevalence of bovine cysticercosis was reported in a study by meat inspection authorities in Germany in 1904; this study found an overall rate of just 0.3%. In 1936 a comparable prevalence rate of 0.4% was reported. After the Second World War the prevalence increased significantly up to 2.0% (Ostertag and Goerttler 1958). Between the beginnings of the fifties till the end of the sixties, the prevalence of bovine cysticercosis in some districts in East Germany (former German Democratic Republic) rose from 2% to 7.9% (Meichsner, 1986). In the administrative district Dresden as an example, there was a significant increase of the prevalence from 4.5% in 1970 to 7.1% in 1983 (Meichsner, 1986).

Another recent study was conducted in Pakistan over a period of 3 years. 736 (7.7%) cattle out of 9501 were found to be infected with *Taenia saginata cysticerci*. Endemic areas with particularly high rates were Noorabad Mammasani (10.7%), Kenareh (10.0%) and Shiraz area (8.5%). This study found seasonal prevalence rate fluctuations; spring and autumn prevalence was significantly higher. The main sites where cysts were found were muscle of tongue, diaphragm, esophagus, shoulder, pharynx and masseter. Cysticercosis was observed to induce, in some cases, necrotic hemorrhaging at sites of infestation. *B. cysticercosis* was the main cause of condemnation of carcasses (34%). Over the timeline of this study (3 yrs.), cysticercosis induce carcass condemnation was estimated to impart 100.1 million Rials of damage to Pakistan’s bovine agronomy (Oryan et al. 1995).

*Cysticercus bovis* is a zoonotic infection that has public health and socioeconomic importance. Northern Turkana District in Kenya was selected as a study area to determine the prevalence of taeniosis in humans (definitive host) and to estimate the risk factors contributing to seropositivity in cattle through meat inspection and serological examination. This study has a great significance for human health and will be supportive in approximating economic losses due to condemnation, refrigeration, and downgrading of diseased slaughtered animals. The area
which was selected for study divided into three grazing zones in the south: Lokichoggio–Mogilla centrally and Kibish in the north for the use of the serological examination and questionnaire (n = 53 herd owners) data. Serum samples which were 188 were acquired from cattle from slaughterhouses of Lokichoggio and Kakuma. Consequences of meat examination were evaluated with these results. The human samples which were collected from each of three grazing zones were 66, 97 and 78. With the use of antigen detection ELISA the prevalence of *Cysticercus bovis* in cattle was observed at 16.7% (95% CI 13–20.9%). The prevalence of *Taenia saginata* by microscopy was obtained as 2.5% (95% CI 0.8–5.6%) (Asaava et al. 2009).

A serological study was carried out between November 2009 and February 2010 in ten abattoirs from the Catalonia area (North-Eastern Spain) on cattle up to two years old, using ELISA to detect *cysticercus bovis* exposure/incidence rates. Antigen was detected in blood (serum) of 23 out of 2073 animals, i.e. a seroprevalence of 1.11% (CI95%: 0.76–1.75%). That seroprevalence was about 50 times greater than that generated in postmortem screenings conducted at the same time. Out of a total of 90,891 animals slaughtered, only 19 animals were found positive (0.02%). The animals which were negative at the time of postmortem were showing positive result with the Ag-ELISA (Allepuz et al. 2012).

Bovine Cysticercosis/Taeniasis is a serious food borne parasitic disease throughout the world. The prevalence of *C. bovis* was 1.6 % and 0.8 % in inspected animals respectively, according to the results of a study performed in Assiut Governorate, in Upper Egypt. The prevalence was higher in female bovines (2.7% and 1.3%) than males (1.4% & 0.5%), respectively. A higher prevalence was found in animals more than 2 years old (2.7% and 0.9%) versus those less than 2 years old (1.1% and 0.6%), correspondingly. Routine meat examination yielded lower rates (1.4%) than detailed checking protocols (1.6%). *Taenia saginata* infection rate was 0.6% in patients having signs of gastroenteritis. Among males the infection rate was 1.6
% while females’ infection rates were nil. Our results indicate that infection rates of cysticercosis are common amongst bovine populations. Comprehensive meat checkup should be performed rather than usual less stringent meat examination protocols currently employed in Pakistan (Basem et al. 2009).

2.10: Disease and Occurrence

The muscles most commonly affected by Cysticercus bovis cysts are the heart, tongue, diaphragm and jaw muscles. The cysts may remain infective for 2 years. About 10-20% of cysts found at abattoirs are still alive. When people eat live cysts, the cysts develop into a tapeworm in their small intestine. Human taeniosis, while unpleasant, are generally asymptomatic or manifests as mild nonspecific gastrointestinal illness, including symptoms of pruritis ani, nausea, weight loss, abdominal pain, diarrhea, and anorexia (Thornton, 1979). More serious complications such as appendicitis have been reported (Pawlowski and Schultz 1972).

C. bovis in cattle does not usually cause noticeable infection, but swine are seriously affected with this disease and strongly manifested the clinical signs (Lloyd, 1998a). Cysticercosis of human with T. solium can result in severe, sometimes fatal neurological disease (neurocysticercosis) and is the important origin of acquired epilepsy (Aubry et al. 1995; White, 2000).

Accurate global prevalence data are not available for T. saginata and T. solium species globally but the causes in developing areas with highest infection rates where poor hygienic conditions, insufficient animal care and lack of education facilitate parasite transmission between humans and domestic cattle and swine. T. solium is endemic in Central and northern South America, west and southern Africa, Southeast Asia, and to a lesser extent in parts of south and Eastern Europe (Pawlowski and Murrel, 2001).

Sporadic cases of human taeniosis and neurocysticercosis, primarily “imported” from endemic areas have been reported elsewhere, and endemic foci from immigration have been
established in the USA with approximately 1000 cases reported annually (Schantz et al. 1992; White, 2000; Sorvillo et al. 2007). Cysticercosis with nervous signs is a rising worldwide infection with approximately 50,000 deaths per year (Roman et al. 2000). *Taenia saginata* is most common in western and eastern central Africa, the Caucasus region and south-central Asia; less so in other parts of Africa, the Mediterranean, south East Asia, Central America, South America and central Europe (Pawlowski and Murrel, 2001).

Circulation of *Taenia Saginata* asiatica is thought to be limited primarily to Southeast Asia (China, Taiwan, Philippines, Vietnam, Indonesia and Korea (Ito et al. 2003). Concerted effort will need to be taken to assess *T. saginata* and *T. saginata asiatica* infection rates and distribution, as these two species are not readily distinguishable (Bowles and McManus, 1994).

#### 2.11: Source and Transmission

*C. bovis* infection of cattle is caused by the larval stage of human *T. saginata*. This disease has human health significance because it spreads via consumption of infected semi-cooked beef. *C. bovis* infestation imparts economic loss due to the rejection, freezing charges and lower quality diseased beef. This infection is common in the bovine populations of a variety of zones in Ethiopia, with infection rate ratios ranging from of 2.2% to 26.25%. These rates may be under reported because conventional meat examination protocols are cursory and are performed only in a rapid, low-stringency manner. Routine practice of consuming raw infected beef dishes, low hygienic standards, illegal slaughtering of animals, inaccessibility of drugs which are used against parasites, and accessibility of surface water to animals all contribute to the spread of disease. Recently, reforms have been attempted in Ethiopia in order to bring Cysticercosis/taeniasis under control (Kumar and Tadesse 2011).

A single person infected with a single *T. saginata* tapeworm is capable of contaminating a significant swath of land and can shed approximately up to half a million eggs; these may
remain viable in the ambient environment for a long time (Pawlowski and Murrel 2001). Since *T. saginata* proglottids have a tendency to spontaneously exit the anus independent of defecation, even when good hygienic facilities are available, inadvertent environmental contamination can occur. Eggs contaminating the environment via defecation or spontaneous discharge of proglottids can be scattered by running water, airstream, and scavenger birds, flies, earthworms, or fomites such as different types machines used at farms (Lawson and Gemmell 1985; Kyvsgaard et al. 1988; Pawlowski and Murrel 2001).

Eggs of infective *Taenia* can remain effective indifferent kinds of atmosphere; cold and humid circumstance. Eggs of *Taenia saginata* in Europe have been demonstrated to remain infective after overwintering on pasture (Ilsoe et al. 1990) and in river water after a month (Pawlowski and Murrel 2001). They can also survive in sewage and in sludge for up to several months, and are resistant to most conventional chemical and disinfecting agents (Pawlowski and Murrel 2001). The locality, the number of cattle which are slaughtered, abundance of grazing lands, access ability of animals to surface water and the closeness of sewage water effluent were important forecasters for *C.bovis* infection (Boone et al. 2007).

Transmission to livestock occurs via food or waterborne ingestion of infective eggs originating from human feces. A major source of infection to pigs is scavenged human feces containing *T. solium* gravid proglottids. This is supported by the usual exercise of open field defecations of people in many village areas, to feed on any available food that may or may not be supplemented by limited rations provided by the farmer. Such a concentrated source of eggs increases the risk of heavy infection, compared to those acquired via contaminated feed or water. Cattle can get infection of cysticercus bovis by accidentally consuming of human feces, contaminated water and infected pastures (William Bradley Scandrett, 2007).
2.12: Diagnosis

There are different methods for the diagnosis of bovine cysticercosis.

2.12.1: Detection of *Taenia saginata* cysticerci by postmortem carcass inspection

Controlling steps had been taken for cysticercus bovis by the CFIA on the bases of detection of cysts in affected slaughtered animals during routine postmortem inspection examination. Cysts were approximately 2.5 mm in diameter which can be viewed grossly by 11 days post-infection (McIntosh and Miller 1960).

The examination protocol involves incision and palpation of diaphragm, heart, esophagus, masseter muscles, tongue and observation of superficial and cut surfaces of the carcass exposed during routine postmortem examination (CFIA Meat Hygiene Manual of Procedures, Section 4.6.1, 2007), USA procedure rules (Snyder and Murrel 1986; Saini et al. 1997) and Europe (Kyvsgaard et al. 1990). The idea of these predilection sites for particular parasite is controversial, and numerous studies have attained conflicting results (Mango and Mango 1972; Juranek et al. 1976; Hammerberg et al. 1978; Sewell and Harrison 1978a, b; 1978b; Pugh and Chambers 1989; Oryan et al. 1995; Maeda et al. 1996). It has been proposed that a variety of factors such as activity of muscle, age, breed, and area of geography may affect presence of cysts (Kearney, 1970).

The heart is widely regarded to be an apparent predilection site for *cysticerci*; paradoxically, cysts in cardiac muscle degenerate earlier, and the resulting lesions may persist longer, than in other skeletal muscle sites (Soulsby, 1963; Gallie and Sewell 1983; Harrison et al. 1984; Smith et al. 1991b; Lloyd, 1998a). Although viable mature cysts elicit minimal host reaction (Silverman and Hulland 1961), degenerating *T. saginata* cysticerci incite a host inflammatory response (Sterba et al. 1979a) that makes them more obvious grossly than viable cysts. Also,
since heart is traditionally one of the more thoroughly examined organ (Procedures of Meat Hygiene Manual by CFIA, 2007), degenerated cardiac lesions are among those most frequently detected by meat inspectors.

Since cattle can harbor both viable (infective) and degenerate cysts concurrently, recovery of only degenerate cysts does not imply that no infective cysts remain in the carcass, or in herd mates. Complete resorption of degenerated cysts may take 3 years or longer (Penfold and Penfold, 1937) and viable cysts may persist for at least 2 to 3 years, and possibly for the life of the host (Penfold, 1937; Dewhirst et al. 1963; Froyd et al. 1964; Urquhart and Brocklesby 1965; Van den Heever, 1967). Therefore, it is important to confirm cysticercosis even in cases where suspect lesions are obviously degenerated and non-infective.

It has been demonstrated that the current inspection protocol has a sensitivity of ≤50 % for detection of lightly-infected carcasses (Dewhirst et al. 1967; McCool, 1979; Walther, 1980), which is the degree of infection often associated with outbreaks in Canada. In addition to possible improvements to the inspection protocol by identifying more reliable target tissues in the carcass, provision to inspection staff of standardized training and reference material could improve the sensitivity of detection of this agent.

During postmortem examination by slicing of liver, heart masseter muscles allocation of *T. saginata (cysticercus bovis)* cysts amongst organs and muscle was found from the half carcass of 21 physically infected Zebu cattle at Morogoro slaughterhouse Tanzania. Fondness sites were musculus triceps brachii, the heart were the sites having high ratio of percentage for cysts (mean 12%, 17% respectively, of the total cysts in the carcass), while low prevalence 5%, 2% and 3%, respectively) were found in psoas muscles, masseter and tongue It was expected that 17% of the cysts would be positioned at an examined site if the rules were followed with care. It was
recommended that the heart should be examined carefully during meat examination (Maeda et al. 1996).

Bovine cysticercosis caused by *Taenia saginata* is disease of public health importance warranting usual examination procedures for the postmortem finding of *cysticerci* (cysts) in beef deliberate for human consumption. 42 marketable beef cattle were alienated into 5 groups of 5–12 animals each and injected with either 10,000, 5000, 1000, 100 or 10 *Taenia saginata* eggs. Usual sites were likewise evaluated for the outstanding 32 animals killed within 117 and 466 DPI. 37 animals were infected. Among them only twenty were detected by routine postmortem examination and seven animals had no cysts in usual sites. When only usual sites for all animals were checked, the heart was infected maximum overall, although it was not significantly dissimilar from the masseter muscle, and was the mainly recurrently exaggerated site. The heart was found as the favorite site for exposure of cysticercus bovis based on elevated cyst density and incidence of disease, and more visibility of gross lesions due to the early inflammatory reaction in heart muscle. Further wide inspection of the heart is suggested to get better finding of diseased animals (Scandrett et al. 2009).

An experimental study was carried on and 51 hearts and 51 masseter muscles of slaughtered cattle were inspected in Dakar. During examination 13.7% of the cardiac and 25.5% of the masseter muscles were found infected. These lesions were examined histologically to confirm *T. saginata cysticerci*. Prevalence rate of disease was 5.8% in hearts and 15.7% in masseter muscles observed. Poorer percentage ratio was recorded after examining 2088 slaughtered cattle by the usual meat check up methods (Schandevyl and Vercruysse1982). An experimental study was carried out in which 55 cattle were used as experimental animals. Those cattle were divided into two groups of naturally (*n* = 25) artificially and (*n* =30) infested. Postmortem was performed and results were obtained. Meat check up insensitively exposed
cysticerci in 12 carcasses in each group compared with 24 and 23 carcasses exposed by total
dissection in natural and artificial infestations, respectively. There was a large difference with the
two groups of cattle at the sites where oncosphere attacked. The head muscles, tongue, heart and
*Triceps brachii* were the favorite sites where cysticerci were found in the predilection sites.
However, nonpredilection areas (neck, lumbar regions, back, hind limbs, chest, pelvic, lungs and
liver) considerably harbored huge numbers of cysticerci. Observations indicated that excluding
for the dead, degenerate or calcified cysticerci a careless meat inspector will most likely miss out
quite a number of feasible cysticerci, which combine the pinkish-red color of the meat and be
passed on for human utilization, becoming the cause of cysticercus bovis (Wanzala et al. 2003).

2.12.2: Hematology and serum biochemistry as an additional approach for diagnosis

Diagnosis of *Taenia saginata* (*T. saginata*) cysticercosis in cattle naturally infected with
the ELISA is the main objective of the present study. Also determining effect of *Cysticercus
bovis* on hematology and serum biochemistry was performed additional approach to diagnosis.
The post-mortem examination of slaughtered cattle infected cases was performed. The results of
ELISA showed more prevalence of positive samples with C. bovis. The optical density (OD) of
the samples tested positive were classified into three categories: high (6-7%) Moderate (4%) and
low (18. 7%). The crude extract was fractionated on diethylaminoethyl-(DEAE) cellulose
column. Two partially purified antigens were obtained [P1 = unadsorbed (-ve) and P2 = adsorbed
(+ ve)]. Electrophoretic separating the crude antigen C. bovis showed 13 bands with a molecular
weight of from 235 to 14 kDa. Then, P1 and P2 were 5 and 8 bands, respectively. Immuno-West
separate antigens against hyper immune serum developed a single band at 57 kDa. The results
showed that there was no effect on hematological parameters. Biochemical studies showed that
there was a significantly decrease in total serum protein (P <0.05) infested livestock. High
albumin and 1-globulin in cattle infested levels were recorded compared to uninfected group.
However, in all positive categories marked (P <0.05) - globulin levels than uninfected cattle was observed. The activity of AST in serum was significantly (P <0.05) decreased down cattle infected versus non-infected. In all groups infestation, total serum cholesterol and urea decreased (P <0.05), especially in heavily infested cattle. On the other hand, creatinine level was significantly increased (P <0.05) in the serum of heavily infested versus non-infested cattle. In conclusion, the performance characteristics of the ELISA show its applicability on the ground in bovine cysticercosis epidemics. As impaired kidney liver function may be considered a marker of *T. saginata* cysticercosis in bovine (Kandil et al. 2012).

2.12.3: Gross and stereomicroscopic examination

A definitive diagnosis can be easily made by an experienced parasitologist, either by gross examination or by using a stereoscopic microscope, if the *cysticerci* are viable, or if an intact or partial metacestode can be seen in a recently degenerated cyst. A diagnosis of cysticercosis in cattle tissue samples implies that the organism is *T. saginata*, based on intermediate host specificity of this parasite. However, degeneration of *cysticerci* can begin within 20 days of infection (Soulsby, 1963) and differentiation of the resultant lesion from chronic inflammatory lesions of various other etiologies can be impossible to confirm by gross, stereomicroscopic, or even histological examination (Schandevyl and Vercruysse 1982).

2.12.4: Molecular methods

Molecular methods for characterizing adult *Taenia*(Gonzalez et al. 2000) have been applied to *cysticerci*, but require further validation before use in regulatory diagnosis can be recommended (Harrison et al. 2005). Once identified as a cysticercus, host species and tissue location of the cysticercus can suggest a more specific etiology.

Experiments had been carried out for the diagnosis of *cysticercus bovis* by using ELISA test with the help of unlike antigens which were prepared by larvae of *T. crassiceps* and *T.*
solium, by the use of different types of sera negative and positive control. There was a low sensitivity observed in the natural conditions of the manifestation of cysticercus bovis by ELISA, whereas higher percentage (up to 90%) in experimental observations. The higher ratio of specificity of the test (81-100%) showed its capability to distinguish itself from other diseases C.bovis. There was no differentiation found in the work of the antigens which were examined .It was resulted that the ELISA test is helpful to identify experimentally diseased animals and to diagnose cysticercosis from other animal diseases, but it is not useful for the detection of anticysticercosis animals to slaughter antibodies (Monteiro et al. 2006).

Laboratory confirmation methods are significant in bovine cysticercosis diagnosis as other diseases can result in morphologically similar lesions and resulting and in false identifications.(Cuttel L. et al. 2013) optimize detection of specific nucleotide sequences. It relies on the availability of appropriate target nucleic acid sequences that flank regions of interest (“primers”) the synthesis of these oligonucleotide primers and a suitable DNA isolation (extraction) technique for test samples.

Cysticercus bovis is a zoonotic infection that has public health and socioeconomic importance. Northern Turkana District in Kenya was selected as a study area to determine the prevalence of taeniosis in the human being the definitive host and to estimate the risk factors that contributes for seropositivity of parasite in cattle through meat inspection and serological examination. This study has a great significance for human health and will be supportive in approximating economic losses due to condemnation, refrigeration, downgrading, of diseased slaughtered animals. The area which was selected for study divided into three grazing zones of or poi to the south, Lokichoggio–Mogilla centrally and Kibish in the north for the use of the serological examination and questionnaire (n = 53 herd owners) data. Serum samples which were 188 were acquired from slaughter cattle from slaughtering areas of Lokichoggio and Kakuma.
Consequences of meat examination were evaluated with these results. The samples of human which were collected from each of three grazing zones were 66, 97 and 78. With the use of antigen detection ELISA the prevalence of cysticercus bovis in cattle was observed at 16.7% (95% CI 13–20.9%). The prevalence of *Taenia saginata* by microscopy was obtained as 2.5% (95% CI 0.8–5.6%) (Asaava et al. 2009).

Diagnostic polymerase chain reaction (PCR) is a molecular method used to amplify and thus (Gottstein, 1994). Genomic libraries are being increasingly generated for more and more parasite species, facilitating development of species-specific DNA sequences for use as primers in diagnostic PCR assays (McManus, 1990; Gottstein, 1994; Singh, 1997).

Bowles and McManus (1994) genetically characterized adult *Taenia saginata* from the closely related human taeniid *T.asiatica* (*Asian Taenia*), by first amplifying a CO1 gene fragment followed by restriction endonuclease Msp 1 digestion to differentiate *T. saginata* from *T. asiatica*. When incubated with Msp, *T. saginata* CO1 remained intact, while that of *Taenia saginata* was cleaved. More recently, protocols utilizing multiplex PCR and RFLP for the molecular differentiation of genomic (gDNA) and rDNA from Echinococcus granulosus and mature *Taenias* (Gonzalez et al. 2000; Gonzalez et al. 2002; Gonzalez et al. 2004). Same research has also been conducted by (Abuseir et al. 2006).

Although most of the current methods have been applied to fresh or frozen samples, they could presumably be adapted for formalin-fixed tissues; however, fixation can result in further degradation of DNA (Mygind et al. 2001), and there are no diagnostic advantages to doing so, unlike those already stated for the *in situ* immunohistochemical assay. Use of both IHC and PCR assays for the diagnosis of a variety of other pathogens indicated that PCR was more sensitive than IHC (Brunnert et al. 1994; Bazler et al. 1999; Held et al. 2000; Tegtmeier et al. 2000). The suggestion that false negative results for degenerated *T. saginata cysticerci* may be due to
insufficient DNA remaining in the specimen (Van der Logt and Gottstein 2000; Geysen et al. 2007) invites a future comparison between the performance of molecular assays which target residual cysticercal DNA with the antigen-based IHC assay.

2.13: Histopathological Changes due to bovine cysticercosis

Histopathological examination of 253 dead cysts out of 416 was performed which had nodular firm whitish lesions, having yellowish material. In these cysts granulomatous lesions were observed by histological exams characterized by caseous and calcareous material, histiocytes in palisade, giant cells, The parasite debris was like non cellular material with spherical and ovoid, a hyaline, colorless, eosinophilic and basophilic corpuscles (Renata et al. 2012).

Histological methods are unreliable for differentiation of degenerated *T. saginata* cysticerci from chronic inflammatory lesions of various other etiologies (Schandevyl and Vercruysse, 1982). No standard criteria have been established for the histological evaluation of *Cysticercus bovis* -suspect lesions. Specimens are formalin-fixed, sectioned and stained, usually with hematoxylin and eosin. Other methods, including procedure of Gomori for reticular fibers, have been promoted to reveal particular parasite characteristics in lesions in advanced phase of degeneration (Slais, 1970); however, Geerts et al. 1980 demonstrated that this stain was no better than hematoxylin and eosin in the identification of 32 degenerate *cysticerci* recovered from 25 bovine hearts. The application of criteria such as de-mineralization, staining technique, and number and region of sections viewed (Pampiglione et al. 1999) depend on the histopathologist conducting the assay (Yves Robinson, personal communication; Mary Sutton, personal communication). Several authors have described (and in some cases developed grading criteria for) the histological characteristics of viable and degenerating bovine and porcine *cysticerci* using a variety of staining techniques (Silverman and Hulland 1961; Slais, 1970; Retzlaff, 1972;
Sterba and Dykova 1978; Sterba et al. 1979a, b; Safranov and Drogun, 1985; Aluga de and Vargas, 1988; Zivkovic, 1996). Silverman and Hulland (1961) were the first to describe in detail histological observations on bovine cysticerci obtained from several hundred naturally or experimentally infected cattle. Cysts were stained with hematoxylin and either eosin or van Gieson. Viable cysts were associated with minimal host inflammatory response with few eosinophils, and no giant cells, whereas degenerate cysts were associated with the formation of granulation tissue in the cyst space, the presence of giant cells around parasite fragments, the breakdown of the cyst wall, and an invasion of granulocytes, including eosinophils, typical of Chronic inflammation. Identification of some lesions was complicated by the onset of degeneration of the cysts at variable stages of development. In general, for a mature cyst, degeneration was initiated via breakdown of the cyst wall and cuticle, followed by disappearance of the subcuticular layers, followed by the eventual dissolution of cestode remnants such as the suckers and rostellum of the scolex (Silverman and Hulland 1961). Since hooklets are not present in *T. saginata*, the parasite remnants most often identified in advanced stages of degeneration are calcareous corpuscles (Silverman and Hulland 1961). Calcareous corpuscles, found in larval cestodes, and located mostly in the scolex-anlage and neck region of cysticerci.

Studies were continued about the oral expressions which are caused by the development of *Taenia saginata* or *T. Solium* in humans, as well as locations in the maxillofacial areas cysticercus cellulosae or C. bovis are very exceptional. With reference to the five cases of cysticercosis with a single location in the oral or in combination with other area locations, the writers talk about the morphology and symptomatic characters of maxilla-facial cysticercosis (Timosca and Gavrili 1974). To diagnose the infection of *Taenia solium*’s oncosphere by intramuscularly inoculums in pigs a new method was developed. Research was conducted by use of 18 animals in five series of experiments. The method of this experiment is simple to carry out,
need a lower number of oncospheres, permits several infections per animal and decreases the deviation inherent in models of oral infection. This experiment intramuscular oncosphere (IMO) can make available a valuable instrument for estimating potential therapeutic or vaccine for cysticercosis (Verastegui et al. 2000).

The experimental study was conducted in which 253 dead cysts out of 416 were differentiated by nodular firm whitish lesions, having yellowish substance; occasionally in calcareous features were scrutinized for histopathology. The histological exams of these cysts capitulated granulomatous lesions, whose centers were characterized by caseous and calcareous matter, multinucleate giant cells, histiocytes in palisade and penetrated composed mainly by lymphoid cells, covered up by fibrosis. Infrequently the lesions margins had granulation tissue and mineralized areas, like linear blade. The parasite wastes were similar to a hyaline, non cellular material with round and ovoid, basophilic, eosinophilic and colorless corpuscles. These corpuscles were observed not often, sometimes, among inflammatory reaction. Fibrous nodules, loaded in lymphoid or mixed infiltrates, were commonly observed (Costa R.F.R et al. 2012).

This study aimed to use BALB/c mice as an animal model to develop T. saginata oncosphere infectivity. The biochemical and histopathological changes in this model were estimated. Cysts resembling with tumors having meta-cysticerci larvae were obtained at the injection sites of mice at the 8 week post-infection which Th confirmed by using polymerase chain reaction PCR assay. The 1,300 base pair (bp) product was detected for T. saginata cyst by PCR, histopathological examination of infected mice heart revealed myocarditis. Biochemical analysis result showed that there was a significantly (P<0.05) increase in serum globulins and marked reduce in A/G ratio in group of mice infested with oncospheres of T. saginata compared to non-infested group. However, there was no significant difference in serum total proteins and albumin of both groups of treatment. Serum of creatinine and total cholesterol levels and ALT
activity in infested group were markedly (P<0.01) increased compared to control non-infected group. In conclusion, female BALB/c mice can be used as experimental animals for studying the host immune response in vaccine development trails. As well as *T. saginata* cysticercosis caused an alteration in liver and kidney functions (Kandil et al. 2013).

Although cattle with cysticercosis are unlikely to exhibit clinical signs, pigs with serious infections may be presumptively diagnosed ante mortem by observation and/or palpation of cysts in the tongue (Gonzalez et al. 1990). In most cases, however, detection is made during postmortem carcass examination in both cattle and pigs. In most parts of the world where regulated postmortem screening for these parasites occurs, examination of so-called predilection “sites” is conducted during routine meat inspection. However, such procedures are insensitive, particularly for lightly infected carcasses (Saini et al. 1997). Viable *cysticerci* can effortlessly be neglected on meat inspection since the translucent cysts merge with the neighboring host tissue. The adequate host inflammatory reaction is provoked after degeneration and death of the parasite for more detection of visible lesion. Moreover, cysticercosis infections can consist of both degenerate and viable *cysticerci* (Juranek et al. 1976). Depending on the extent of degeneration, the end result of which is a mineralized or fibrotic lesion, definitive parasite characteristics may not be evident on gross examination or histology. The recent development of an immunohistochemical assay for parasite excretory-secretory antigen in degenerate C. bovis lesions will help in this regard (Ogunremi et al. 2004a). Molecular methods for characterizing adult *Taenia* (Gonzalez et al. 2000) have been applied to *cysticerci*, but require further validation before use in regulatory diagnosis can be recommended (Harrison et al. 2005). Once identified as a cysticercus, host species and tissue location of the cysticercus can suggest a more specific etiology.
Immunity to taeniids is predominately antibody mediated (Ferrer et al. 2003). Immunity in calves against cysticercus bovis (Cestoda) can be generated by injecting intramuscularly artificially hatched heterologous or homologous oncospheres. Such treatment rendered calves immune to *T. saginata* eggs infestation upon ingestion (Wikerhauser et al. 1971). However; serological assays for bovine and porcine cysticercosis have not met with the same success as similar assays for human *T. solium* cysticercosis. Even though commercially available ELISA and EITB have high specificity and sensitivity when applied to human serum or CSF samples for *T. solium*, their trustworthiness has been low for naturally diseased animals (Sloan et al. 1995; Sciutto et al. 1998). Similarly, in spite of ongoing research on the development of serological assays for bovine cysticercosis, using homologous or heterologous (e.g *T. Solium, T. hydatigena, T.crassiceps*) antigens or synthetic peptides (Ferrer et al. 2003) to detect circulating parasite antigen (AG-ELISA) or antibody (AB-ELISA) has proven to be inadequately sensitive and/or specific (Geertz et al. 1981; Harrison and Sewell 1981; Harrison et al. 1989; Hayunga et al. 1991; Smith et al. 1991a; Bogh et al. 1996; Lloyd, 1998a; Van Kerckhoven et al. 1998 ; Dorny et al. 2000; Monteiro et al. 2006). Thus, there is no serological assay available commercially for use in animals (Lloyd, 1998b; OIE, 2004c). Currently, such an assay may have value as an epidemiological tool for screening herds for cysticercosis but would not be applicable for individual animal diagnosis (Wanzala et al. 2002; Monteiro et al. 2006).

A study carried out on four Zebu calves aged 1–1.5 years sought to the efficacy of vaccination using hatched ova of *Taenia saginata* administrated subcutaneously. There was no consequent oral infectivity with this tapeworm as immunity elicited protected thee animals and the premature metacestodes deterioration was manifested in which consequences three of four animals could not attain maturity. In the fourth calf three viable cysts were found which had
more than 300 specimens’ when compared to non-vaccinated controls (Sheiba Babiker and Zein Eldin 1987).

Dependable techniques for recovering *Taenia* eggs from various environmental matrices, including livestock feed and water, are not available. In most sporadic outbreaks of bovine cysticercosis in low prevalence regions such as North America, a definitive source is not often identified. Even if a particular feed or water source is suspected, processing of relatively large volumes contaminated at low levels is problematic. Modified flotation methods have been attempted in such cases, but the high specific gravity of *Taenia* eggs, and confounding artifacts and adherent debris in the assayed matrix negatively impact sensitivity (Scandrett and Gajadhar 2004).

As well, since there is usually a minimum of several months after presumed exposure before the first index animal is detected at slaughter; the contaminated source may no longer remain, or if so, may have undergone degeneration, further confounding recovery of the agent and interpretation of results. Since taeniid eggs cannot be speciated based on morphology, and there is no baseline data available for levels of environmental contamination with other domestic and wildlife taeniid species, any positive findings must be interpreted with caution. Reliable molecular methods for detecting low numbers of *Taenia* eggs are still being developed (Gonzalez et al. 2000; Nunes et al. 2005).

Globalization poses an increasing threat of incursions of cysticercosis and taeniosis via the increased international movement of people and importation of animals, their products, and potentially contaminated produce or other fomites from endemic regions. In theory, these infections can be eradicated (Flisser et al. 2003). Human taeniosis is the only source of cysticercosis and is easily and inexpensively treated with anthelmintics (such as niclosamide or praziquantel); pigs and cattle are the only significant reservoir of cysticercosis, and simple
cooking or freezing measures render *cysticerci* non-infective. Most eradication efforts have been aimed at *T. Solium* due to the severe consequences of human neurocysticercosis. Bovine cysticercosis, in addition to having a lesser public health concern, is less amenable to eradication due to the greater biological potential of the *T. saginata* tapeworm, greater difficulty in detecting animals that are often lightly infected, and a global propensity to consume raw or semi-cooked beef (Pawlowski and Murrel 2001).

An animal model of *T. saginata asiatica* used for oncosphere infection by using normal strain of C3H/HeN female mice was implemented to asses’ cytokine responses. Cytokine enzyme-linked immunosorbent assay (cytokine ELISA) and flow cytometry were used in this model to analyze the host cellular immune response. There was appearance of tumor-like cysts containing *cysticerci* at the inoculation sites of female mice after 7 weeks of infection with the *T. saginata asiatica* oncospheres. It was observed by the results that the Th1 response was not induced during the stage of cysticercus formation and Th1 cells performed a main role in the immune response in C3H/HeN mice during the early stages of the oncosphere infection (Peng et al. 2009).

In spite of its low sensitivity, regulated postmortem inspection of cattle and pig carcasses at slaughter for cysticercosis helps to reduce transmission of these parasites. Affected carcasses are condemned, or treated by cooking or freezing to kill the parasite (Hird and Pullen 1979; Saini et al. 1997). Disease control regulations often dictate that epidemiological investigation and quarantine of suspect herds may be conducted, with significant economic impact on both regulators and affected producers. Antihelmintic treatment of livestock is effective but does not reliably eliminate cysticerci, and is often not practical, particularly for cattle (Gallie and Sewell, 1983; Harrison et al. 1984; Gonzalez et al. 1998; Gonzalez et al. 2001). Vaccination of animals at risk holds more promise. Immunization of cattle with preparations of crude parasite antigen
reduced infection in animals exposed to sewage on pasture (Rickard et al. 1981). More recently, recombinant subunit vaccines based on oncosphere antigens have proven highly effective in reducing infection rates. A synthetic peptide protecting cattle and pigs from experimental challenge with *T. saginata* and *T. Solium* eggs has also been developed (Lightowlers et al. 1996; Gonzalez et al. 2005). Vaccines against *T. Solium* cysticercosis significantly reduced prevalence and intensity of infection in pigs exposed to natural challenge (Huerta et al. 2002).

Since *Taenia* eggs are inherently resistant to many environmental conditions and most practical and conventional chemical treatments, efforts should be aimed at preventing environmental contamination with eggs. This will entail reducing the overall prevalence of human taeniasis, and preventing exposure of livestock to human feces and sewage. If sewage must be used as fertilizer, measures such as delayed grazing of cattle on treated pastures can be used to reduce the number of viable eggs in the applied sludge (Cabaret et al. 2002).

As humans are ultimately responsible for maintaining the parasite cycle, more data on the prevalence of human taeniosis is needed. This requires increased surveillance and mandatory reporting by public health agencies. Control programs for *T. Solium* have included mass taeniacidal treatment (Allan et al. 1997). Education on the parasite cycle and on mitigating measures such as proper hygiene and latrine use, preventing access of livestock to human feces, and thorough cooking of meat, are important in reducing overall parasite transmission.

Autopsy of Soay Sheep on St. Kilda revealed the presence of *cysticerci* of *Taenia hydatigena* despite the absence of the definitive host on the island. Both the intensity of infection and the prevalence increased with age implying that the sheep did not acquire immunity to re-infection or super infection. The sheep on average ingested approximately 2.4 eggs per annum. This is far below that expected if an infected dog had visited the island. The data provide
evidence that taeniid eggs are being transported by wildlife from at least the nearest inhabited land mass to St Kilda some 60 km distant (Torgerson et al. 1995).

One recent study involved introducing viable *T. saginata* eggs into calf stomachs with the help of stomach tube. These calves were slaughtered after 12, 16 or 24 weeks. The carcasses infected with *cysticerci* were frozen at six different temperatures for different times. *Cysticerci* were isolated manually from the sets of infected carcasses after thawing them at room temperature. Scolex evagination and peristaltic movements of parasite bladder wall were criteria of viability of the metacestodes. 12-16 week-old *cysticerci* were found more susceptible to the harmful effects of freezing than were 24-week-old *cysticerci*. The temperature and time combinations required to guarantee death of all *cysticerci* were 144 h at −15°C, 216 h at −10°C and 360 h at −5°C or less (Ronald et al. 1978).

**2.14: Risk factors to infection**

*Cysticercus bovis* is of economic importance and an important public health problem particularly in East Africa. A cross-sectional survey on bovine cysticercosis was carried out from October 2012 to July 2013. To estimate the prevalence and risk factors to infection 400 zebu cattle slaughtered at an Indasilassie municipal abattoir were examined. Samples were taken from 270 residents for a questionnaire surveys to estimate the zoonotic potential and its significance in humans. The prevalence of *Cysticercus bovis* was found to be 15.60% (95% CI: 12.60, 18.40). The likelihood of acquiring bovine cysticercosis infection was higher in Indabaguna (OR = 2.50, 95% CI: 1.25, 5.0, P = 0.01) than in Shire Indasilassie, animals living in rural area (OR = 3.74, 95% CI: 1.60, 8.80, P = 0.002) showed higher rates than animals living in urban area, animals consuming river and pond water (OR = 2.6, 95% CI: 1.17, 5.84), P = 0.02) than animals drinking treated water. Moreover, a total of 120 cysts were randomly collected of which, 42 (35.25%) were found to be viable
while the rest 78 (65%) were found to be non-viable (degenerated) cysts. The heart, liver, diaphragm muscle, tongue, shoulder muscles, masseter muscles, intercostals muscle and thigh muscles showed rates of infection at 11.69%, 6.49%, 1.30%, 12.99%, 25.97%, 3.90% and 23.38% respectively. The questionnaire survey clearly indicated that place of residents, age, sex and raw meat consumption were significantly associated with higher taeniasis infection rates in human (P < 0.05). Therefore, it is highly imperative that efforts aimed at treating taeniasis and controlling exposure of animals and humans to all T. saginata life stages be focused on regions known to be highly endemic for this parasite (Dawit Gebremichael and Temesgen Mohammed 2013).

2.15: Prevention and control

Lack of and improper use of latrines or open field defecations leads to contamination of grazing lands. The use of latrine reduces spread of T. saginata eggs. Controlled grazing, avoiding use of sewage effluent to fertilize pasture, prevents infection in cattle (Symth, 1994). Adequate meat inspection, abstinence from eating raw or inadequately cooked beef (thorough cooking of meat at a temperature of 56 - 600c) and freezing infected carcass at -100 c for 10 days are all practical measures to help reduce human infection rates. Chemotherapy in humans reduces the spread of eggs and infection in cattle (Solusby, 1982).

2.15.1: Vaccination

In sheep and cattle recombinant vaccines against cysticercosis and hydatidosis have been produced. Recombinant vaccines play important role in control of taeniasis /cysticercus and hydatidosis in cattle and sheep. Recombinant vaccines are non-living vaccines against various parasitic infestations. Recombinants vaccines were developed through various molecular techniques that include sequencing antigen-coding genes, then using techniques such as PCR and
cloning to generate expression systems which can efficiently generate large quantities of desired antigens to be deployed as vaccines for testing and for use (Marshall and Charles 2001).

2.15.2: Treatment

Praziquantel is used as a treatment for human cysticercosis. Five to ten mg/kg praziquantel is suggested as a single dose in human taeniasis. Field studies confirm that for exclusion of *T. Solium* tapeworms in man a dose of b 3.4 -- 7.5 mg/kg was effective. Several intestinal cestodiasis can be effectively treated by doses of one to five mg of praziquantel / kg (Pawłowski. 1990).

There is a large number of taenicidal drugs available in the market. However the drug of choice in treating Taeniasis is niclosamide (Niclocide, Yomesan). An adult dose rate of 2000 mg is effective in damaging the worm to such an extent that a purge following therapy often eliminates the scolex. Praziquantel with a dose of 5 to 10 milligram / kilogram body weight also has been reported highly effective (Doyle et al.1997), but the scolex is partially digested and often not recovered (Symth, 1994). Other drugs used in the treatment of *T. saginata* are mebendazole (Soulsby, 1982; Doyle et al. 1997) followed by purgative, such as magnesium sulphate (MgSO4) to expel the dead worms in toto (Soulsby, 1982).

In animals treatment with compounds such as albendazole (50mg per kg), praziquantel (50mg per kg), mebendazole (50mg per kg) can be given but they are considered not to be fully effective (Symth, 1994; Soulsby, 1982). Praziquantel is effective at 50mg/kg/day for four days but this treatment is impractical because of its high cost (Reinecke, 1983). *C. bovis* infection can be controlled with the help of recombinant vaccines. This vaccine has already been used in cattle (Lightowlers et al.1996).

2.16: Economic importance of *C. bovis* / Cost of Treatment
Attempts to reduce the prevalence of *Taenia saginata* and *T. solium* in human beings and their cysticerci in animals (cattle, pigs) may have a considerable impact on the economics of meat production industries. There are economic losses to food industry due an important food safety issue of Cysticercosis in domestic animals. This will be particularly important where export industries are involved, since most importing countries have stringent regulation designed to prevent the importation of infected meat (Harrison and Sewell 1991). Cysticercosis costs the bovine agronomy of South Africa an estimated 3.3 million $ US annually. (Dorny et al. 2002). These costs include treatment of human taeniasis, expenses in infected animal processing and inspection of facilities, and revenue loss (Mann, 1984). Losses/year due to treatment were recently estimated by Abdusslam (1975). There were annual losses of USD 4 million and USD 2 million respectively in Kenya and Botswana due to bovine cysticercosis (Grindle, 1978). This mainly arose from the loss of value in abattoirs resulting from boiling the meat to kill the cyst, as the presence of *cysticerci* in the meat would be a serious obstacle to meet the import regulations of the recipient countries (Gracey, 1981). In feedlot cattle, incidence rates range from 40% to 3% (Reinecke, 1983).

Heavy bovine cysticercosis infestation often induces changes in the consistency or appearance of tissues. When infestation signs are readily apparent, meat processing facilities will condemn tissues, or even whole carcasses (Hubert, 1974). (Dewhirst et al. 1967; Yoder et al. 1994; Onyango-Abuje 1996b; Giesecke, 1997). Cysticercosis is a significant cause of production losses in Europe and other western countries and is an impediment to the export of beef from many developing countries (Harrison, 1996).

In one recent study on slaughterhouse records indicated totals of 115,186 cattle, 61,551 sheep, 37,850 goats and 13,310 pigs processed. Disease induced condemnation figures were 39 (0.063%), 125 (0.108%) 132 (0.992%), 40 (0.106%) for sheep, cattle, pig, goat carcasses
respectively. The most important cause of total carcass condemnations was cysticercosis in cattle (0.051%) and pigs (1.397%). Among the four species lungs and livers were found to have the highest (larval) parasite loads. In cattle fasciolosis of liver was (8.6%). Infected lungs ratio was 2.43%, 3.99%, and 2.83 in cattle, goats and sheep respectively. Due to their zoonotic nature, there was a great risk for humans for occurrence of fasciolosis, hydatidosis, tuberculosis and cysticercosis (Mellau et al. 2010).

Due to bovine cysticercosis infection condemnation of carcasses was 34.6%. Over the 3-year period, the economic impact of cysticercosis infection was estimated to be 100 Million Rials (Oryan et al. 1995). There is a close relationship between infection and economical losses. Cysticercosis is a significant cause of production losses in Europe and other western countries and is an impediment to the export of beef from many developing countries. In South America, the total annual loss reaches $ 420 million (Sérgio de Arruda Pinto et al. 2002). Accurate estimations of economic losses in other countries are lacking, as there is no reporting system for this infection. T. Saginata is a vital cestode parasite which plays important role in human health and economics. Cattle perform as intermediate host in its life cycle after ingesting T. saginata eggs (proglottids) from diseased and carrier human beings. After 10 weeks of life cycle in cattle muscles Cysticerci become able to infect humans (Flisser et al. 2005; McFadden et al. 2011). Many countries have adopted measures that condemn carcasses with high levels of C. bovis infestation (if cysts are found at least two sites viz. forelimbs, esophagus, heart, diaphragm, and the tongue and masseter muscles). Animals with lower levels in infestation should also be rejected or should be refrigerated prior to human consumption (Ashwani Kumar et al. 2011).

In a recent study in Ethiopia focused on a routine meat inspection showing 824 (18.49%) sample infestations with Cysticercus bovis out of a total 4456 organ samples from slaughtered cattle. The main predilection areas for cysts were thigh, triceps, cardiac muscle, masseter muscle
and tongue. Postmortem examination showed that 768 (18.72%) out of a surveyed 4102 male cattle had cysts of *Cysticercus bovis* and 56 (15.82%) out of 354 female cattle were found to be infected. All the adult animals were slaughtered. No significant difference was noted between the two sexes. There was monthly increase observed in infection rates during the dry season (Nigatu Kebede, 2008). While cysticercosis is found throughout the world, it is an acute problem in tropic and sub tropic regions (Basem et al. 2009). A two year retrospective study conducted in Morogoro region in the eastern part of Tanzania found that many cattle carcasses destined for human consumption had to be condemned due to a variety of animal diseases. The study’s results showed the major causes to be tuberculosis (44%), weight loss (28%) and *Cysticercus bovis* 16% (Kambarage et al. 1995).

Economic losses of beef industry depend upon the nature of infection. Highly infected carcasses are usually destroyed while carcasses having low parasite loads are kept under treatment in cold storage to eliminate of parasite activity (Kandil, O.M et al. 2004). The infection of *bovine Cysticercus* is found all over the world, mostly in the developing countries, where there are not stringent inspection protocols and management practices are very poor (Carlos et al. 2003). In the countries of Asia and Africa livestock play a major role on the social life and economy of the people. Bovine cysticercosis is a rising public health issue in the rural areas of the world as well as in metropolitan areas where infected animals are transported and consumed (Carlos et al. 2003).

2.17: Statement of Problem:

Keeping in view the importance of bovine agronomy in Pakistan and public health hazards of cysticercosis, the present study was designed with the following objectives.

1. Prevalence of *bovine cysticercosis* in cattle and buffaloes in Punjab.

3. Effect of *bovine Cysticercosis* on hemogram and serum biochemistry as an additional approach for diagnosis in cattle and buffaloes.

4. Histopathological changes induced by *bovine Cysticercosis* in different organs of cattle and buffaloes.

5. Approximate the economic losses to bovine agronomy due to *Cysticercosis* infection in Punjab.
CHAPTER 3
MATERIALS AND METHODS

3.1. Prevalence of *bovine cysticercosis* in cattle and buffaloes in Punjab

3.1.1. Geo-Location of Study Area

The total area of Punjab province is 205,344 square kilometers and holds 60% of human population of the country. Punjab contains five rivers; Jhelum, Sutlej, Indus, Ravi and Chenab. Punjab is the only province that has boundaries with other provinces viz. Sindh, Baluchistan, Khyber-Pakhtoon-Khawa, Gilgit-Baltistan, and Azad Jammun and Kashmir. It has features the most fertile agriculture lands where various types of fodder are grown as animal feed. The province is divided into three ecologically different zones; Southern, Central and Northern Punjab. Punjab province has four clearly defined seasons viz. summer, winter, autumn and spring.

Map of Punjab province
(www.goodnewspunjab.com.pk)
3.1.2. Study Site

The study was conducted at a number of public and private abattoirs in Punjab Province. All these abattoirs were HACCP certified and had strict rules and regulations regarding veterinary inspection for the production of wholesome meat. The main purpose of these abattoirs was processing of one or several classes of livestock into fresh meat for human consumption through hygienic processing and storage including edible byproducts. In these Abattoir cattle, buffaloes sheep and goats are processed as Halal meat for human consumption. The animals for slaughter were brought from various constituencies of the Punjab. An average 350 cattle / buffaloes, 5500 sheep/goats were being slaughtered on daily basis in these abattoirs.

The public sector abattoirs provide healthy meat at various organizations such as restaurants, healthcare centers, butcher shops and different grocery stores. On the other hand, private sector abattoirs are processing one or several classes of livestock into fresh, chilled, hygienic meat and edible by-products for export to gulf countries. Major destinations for export are Saudi Arabia, Masqat, Oman, Dubai, and Abu Dhabi. In these abattoirs, variety of livestock including cattle and buffaloes are brought from all over the province of Punjab.

3.1.3. Study Animals

The study was conducted from November, 2012 to October, 2013. The cattle and buffaloes brought to the public and private abattoirs for slaughter were included in this study. These animals were procured from live animal markets and household farmers throughout the three zones of Punjab province.

3.1.4. Study Design

A total of 2400 animals (n=800 animals from each zone, where, n=400 cattle; n=400 buffaloes) were randomly selected, tagged and included in this study. Data on each animal was
recorded in a “data capture form” where entries included species, sex, age, breed, zone etc (Annexure-I).

3.2. Ante-mortem examination

Each animal included in this study was subjected to ante-mortem inspection. During this inspection, body temperature, respiration rate, heart rate, apparent anomalies, blemishes, other clinical abnormalities and body condition scoring were recorded very carefully as per routine criteria.

3.2.1. Procedure for body condition scoring

Body condition scoring was conducted as the method described by Nicholson and Butterworth (1986).

3.2.2. Collection of Blood Samples

Once the ante-mortem inspection was finished two 5 mL blood samples were collected from each animal using 18Gx1.5” hypodermic needle attached to disposable syringe. The first sample was poured into anticoagulant supplemented, heparinized, (green toped) vacutainer (Chengdu Rich Science Industry Co. Ltd. China) for hematological evaluation. The second blood sample was drawn into sterile plain, clean, dry centrifuge tube and was allowed to clot for serum separation. Blood samples were centrifuged for serum collection and then stored at 4°C for biochemical analyses and at -20°C until use in serological (ELISA) analyses.

3.3. Postmortem Examination

After ante-mortem inspection cattle and buffaloes were slaughtered; postmortem examination followed immediately at the same time in the same facility. During postmortem examination liver, kidneys, lungs, heart, tongue, esophagus, skeletal muscles and masseter (external and internal) muscles were examined for the presence of larvae and cysts of *C. bovis*. 
Postmortems were performed according to standard inspection procedures for *T. saginata* cysticercosis (Ostertag, 1902) consisting the following points.

Examination of the muscle surfaces of the carcass, inspection of heart and tongue and of surfaces after incision of the muscles of mastication (Gracey, 1986).

1. Myocardium and pericardium were incised through longitudinal incision by opening ventricles and incising the septum.

2. Two further incisions were made in the heart from auricles to apex.

3. Tongue was removed and longitudinal incision was made through the lower surface of the tongue.

4. Two incisions equivalent with the lower jaw through the internal and external masseter muscles were made (eight incisions in all).

5. The muscular segment of the diaphragm was examined after removal of its serous layer.

6. Observable mucous surfaces and esophagus were examined.

The animals were inspected for the presence of cysts of bovine cysticercosis and nature of cyst i.e. live or calcified. Overall, zone-wise, abattoir-wise, sex-wise, breed-wise, age-wise, body condition-wise and tissue-wise prevalence of *C. bovis* in cattle and buffaloes was examined and calculated using following formula (Thrusfield, 2005).

\[
\text{Prevalence} \% = \frac{\text{No. of individuals having a disease at a particular point in time}}{\text{No. of individuals in the population at risk at that point in time}} \times 100
\]
3.4. Serological methods for the detection of bovine cysticercosis/Taeniasis

ELISA (enzyme linked immuno-sorbent assay) test was used for the detection of Cysticercus bovis antibodies by using Bovine Cysticercosis antibody CYT Ab ELISA Kit manufactured by Biotang inc. company Massachusetts (USA) with cat No.BV1919. This kit was kept at 2-8 °C before use.

3.4.1. Materials Provided with the Kit.

<p>| | | | | | |</p>
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Seal film</td>
<td>2 pieces</td>
<td>5</td>
<td>Stop Solution</td>
<td>12 ml * 1 bottle</td>
</tr>
<tr>
<td>2</td>
<td>10 * Sample Buffer</td>
<td>12ml * 1 bottle</td>
<td>6</td>
<td>20 * wash Solution</td>
<td>50 ml*1 bottle</td>
</tr>
<tr>
<td>3</td>
<td>Negative /Positive Control</td>
<td>1bottle for each</td>
<td>7</td>
<td>Substrate(TMB) Solution</td>
<td>12 ml*1 bottle</td>
</tr>
<tr>
<td>4</td>
<td>Microplate(precoated)</td>
<td>96 wells</td>
<td>8</td>
<td>Enzyme Conjugate</td>
<td>12 ml*1 bottle</td>
</tr>
</tbody>
</table>

3.5. Collection of serum samples for ELISA

Serum samples from 180 animals (n=90 cattle; n=90 buffaloes) were used for serological (ELISA) analyses. The following method was adapted to detect the prevalence of bovine cysticercosis antibodies by using ELISA kit and results were noted by ELISA reader at University Diagnostic Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan.
3.6. Bovine Cysticercosis Antibody ELISA

3.6.1. Purpose
This kit is used for the determination the existence of bovine cysticercosis Antibody (CYT-Ab) in bovine serum, plasma, cell culture supernatants, and other biological fluids.

3.6.2. Principle of the assay
The kit assays bovine CYT-Ab in the samples using the method of purified CYT antigen pre-coated enzyme-labeled SPA ELISA. Microplate was coated with CYT antigen a solid-phase antigen, and was prepared by purified bovine CYT antigen. The CYT-Ab in the controls or samples binds with the coated antigen after loading of samples. After this step Staphylococcal protein A (SPA)-enzyme immunoglobulin conjugate was added into wells. Unbound reagents were removed after completing washing, and then tetramethylbenzidine (TMB) substrate was added. Blue color was produces by addition of TMB substrate, which serves as a catalyst for the HRP enzyme. The reaction was terminated by adding an acidic Stop solution after reaching the desired color intensity. Absorbance (OD Value) at 450 nm was determined by the microwell reader, and the levels of CYT-Ab were calculated according to OD from positive or negative controls.

3.6.3. Assay procedures
1. 100 μl of negative or positive control were added to each well after addition of sample without touching the well wall then was mixed gently.
2. Plate was incubated at 37 ºC for 30 min.
3. The solution was discarded and then 200μl washing buffer was added to each well. For good performance liquid was completely removed at each step. This process was repeated 5 times.
After the last washing, decanting was done to remove any remaining wash solution. Plate was reverted and blotted against clean paper towels each time.

4. Hundred μl of Enzyme Conjugate was shifted to every well. The plate was incubated at 37 ºC for 30 min.

5. Again washing was done as above.

6. Plates were again incubated for third time at 37 ºC for 10 min after adding 100 μl of Substrate (TMB) Solution.

7. 100 μl of Stop Solution was added to each well and mixed.

8. Absorbance was read at 450 nm within 30 min after adding Stop Solution.

3.6.4. Calculation

1. Duplicate readings for each control and sample were averaged then the average of blank well was subtracted (1 x sample buffer only).

2. Cutoff value was determined as: Cutoff = OD_\text{negative} x 3.1. If OD_\text{negative} which was less than 0.10, 0.10 was used as the value. OD_\text{positive} or OD_\text{negative} was the OD_{450} from positive or negative control.

3. When OD_{\text{sample}} ≥ Cutoff, then testing sample was CYT-Ab positive; and If OD_{\text{sample}} < Cutoff, the testing sample was CYT-Ab negative.

4. The range of normal OD_{\text{negative}} was less than 0.1.5, and for OD_{\text{positive}} it was more than 0.50.5. Whenever the OD_{\text{sample}} was less than 0.1 or more than 2.5, it was considered out of the optimal test range. For accuracy dilution of sample was adjusted.

3.7. HEMATOLOGICAL STUDIES

Blood samples from 60 animals (30 cows and 30 buffaloes) which tested positive for cysticercosis through ELISA were used. A further blood samples from 60 animals (30 cows and 30 buffaloes) negative for cysticercosis were also collected and used as control negative group.
These blood samples were processed using an automated hematology analyzer (UDL/GNL/E002). Serial No. 130076 at University Diagnostic Lab.

### 3.8.1. Blood parameters studied

Different blood parameters e.g. Mean corpuscular Hemoglobin Concentration (MCHC), *Mean Cell Volume* MCV, Mean Corpuscular Hemoglobin (MCH), *Packed Cell Volume* (PCV), Hemoglobin (Hb) concentration, Leukocytes count were determined.

### 3.9. SERUM BIOCHEMICAL STUDIES

#### 3.9.1. Collection of serum samples

A total 60 of serum samples (30 cows and 30 buffaloes) positive for cysticercosis through ELISA were collected and analyzed. 60 more serum samples (30 cows and 30 buffaloes) testing negative were also collected and were used as a control group. These serum samples were processed by Biochemistry Analyzer (UDL/GNL/E003, M.1600P. Serial No. 06031602) in University diagnostic laboratory at University of Veterinary and Animal Sciences, Lahore. The following biochemical parameters were studied.

#### 3.9.3. Alanine Aminotransferase

Serum concentrations of alanine aminotransferase (ALT) were measured by using a commercially available kit (Crescent diagnostics, Saudi Arabia, Cat # CZ902L). This kit measures or detects ALT-mediated conversion of alanine to an alpha-keto acid (glutamine), indicating the presence of this enzyme’s. KG plus L-alanine are the substrates in the kit. The addition of a preset amount (concentration) of NaOH induces treated samples to turn red in the presence of glutamine, thus providing a colorimetric indicator of ALT activity. Results from samples (along with premade standards) treated with this kit can be read on a spectrophotometer, allowing for accurate quantitative measurements of ALT activity.
3.9.4. Aspartate transaminase

Serum levels of aspartate transaminase (AST) were determined by a modified +IFCC technique using a kit available in the market (Crescent diagnostics, Saudi Arabia, Cat # CZ904L). AST catalyzes the transfer of an amino group between 2-Oxoglutarate and L-Aspartate, which results in glutamine and oxaloacetate as products Oxaloacetate in the presence of Malate Dehydrogenase reduces NADH to NAD. The rate of oxidation of NADH to NAD can be measured colorimetrically as a decrease in absorbance, reflecting the amount of AST activity in a given sample.

3.9.5. Alkaline Phosphatase

Serum activity of alkaline phosphatase (ALP) was determined by DGKC method using a commercially available kit (Crescent Diagnostics, Saudi Arabia, Cat # CZ901U). ALP at an alkaline pH hydrolyses p-Nitrophenylphosphate to form Phosphate and p-Nitrophenol. The formation of p-Nitrophenol registers as a color change in tested samples. ALP activity in the sample is directly proportional to the intensity of the color change, which is measured as an increase in absorbance.

3.9.6. Cholesterol

Cholesterol level in serum samples was measured by CHOD / PAP method using commercially available kit “Global,s Cholesterol Kit” (Global in vitro LLP, London, UK, Cat # CHO6075). In this method cholesterol esterase hydrolyses esterified cholesterols to free cholesterol. The free cholesterol is oxidized to form Hydrogen ptheneroxide; a red colored quinoneimine dye complex is formed as a result, further reacting with phenol and 4-aminoantipyrine by the catalytic action of peroxidase. The amount of cholesterol present in the sample is directly proportional to intensity of the color formed.
3.9.7. **Total protein**

Serum total proteins were determined by the Biuret technique using the handy kit “Total Protein FS” (DiaSys Diagnostic Systems GmbH, Germany Ref. # 123119910021) available in the market. In this method serum protein binds with cupric ions present in the biuret reagent to form a violet blue color complex in alkaline solution. The protein concentration is directly proportional the absorbance of color.

3.9.8. **Albumin**

BCG technique was used to measure the albumin concentration from the blood samples by using commercially available kit “Albumin FS” prepared by DiaSys Diagnostic Systems GmbH, Germany (Ref. # 102209910021). The presence of Albumin induces a change in the color of reagent indicator from yellow-green to green-blue in the presence of bromocresol green at slightly acidic pHs. The optical density of the green-blue color is directly proportional to concentration of albumen present in the samples.

3.9.9. **Urea**

Serum urea levels were measured by Urease-GLDH enzymatic UV test using a commercially available kit “Urea UV” (Merck Pvt. Limited, France, Ref. # 5.17610.0001)). In this method urea was hydrolyzed into NH$_4^+$ and CO$_2$ in the presence of urease which reacted with a ketoglutarate and NADH in the presence of glutamate dehydrogenase to form glutamate and NAD resulting in a color change. The difference in the absorbance of color at fixed time interval during conversion was proportional to the urea concentration in the sample.

3.10.10. **Creatinine**

Serum creatinine was determined by Kinetic Method without deproteinization (Jaffe reaction) using commercially available kit “Creatinine Test Kit” (Crescent Diagnostics, Saudi
Arabia; Cat #CS604-8). In this method picric acid in an alkaline medium reacted with creatinine to form a reddish-orange colored complex with the alkaline picrate. Intensity of the color formed during the fixed time was directly proportional to the amount of creatinine present in the sample. Serum samples for the detection of Ca, K, Na and phosphorus were processed by using automated biochemistry analyzer (UDL/GNL/Eoo2). Serial No.130076 at University Diagnostic Lab.

3.11. Histopathological studies

For this study affected organs from different cattle and buffaloes from a variety of abattoirs were collected carefully due to the zoonotic risk. All the precautionary measures were adopted at the time of organ collection.

3.11.1. Collection of Organs

At the time of postmortem, affected organs (Liver, Lung, heart, tongue, esophagus and skeleton muscle, masseter muscles) were collected from the infected cattle and buffaloes for histopathological examination. Tissue samples of liver, heart, esophagus and tongue were fixed in 10% buffered formalin and were processed for histopathological examination.

3.11.2 Preparation of Slides

The slides from affected organs were prepared by using a standard method of dehydration in ascending series of ethanol, clearing with xylene and embedding in paraffin. Sections of 5 µm thickness were sliced using microtome and were stained with Hematoxylin and Eosin stain (Bancroft and Gamble 2007).

These samples were processed by routine processing in descending dilutions of alcohol. These slides were studied in pathological lab with the help of microscope (Olympus-BX-40) in
Diagnostic Laboratory at the Department of Veterinary Population Medicine, at University of Minnesota (USA).

3.12. Estimation of Economic losses due to Bovine Cysticercosis

Economic losses due to bovine cysticercosis were estimated according to the method of Mellau et al. (2010). In Pakistan previously no such data is available that can figure out the economic losses due to bovine cysticercosis. A yearlong record of animals slaughtered in abattoirs during the period from November, 2012 to October, 2013 was compiled. Losses due to bovine cysticercosis were studied and examined from different angles. To estimate the economic losses the study covered both private and public slaughter houses at Lahore and their surroundings. Losses in terms of condemnation, refrigerating and downgrading of carcasses were estimated in these abattoirs. Flisser et al. 2005 and McFadden et al. 2011 concluded that accurate estimations of economic losses in other countries are lacking, as there is no system available for reporting this infection. So we can say that generating estimates is a complicated task because the required data regarding losses and cooperation by the administration of abattoirs on this infection in cattle/buffalo is not available in the country.

3.13. Statistical Analysis

The data on prevalence was analyzed using Chi square test and one way analysis of variance while the data on blood parameters of diseases and non-diseased animals was tested through student’s independent T-test. The probability level of $P<0.05$ was considered as statistically significantly different. A statistical software package “SPSS13.00” was used for statistical analysis.
4.1: **Prevalence of Bovine Cysticercosis in Punjab**

During the study period a total of 2400 animals (1200 cattle and 1200 buffaloes) were examined. Postmortem was performed and animals were inspected for the presence of cysts of bovine cysticercosis.

Overall prevalence of bovine cysticercosis in cattle and buffaloes is given in Table 4.1. The prevalence of bovine cysticercosis in cattle and buffaloes was 2.92 and 3.17 percent, respectively. Statistically the difference in prevalence of bovine cysticercosis between cattle and buffaloes was not significant (P>0.05).

Data on zone-wise prevalence of *Cysticercus bovis* in cattle and buffaloes is shown in Table 4.2. The highest prevalence of bovine cysticercosis was observed in cattle (3.75%) and buffaloes (3.5%) of North Punjab followed by South Punjab where prevalence of bovine cysticercosis was 2.75 and 3.25% in cattle and buffaloes, respectively. On the other hand, lowest prevalence of *Bovine Cysticercosis* was found in cattle (2.25%) and buffaloes (2.75%) in Central Punjab (Fig. 4.2).

Data on abattoir-wise prevalence of bovine cysticercosis in cattle and buffaloes is shown in Table 4.3. The prevalence of bovine cysticercosis was higher in cattle (4.63%) and buffaloes (5%) at public sector abattoirs than private sector abattoirs, where prevalence was 1.56% and 1.67% in cattle and buffaloes, respectively (Fig. 4.3). The prevalence of bovine cysticercosis in
both cattle and buffaloes was significantly higher (P<0.05) in public sector abattoirs compared to private sector abattoirs.

Data on sex-wise prevalence of bovine cysticercosis in cattle and buffaloes is given in Table 4.4. The prevalence of bovine cysticercosis was higher in female cattle (3.75%) and female buffaloes (3.83%) than male animals (2.53% in cattle, 2.80% in buffaloes) (Fig. 4.4). Statistically the difference in prevalence of bovine cysticercosis between male and female cattle and buffaloes was non-significantly different (P>0.05).

Breed-wise data for prevalence of bovine cysticercosis in cattle and buffaloes is shown in Table 4.5. The highest prevalence of bovine cysticercosis was observed in Cholistani cattle (3.15%) and NiliRavi buffaloes (3.17%). While prevalence of bovine cysticercosis was 3.05%, 2.94%, 2.78% and 2.27% in Sahiwal, Dhani, Lohani and cross bred cattle, respectively. No positive case was found in Dajal cattle and Kundi buffalo breeds (Fig. 4.5). When compared statistically, a non significant difference (P>0.05) in prevalence of bovine cysticercosis in different cattle breeds was observed.

Age-wise prevalence of bovine cysticercosis in cattle and buffaloes is shown in Table 4.6. Animal of 3 years and above age group were highly affected with bovine cysticercosis. Prevalence was 6.67% and 6.98% in cattle and buffalos of 3-5 years old, respectively. Younger animals less than one year old had the lowest prevalence which was 0.65 % for cattle and 0.97% for buffalo. In age group of 1 to 3 years prevalence was 2.06% in cattle while it was 1.90% in buffalo (Fig. 4.6). The prevalence of bovine cysticercosis was significantly different (P<0.05) among different age groups of cattle and buffaloes.

Data on body condition-wise prevalence of bovine cysticercosis in cattle and buffaloes is given in Table 4.7. Highest prevalence of bovine cysticercosis was observed in cattle (3.26%) and buffaloes (3.3%) found to be in poor physical health. Cattle scoring highly in terms of body
health showed a 2.79% rate of infection while 2.93% of those having a medium score were infected. In buffalo 3.04% of the animals classified as healthy were infected; rates for those with a medium and poor body health score were 3.28%. (Fig. 4.7). Statistically, a non significant difference (P>0.05) was observed in prevalence of bovine cysticercosis in cattle and buffaloes having different body condition scores.

Tissue-wise prevalence of bovine Cysticercosis in cattle and buffalo is shown in Table 4.8. The highest prevalence of infestation was observed in the liver of both cattle and buffalo as 31.4 and 31.6 percent, respectively. In cattle other organs like heart, lungs, tongue and esophagus showed prevalence as 22.9, 2.9, 11.4 and 8.6 percent, respectively. While in buffalo heart and tongue showed 23.7% prevalence and lungs were 2.6% affected with bovine cysticercosis (Fig. 4.8). Statistically, the difference in prevalence of bovine cysticercosis cysts in different organs of cattle and buffaloes was significantly different (P<0.05).
Table 4.1: Overall prevalence of *Cysticercus bovis* in cattle and buffalo in Punjab

<table>
<thead>
<tr>
<th>Species</th>
<th>No. examined</th>
<th>No. positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>1200</td>
<td>35</td>
<td>2.92*</td>
</tr>
<tr>
<td>Buffalo</td>
<td>1200</td>
<td>38</td>
<td>3.17*</td>
</tr>
<tr>
<td>Overall</td>
<td>2400</td>
<td>73</td>
<td>3.045</td>
</tr>
</tbody>
</table>

*indicates that the differences of values in the same column are statistically non significant (P>0.05)

![Graph showing comparison of prevalence of Bovine Cysticercosis in cattle and buffalo](image)

**Fig. 4.1: Comparison of prevalence of Bovine Cysticercosis in cattle and buffalo**
Table 4.2: Zone/Area-wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab

<table>
<thead>
<tr>
<th>Zone</th>
<th>No. examined</th>
<th>No. positive</th>
<th>Prevalence (%)</th>
<th>No. examined</th>
<th>No. positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Punjab</td>
<td>400</td>
<td>11</td>
<td>2.75</td>
<td>400</td>
<td>13</td>
<td>3.25</td>
</tr>
<tr>
<td>Central Punjab</td>
<td>400</td>
<td>9</td>
<td>2.25</td>
<td>400</td>
<td>11</td>
<td>2.75</td>
</tr>
<tr>
<td>North Punjab</td>
<td>400</td>
<td>15</td>
<td>3.75</td>
<td>400</td>
<td>14</td>
<td>3.50</td>
</tr>
</tbody>
</table>

Figure 4.2: Percentage of positive cattle and buffalo in different area of Punjab province
Table 4.3: Abattoir-wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab

<table>
<thead>
<tr>
<th>Type of Abattoir</th>
<th>Cattle</th>
<th>Buffaloes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>No. positive</td>
</tr>
<tr>
<td>Public sector abattoir</td>
<td>540</td>
<td>25</td>
</tr>
<tr>
<td>Private sector abattoir</td>
<td>660</td>
<td>10</td>
</tr>
</tbody>
</table>

**indicates that values in the same column are statistically significantly different (P<0.05)

Fig. 4.3. Percentage of positive cattle and buffalo in public and private sector abattoir
Table 4.4. Sex-wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cattle</th>
<th>Buffaloes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>No. positive</td>
</tr>
<tr>
<td>Male</td>
<td>827</td>
<td>21</td>
</tr>
<tr>
<td>Female</td>
<td>373</td>
<td>14</td>
</tr>
</tbody>
</table>

*indicates that values in the same column are statistically non significantly different (P>0.05)

Fig. 4.4. Percentage of positive animals among different sex in cattle and buffalo
Table 4.5. Breed-wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab

<table>
<thead>
<tr>
<th>Breed</th>
<th>No. examined</th>
<th>No. positive</th>
<th>Prevalence (%)</th>
<th>Breed</th>
<th>No. examined</th>
<th>No. positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sahiwal</td>
<td>360</td>
<td>11</td>
<td>3.05*</td>
<td>NiliRavi</td>
<td>960</td>
<td>38</td>
<td>3.17**</td>
</tr>
<tr>
<td>Cholistani</td>
<td>444</td>
<td>14</td>
<td>3.15*</td>
<td>Kundi</td>
<td>240</td>
<td>0</td>
<td>0**</td>
</tr>
<tr>
<td>Dhani</td>
<td>204</td>
<td>6</td>
<td>2.94*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lohani</td>
<td>36</td>
<td>1</td>
<td>2.78*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cross breed</td>
<td>132</td>
<td>3</td>
<td>2.27*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dajal</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*indicates that values in the same column are statistically non significantly different (P>0.05)

**indicates that values in the same column are statistically significantly different (P<0.05)

Fig. 4.5. Breed-wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab
Table 4.6. Age-wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab

<table>
<thead>
<tr>
<th>Age</th>
<th>Cattle</th>
<th></th>
<th></th>
<th>Buffaloes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>No. positive</td>
<td>Prevalence (%)</td>
<td>No. examined</td>
<td>No. positive</td>
<td>Prevalence (%)</td>
</tr>
<tr>
<td>6 Month – 1 Year</td>
<td>310</td>
<td>2</td>
<td>0.65 **</td>
<td>310</td>
<td>3</td>
<td>0.97 **</td>
</tr>
<tr>
<td>1-3 Year</td>
<td>630</td>
<td>13</td>
<td>2.06 **</td>
<td>630</td>
<td>12</td>
<td>1.90 **</td>
</tr>
<tr>
<td>3-5 year and above</td>
<td>360</td>
<td>20</td>
<td>6.67 **</td>
<td>360</td>
<td>23</td>
<td>6.98 **</td>
</tr>
</tbody>
</table>

**indicates that values in the same column are statistically significantly different (P<0.05)

Fig.4.6 Age-wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab
Table 4.7 Body condition-wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab

<table>
<thead>
<tr>
<th>Body condition</th>
<th>Cattle</th>
<th></th>
<th></th>
<th>Buffaloes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>No. positive</td>
<td>Prevalence (%)</td>
<td>No. examined</td>
<td>No. positive</td>
<td>Prevalence (%)</td>
</tr>
<tr>
<td>Good</td>
<td>610</td>
<td>17</td>
<td>2.79*</td>
<td>625</td>
<td>19</td>
<td>3.04*</td>
</tr>
<tr>
<td>Medium</td>
<td>375</td>
<td>11</td>
<td>2.93*</td>
<td>365</td>
<td>12</td>
<td>3.28*</td>
</tr>
<tr>
<td>Weak</td>
<td>215</td>
<td>7</td>
<td>3.26*</td>
<td>210</td>
<td>7</td>
<td>3.3*</td>
</tr>
</tbody>
</table>

*indicates that values in the same column are statistically non significantly different (P>0.05)

Fig. 4.7.Body condition-wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab
Table 4.8. Tissue wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No of cysts found</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cattle</td>
<td>Buffaloes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. Positive</td>
<td>Prevalence (%)</td>
<td>No. Positive</td>
</tr>
<tr>
<td>Liver</td>
<td>11</td>
<td>31.4**</td>
<td>12</td>
</tr>
<tr>
<td>Heart</td>
<td>8</td>
<td>22.9**</td>
<td>9</td>
</tr>
<tr>
<td>Lungs</td>
<td>1</td>
<td>2.9**</td>
<td>1</td>
</tr>
<tr>
<td>Tongue</td>
<td>4</td>
<td>11.4**</td>
<td>9</td>
</tr>
<tr>
<td>Esophagus</td>
<td>3</td>
<td>8.6**</td>
<td>2</td>
</tr>
<tr>
<td>Skeletal Muscles</td>
<td>3</td>
<td>8.6**</td>
<td>2</td>
</tr>
<tr>
<td>Masseter Muscles</td>
<td>3</td>
<td>8.6**</td>
<td>1</td>
</tr>
<tr>
<td>Multiple tissues</td>
<td>2</td>
<td>5.7**</td>
<td>2</td>
</tr>
</tbody>
</table>

**indicates that values in the same column are statistically significantly different (P<0.05)

The tissue based prevalence of bovine cysticercosis in different organs of infected cattle and buffalo were significantly high in liver, heart, tongue and esophagus as compared to skeletal muscles, Masseter muscles and other body tissues.
Figure 4.8: Tissue-wise prevalence of Bovine Cysticercosis is cattle and buffalo in Punjab
4.4: Prevalence of Bovine Cysticercosis through ELISA

Data on prevalence of bovine cysticercosis in cattle and buffalo by ELISA is given in Table 4.13. The prevalence of bovine cysticercosis through ELISA was 13.33 and 14.44 percent in cattle and buffalo, respectively. The difference was non-significant between cattle and buffalo. A significantly higher (P<0.05) prevalence was obtained using ELISA than with postmortem.

Zone wise prevalence data for bovine cysticercosis in cattle and buffalo by ELISA is given in Table 4.14 which shows that there was no significant difference among prevalence of bovine cysticercosis in cattle of different zones of Punjab. The prevalence of bovine cysticercosis detected through ELISA testing was 13.33, 10 and 16.66 percent in south, central and north Punjab respectively. Similar effects were seen in the prevalence of bovine cysticercosis in buffalo. In south Punjab prevalence of bovine cysticercosis was 13.33, in central and North Punjab prevalence was 10% and 20%, respectively.

Abattoir wise prevalence data for bovine cysticercosis in cattle and buffalo detected by ELISA is given in Table 4.15 which shows that there was no significant difference among prevalence of this disease in cattle of different abattoirs of Punjab. The prevalence of bovine cysticercosis observed through ELISA was 16 and 10 percent in public and private abattoirs, respectively. Similar results were seen in the prevalence of bovine cysticercosis in buffalo. In private abattoirs prevalence of bovine cysticercosis was 15% while in public abattoirs prevalence was 13.33%.

Sex wise prevalence data for bovine cysticercosis in cattle and buffalo using ELISA testing is given in Table 4.16, which shows that there was no significant difference between male and female cattle. ELISA testing showed prevalence of 11.11 and 15.55 percent in males and females, respectively. Similar results were seen in the prevalence of bovine cysticercosis in
buffaloes. Males showed a prevalence of 13.33, females' bovine cysticercosis prevalence was 15.55%.

Data for bovine cysticercosis in cattle and buffalo using ELISA is given in Table 4.17, which shows that there was no significant difference among prevalence of bovine cysticercosis in different breeds of cattle. The prevalence of bovine cysticercosis through ELISA was 13.33, 13.33, 10, 20 and 13.33 percent in Sahiwal, Cholistani, Dhani, Lohani and crossbreeds, respectively. Significant differences were seen in the prevalence of bovine cysticercosis in buffalo breeds. In NiliRavi prevalence of bovine cysticercosis was 17.14 and in Kundi prevalence was 5%.

ELISA results for bovine cysticercosis rates versus age are given in Table 4.18. The data showed that the prevalence of bovine cysticercosis was significantly different across different age groups of cattle. The prevalence of bovine cysticercosis was measured at 6.66, 10, and 23.33 percent in 6-12 months, 1-3 year and 3–5 year old cattle, respectively. Similar results were obtained in buffalo age groups: 6.66, 10, and 26.66 percent in 6-12 months old, 1-3 year old and 3–5 year old animals, respectively.

Results generated though ELISA are presented in Table 4.19. The data shows that there were significant differences among prevalence rates for bovine cysticercosis in different body conditions of cattle and buffalo. The prevalence of *Bovine Cysticercosis* measured using ELISA was 3.33, 13.33, and 23.33 percent in cattle with body health scores of high, medium, and low, respectively. The prevalence data for buffalo showed a similar pattern when correlated against body health scores: 13.33, 6.66, and 23.33 percent in animals with high, medium and low scores, respectively.
Table 4.9. Overall prevalence of bovine cysticercosis in cattle and buffalo by ELISA

<table>
<thead>
<tr>
<th>Species</th>
<th>No. examined</th>
<th>No. positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>90</td>
<td>12</td>
<td>13.33*</td>
</tr>
<tr>
<td>Buffalo</td>
<td>90</td>
<td>13</td>
<td>14.44*</td>
</tr>
<tr>
<td>Overall</td>
<td>180</td>
<td>25</td>
<td>13.89</td>
</tr>
</tbody>
</table>

*indicates that values in the same column are statistically non significantly different (P>0.05).

Table 4.10. Zone/Area-wise prevalence of *Bovine Cysticercosis* in cattle and buffaloes in Punjab through ELISA

<table>
<thead>
<tr>
<th>Zone</th>
<th>Cattle</th>
<th></th>
<th></th>
<th>Buffaloes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>No. positive</td>
<td>Percentage</td>
<td>No. examined</td>
<td>No. positive</td>
<td>Percentage</td>
</tr>
<tr>
<td>South Punjab</td>
<td>30</td>
<td>4</td>
<td>13.33*</td>
<td>30</td>
<td>4</td>
<td>13.33*</td>
</tr>
<tr>
<td>Central Punjab</td>
<td>30</td>
<td>3</td>
<td>10.00*</td>
<td>30</td>
<td>3</td>
<td>10*</td>
</tr>
<tr>
<td>North Punjab</td>
<td>30</td>
<td>5</td>
<td>16.66*</td>
<td>30</td>
<td>6</td>
<td>20*</td>
</tr>
</tbody>
</table>

*indicates that values in the same column are statistically non significantly different (P>0.05)
Table 4.11: Abattoir-wise prevalence of Bovine Cysticercosis in cattle and buffalo by ELISA

<table>
<thead>
<tr>
<th>Type of Abattoir</th>
<th>Cattle</th>
<th></th>
<th></th>
<th>Buffaloes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>No. positive</td>
<td>Percentage</td>
<td>No. examined</td>
<td>No. positive</td>
<td>Percentage</td>
</tr>
<tr>
<td>Public sector abattoir</td>
<td>50</td>
<td>8</td>
<td>16*</td>
<td>60</td>
<td>9</td>
<td>15*</td>
</tr>
<tr>
<td>Private sector abattoir</td>
<td>40</td>
<td>4</td>
<td>10*</td>
<td>30</td>
<td>4</td>
<td>13.33*</td>
</tr>
</tbody>
</table>

*indicates that values in the same column are statistically non significantly different (P>0.05)

Table 4.12. Sex-wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab through ELISA

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cattle</th>
<th></th>
<th></th>
<th>Buffaloes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>No. positive (%)</td>
<td>Percentage</td>
<td>No. examined</td>
<td>No. positive</td>
<td>Percentage</td>
</tr>
<tr>
<td>Male</td>
<td>45</td>
<td>5</td>
<td>11.11*</td>
<td>45</td>
<td>6</td>
<td>13.33*</td>
</tr>
<tr>
<td>Female</td>
<td>45</td>
<td>7</td>
<td>15.55*</td>
<td>45</td>
<td>7</td>
<td>15.55*</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>12</td>
<td>13.33*</td>
<td>90</td>
<td>13</td>
<td>14.44*</td>
</tr>
</tbody>
</table>

*indicate that values in the same column are statistically non significantly different (P>0.05)
Table 4.13. Breed wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab through ELISA

<table>
<thead>
<tr>
<th>Breed</th>
<th>Cattle</th>
<th></th>
<th></th>
<th>Buffaloes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>No. positive</td>
<td>Percentage</td>
<td>Breed</td>
<td>No. examined</td>
<td>No. positive</td>
</tr>
<tr>
<td>Sahiwal</td>
<td>30</td>
<td>4*</td>
<td>13.33</td>
<td>NiliRavi</td>
<td>70</td>
<td>12**</td>
</tr>
<tr>
<td>Cholistani</td>
<td>15</td>
<td>2*</td>
<td>13.33</td>
<td>Kundi</td>
<td>20</td>
<td>1**</td>
</tr>
<tr>
<td>Dhani</td>
<td>10</td>
<td>1*</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lohani</td>
<td>5</td>
<td>1*</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cross bred</td>
<td>30</td>
<td>4*</td>
<td>13.33</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*indicates that values in the same column are statistically non significantly different (P>0.05) **indicates that values in the same column are statistically significantly different (P<0.05)

Table 4.14. Age-wise prevalence of Bovine Cysticercosis in cattle and buffalo in Punjab through ELISA

<table>
<thead>
<tr>
<th>Age</th>
<th>Cattle</th>
<th></th>
<th></th>
<th>Buffaloes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>No. positive</td>
<td>Percentage</td>
<td>No. examined</td>
<td>No. positive</td>
<td>Percentage</td>
</tr>
<tr>
<td>6 Month - 1 Year</td>
<td>30</td>
<td>2**</td>
<td>6.66</td>
<td>30</td>
<td>2**</td>
<td>6.66</td>
</tr>
<tr>
<td>1-3 Year</td>
<td>30</td>
<td>3**</td>
<td>10</td>
<td>30</td>
<td>3**</td>
<td>10</td>
</tr>
<tr>
<td>3 year and above</td>
<td>30</td>
<td>7**</td>
<td>23.33</td>
<td>30</td>
<td>8**</td>
<td>26.66</td>
</tr>
</tbody>
</table>

**indicates that values in the same column are statistically significantly different (P<0.05)
Table 4.15. Body condition-wise prevalence of Bovine Cysticercosis in cattle and buffalo through ELISA

<table>
<thead>
<tr>
<th>Body condition</th>
<th>Cattle</th>
<th></th>
<th></th>
<th>Buffaloes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>No. positive</td>
<td>Percentage</td>
<td>No. examined</td>
<td>No. positive</td>
<td>Percentage</td>
</tr>
<tr>
<td>Good</td>
<td>30</td>
<td>5</td>
<td>3.33**</td>
<td>30</td>
<td>4</td>
<td>13.33**</td>
</tr>
<tr>
<td>Medium</td>
<td>30</td>
<td>3</td>
<td>13.33**</td>
<td>30</td>
<td>2</td>
<td>6.66**</td>
</tr>
<tr>
<td>Weak</td>
<td>30</td>
<td>4</td>
<td>23.33**</td>
<td>30</td>
<td>7</td>
<td>23.33**</td>
</tr>
</tbody>
</table>

**indicates that values in the same column are statistically significantly different (P<0.05)

4.5. Cyst Morphology

4.5.1. Size of the cysts

Size range of cysts was primarily between 1.5cm to 3.0 cm; these were detected in tissues from animals processed in abattoirs during postmortem exams. In general, the largest cysts were easily detected visually. Presented here are images of organs affected with bovine cysticercosis. These organ samples were collected from abattoirs during postmortem examinations.
Fig. 4.9. Cysticercci in Cross section of Liver

Fig. 4.10. Cysticercci on surface of Liver
**Fig. 4.11.** Cysticerci in Cross section of Tongue (bovine)

**Fig. 4.12.** Cysts in body muscles (bovine)
**Fig. 4.13.** Cyst in Liver

**Fig. 4.14.** Cysts in body muscles
4.6. Hematological Parameters

Data on hemogram of healthy and bovine cysticercosis infected cattle is shown in Table 4.9. The values of hemoglobin and platelets count were significantly different (P<0.05) in healthy and bovine cysticercosis infected cattle. On the other hand, WBCs, PCV, MCV, MCH and MCHC values were not significantly different (P>0.05) between healthy and bovine cysticercosis infected cattle (Fig.4.9)
Table 4.16: Blood values of healthy and infected cattle

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cattle (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.73**</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>33.83*</td>
</tr>
<tr>
<td>RBC (x10^6/μL)</td>
<td>6.40*</td>
</tr>
<tr>
<td>WBC count (x10^3/μL)</td>
<td>8.30*</td>
</tr>
<tr>
<td>Platelets (x10^3/μL)</td>
<td>228.45**</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>55.11*</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>17.32*</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>31.67*</td>
</tr>
</tbody>
</table>

**indicates that values of hematology in the same row are statistically significantly different (P<0.05)

*indicates that values of hematology in the same row are statistically non-significant (P>0.05)

Figure 4.17: Blood values of healthy and infected cattle
Data on Hemogram of healthy and bovine cysticercosis infected buffalo is shown in Table 4.10. The values of hemoglobin and platelets count were significantly different (P<0.05) in healthy and bovine cysticercosis infected buffalo. On the other hand, WBCs, PCV, MCV, MCH and MCHC values were not significantly different (P>0.05) between healthy and bovine cysticercosis infected buffaloes (Fig. 4.10)
Table 4.17. Blood values of healthy and infected buffalo

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Buffaloes (n=30)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Infected</td>
<td>Normal range</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.59**</td>
<td>12.02**</td>
<td>8-15</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>33.077*</td>
<td>33.418*</td>
<td>24-48</td>
</tr>
<tr>
<td>RBC (x10^6/uL)</td>
<td>7.65*</td>
<td>7.31*</td>
<td>5-10</td>
</tr>
<tr>
<td>WBC count (x10^3/uL)</td>
<td>8.59*</td>
<td>8.82*</td>
<td>4-12</td>
</tr>
<tr>
<td>PLT (x10^3/uL)</td>
<td>278.44**</td>
<td>252.68**</td>
<td>50-750</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>51.45*</td>
<td>51.07*</td>
<td>40-60</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>16.5*</td>
<td>17.08*</td>
<td>11-17</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.72*</td>
<td>34.23*</td>
<td>30-36</td>
</tr>
</tbody>
</table>

**indicates that values of hematology in the same row are statistically significantly different (P<0.05)

*indicates that values of hematology in the same row are statistically non-significantly different (P>0.05)

Figure 4.18: Blood values of healthy and infected buffalo
4.7. Serum Biochemistry

Data on different serum biochemical values of healthy and bovine cysticercosis infected cattle and buffalo are given in Table 4.11 and 4.12 respectively. When compared serum protein, AST, Cholesterol, ALT, creatinine and blood urea levels in profile of healthy and infested groups of cattle and buffalo, the difference was significant ( \( P<0.05 \)).
Table 4.18: Blood chemistry of healthy and Bovine Cysticercosis infected cattle

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cattle (n=30)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Infected</td>
<td>Normal Values</td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>139*</td>
<td>140.1*</td>
<td>136-144</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>133.4**</td>
<td>47.11**</td>
<td>62-193</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>71.97*</td>
<td>72.34*</td>
<td>45-75</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>113**</td>
<td>56**</td>
<td>78-132</td>
</tr>
<tr>
<td>ALKP (U/L)</td>
<td>43.35**</td>
<td>48.13**</td>
<td>18-153</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>11.53**</td>
<td>29.47**</td>
<td>6.9-35</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>9.78*</td>
<td>9.59*</td>
<td>8-11.4</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>6.76*</td>
<td>6.71*</td>
<td>5.6-8</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.38**</td>
<td>4.7**</td>
<td>6.2-8.2</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>5.01*</td>
<td>4.8*</td>
<td>3.9-5.8</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>5.57**</td>
<td>4.02**</td>
<td>6-27</td>
</tr>
<tr>
<td>ALB (g/dl)</td>
<td>3.04**</td>
<td>2.08**</td>
<td>2.8-3.9</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.95**</td>
<td>3.23**</td>
<td>0.5-2.2</td>
</tr>
</tbody>
</table>

** indicates that values of serum biochemistry in the same row are statistically significantly different (P<0.05)
* indicates that values of serum biochemistry in the same row are statistically non-significantly different (P>0.05)
Fig. 4.19(1) Comparison of serum biochemistry values of healthy and Bovine Cysticercosis infected cattle

Fig. 4.19 (2) Comparison of serum biochemistry values of healthy and Bovine Cysticercosis infected cattle
Table 4.19: Serum biochemistry of healthy and Bovine Cysticercosis infected buffalo

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy</th>
<th>Infected</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mEq/L)</td>
<td>143.1*</td>
<td>140.16*</td>
<td>136-144</td>
</tr>
<tr>
<td>Cholesterol( mg/dl)</td>
<td>93.40**</td>
<td>43.07**</td>
<td>62-193</td>
</tr>
<tr>
<td>Glucose(mg/dl)</td>
<td>65.91*</td>
<td>65.55*</td>
<td>45-75</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>98**</td>
<td>60.13**</td>
<td>78-132</td>
</tr>
<tr>
<td>ALKP (U/L)</td>
<td>48.35**</td>
<td>51.7**</td>
<td>18-153</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>11.53**</td>
<td>21.97**</td>
<td>6.9-35</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>9.86*</td>
<td>9.7*</td>
<td>8-11.4</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>7.24*</td>
<td>7.29*</td>
<td>5.6-8</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.93**</td>
<td>4.86**</td>
<td>6.2-8.2</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>5.11*</td>
<td>5.31*</td>
<td>3.9-5.8</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>5.63**</td>
<td>4.50**</td>
<td>6-27</td>
</tr>
<tr>
<td>ALB (g/dl)</td>
<td>3.04**</td>
<td>2.03**</td>
<td>2.8-3.9</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.79**</td>
<td>4.32**</td>
<td>0.5-2.2</td>
</tr>
</tbody>
</table>

**indicates that values of serum biochemistry in the same row are statistically significantly different (P<0.05)

*indicates that values of serum biochemistry in the same row are statistically non-significantly different (P>0.05)
Fig. 4.20(1) Comparison of serum biochemistry values of healthy and Bovine Cysticercosis infected buffalo

Fig. 4.20 (2) Comparison of serum biochemistry values of healthy and Bovine Cysticercosis infected buffalo
4.8. Histopathological changes induced by Bovine Cysticercosis in different organs of cattle and buffalo

The effected organs of cattle and buffalo were collected postmortem from abattoirs. Histopathological changes induced by *Cysticercus bovis* in organs of cattle and buffaloes were examined. For this purpose slides were prepared to view histopathological changes induced by the bovine cysticercosis. These slides were studied in a pathology laboratory by using an microscope (Olympus-B.X-40) under the supervision of diplomat of American College of Veterinary Pathologists in the veterinary diagnostic laboratory in the department of Veterinary Population Medicine, University of Minnesota, USA.

4.8.1 Histopathological changes in the Liver:

There was prominent portal fibrosis that often bridged between triads. Affected triads contain aggregates of lymphocytes (and small numbers of macrophages containing tan pigment) along with a mild degree of biliary hyperplasia. Several large and irregular hyper plastic bile ducts were also observed. The hepatic parenchyma was subdivided into islands of irregular hepatocytes, bordered by fibrous connective tissue and in various stages of vacuolar degeneration. Hepatocytes w somewhat distorted by fibrous connective tissue. In several areas of prominent fibrosis the hepatocytes were absent or lost.

4.8.2 Histopathological changes in the Tongue:

When the slides of tongue were examined under microscope changes were observed within myofibers of the deep muscle layer. Five intracellular sarcocysts of varying diameter were observed. The sarcocysts had a thick, hyperseosinophilic wall surrounding hundreds of crescent-shaped organisms.
4.8.3 **Histopathological changes in the Esophagus:**

Within the tunica muscularis were sixteen intracellular sarcocysts of varying size, similar to those described in the tongue.

![Section of the liver from buffalo showing histopathological changes](image)

**Fig.4.21. Section of the liver from buffalo showing histopathological changes**
Fig. 4.22. Section of the liver from cattle showing histopathological changes

Fig. 4.23. Section of the tongue from buffalo showing histopathological changes
Fig. 4.24. Section of the tongue from cattle showing histopathological changes

Fig. 4.25 and 4.26. Sections of the esophagus from buffalo showing histopathological changes
4.9: Estimation of Economic losses due to *Bovine Cysticercosis* in Punjab Province

Economic losses due to *bovine cysticercosis* can be estimated by disease prevalence, condition of animals infested, prospective markets and prices of cattle / buffalo and treatment costs for apprehended carcasses. Economic losses due to bovine cysticercosis have also been estimated according to the method of Mellau et al. 2010. In Pakistan, unfortunately such data is not available; economic losses due to bovine cysticercosis are very difficult to estimate. A yearlong record of animals slaughtered (November, 2012 to October, 2013) was compiled and examined. To estimate the economic losses, the study included records from facilities registering the highest number of animals slaughtered in Punjab Province. All these abattoirs were HACCP certified and having strict rules and regulations regarding Veterinary Inspection for the production of saleable meat. Exact records for condemnation, refrigerating and downgrading of carcasses were not available. Therefore, approximating the economic losses is a problem due to lack of detailed records.

\[
\text{Total cattle slaughtered in the abattoirs} = 46310 \\
\text{Total buffaloes slaughtered in the abattoirs} = 18552 \\
\text{Number of animals affected} = 1352 \text{ cattle} + 588 \text { buffaloes}
\]
Number of whole cattle carcass rejected out 1352 = 135 Animals

Average whole cattle carcass weight = 120

Rate per kg (in PKR) = 300

**Losses of whole cattle carcass rejection = PKR 4860000**

Number of whole buffaloes carcass rejected out 588 = 58

Average weight of whole buffaloes carcass (kg) = 100

Rate per kg (in PKR) = 300

**Losses of whole buffalo’s carcass rejection: PKR 1740000**

**Total losses due to whole carcass rejection (cattle & buffaloes)**

\[ = 1740000 + 4860000 = (6600000) \]

**Now expenses due to freezing (treatment) of infected carcasses:**

Remaining cattle = 1217 x 120 = 146040 kg

Remaining buffaloes = 530 x 100 = 53000 kg

Total weight in kg = 199040 kg

Freezing cost @ 5/kg = 199040 x 5 = 995200

**Now to calculate down grading at 100 rupee rate is less for frozen carcasses due to Bovine Cysticercosis**

Cattle = 146040 x 100 = 14604000

Buffalo = 53000 x 100 = 5300000

Total downgrading losses = 19904000 + 995200 = 20899200

Total losses (in PKR) = Loss carcass condemned + Total downgrading losses Net Losses

(in PKR) = 20899200 + 6600000 = 27499200
Losses during one year (in PKR) = 27499200

Losses during one year (in US $) = 274992.00

These are approximate estimates. So the total economic losses due to bovine cysticercosis estimated in one year study at different abattoirs of Punjab Province are US$0.274 million or PKR 27.499 million.
5.1. Prevalence and Diagnosis of Bovine Cysticercosis

Taeniasis is a crucial worldwide food borne parasitic disease with serious public health implications. Man is the final host of *Taenia saginata* while larval stage of this parasite causes muscles infestation in cattle and buffalo a condition known as bovine cysticercosis (Minozzo et al. 2002; Hancock et al. 1989). On the other hand, *Taenia* infection in humans is named as taeniasis, caused by adult stage of *Taenia saginata* (Hancock et al. 1989). The occurrence of *Taenia saginata* is more common in developing countries due to poor hygiene, and habitual consumption of sun-cured, rare or un-cooked beef (Florova, 1982; Symth, 1994). The disease is also a problem in developed countries where “rare” (semi-cooked) beefsteak is consumed. It is worth noting that tape worm/parasite eggs have been known to be resistant to all stages of sewage treatment. Moreover, it is also important to note that stringent meat inspection protocols, even in technologically developed countries, have proven not to be 100% effective. As a result, while developed industrialized nations have significantly lower taeniasis rates than developing ones; this disease is well established and is a problem in the former (Florova, 1982; Symth, 1994).

The major objective of the current study was to determine the prevalence of bovine cysticercosis through postmortem inspection and detection of serum antibodies against bovine cysticercosis using ELISA. The effects of *Cysticercus bovis* on hemogram and serum biochemistry can be used as additional diagnostic tools. Affected organs were collected at the time of postmortem and slides were prepared from infected organs of animals for
histopathological examination to observe the changes. The economic impact of bovine cysticercosis on the meat industry in Pakistan was also estimated.

The purpose of meat inspection is to insure public health, monitor animal health and welfare as well as assisting in herd health management (SCVPH, 2003). The traditional methods of meat inspection alone do not fulfill these requirements. As a matter of fact, there is lack of reliable and effective alternative methods that can fully or even partially replace traditional meat inspection procedures, particularly with regard to the detection of *T. saginata*, or cysticercosis. Furthermore, this infection can only be eliminated through hygienic care of livestock and promoting public awareness for cleanliness and food hygiene (Gonzales et al. 2006). The study design of this thesis is multidirectional, and reflects the positive and negative impacts of different methods of meat inspection and detection of cysticercosis.

In current study, out of 2400 animals examined at multiple abattoirs, overall 73(3.03%) cattle and buffalo were tested positive through postmortem inspection procedure. Previous reports on bovine cysticercosis indicated low infection rate as described by Hariday et al. (1999) (0.23%), Rodriguez- Hidalgo et al. (2003), (0.37%) and Abdo et al. (2009) (1.65%) which is not congruent with the results of this study. Likewise, the prevalence of *C. bovis* reported in current study is lower than those recorded by Oryan et al. (1995) (7.7%), and Kandil et al. (2012) (4.4%). These differences in the reported prevalence rates are predictable due to various reasons like climatic dissimilarity among the localities, management of animals, number of collected samples, in addition to control measures and elimination programs in such countries. The prevalence of *C. bovis* through ELISA was 13.33 and 14.44 percent in cattle and buffaloes, respectively. The difference was non-significant between cattle and buffalo. When compared, significantly higher (P<0.05) prevalence was observed by ELISA than postmortem inspection. This indicates that ELISA is more sensitive and can detect the antibodies of an organism present
in the blood (serum). Acceptance of ELISA for testing large numbers of animals against cysticercosis has already been established (Kandil2005). According to Kandil et al. (2012), 29.4% and 61.76% of examined sera were positive for *C. bovis* and *T. Saginata* when used crude antigens, respectively.

In current study, the prevalence of cysticercosis was higher in public abattoirs (5%) than private abattoirs (1.67%). This difference in prevalence could be due to animal selection procedures for slaughter. The private sector abattoirs usually have developed strict SOPs regarding animal selection and slaughtering, which are not generally followed by public sector abattoirs. Gender-wise, prevalence of cysticercosis was more prevalent in female animals (3.83%) than males (2.8%). Furthermore, this study revealed that prevalence of cysticercosis was high in North Punjab (3.50%) compared to South Punjab (3.25%), while the Central Punjab had the lowest (2.75%). This difference could be attributed to life style of the people and tradition of keeping animals (animals are feed on pastures) and open field defecation.

Age of the animal was also determined as risk factor for cysticercosis. Prevalence of *Cysticercus bovis* was higher (6.8%) in 3-5 years age group of cattle and buffalo than in the 1-3 years age group (1.98%) and 6 month-1year (0.81%) group. The findings of this study were supported by others. The proportion of carcasses found to be infected with cysts was significantly different for species (cattle: 2.1 %, buffalo: 4.7 %), gender (higher in females than males) and age (higher in animals >2.5 years of age) (Nauman et al. 2013). In detail, the percentages were in cattle and buffalo 2.6 % and 6.0 % for females vs. 1.9% and 4.1% for males, and 3.8% and 7.4% for animals >2.5 years of age vs. 1.4% and 2.8% for animals <2.5 years of age (Nauman et al. 2013).

5.2. Effects of Bovine Cysticercosis on Hemogram and Biochemistry
The results of present study revealed that there was no effect of cysticercosis on blood parameters except the values of hemoglobin and platelets count were significantly different (P<0.05) in healthy and *C. bovis* infected cattle and buffalo. On the other hand, WBCs, PCV, MCV, MCH and MCHC values were not significantly different (P>0.05) between healthy and *C. bovis* infected cattle and buffalo. According to Kandil et al. (2012) there was no statistical difference in PCV%, hemoglobin, RBCs and WBCs in the control (non-infected) and infected groups.

There was a significant decrease in total serum proteins (P<0.05) of infested animals (buffalo/cattle) than non-infected. Decreased levels of albumin in infested cattle and buffalo were observed compared to non-infested animals. Decreased levels of albumin could be attributable from serum globulin concentration with an associated decrease in serum albumin concentration in calves experimentally infected with *T. saginata* (Gallie and Sewell 1974; Evranova and Mosina 1965). Results of present study described significant decrease (P<0.05) in AST while ALT levels increased. Large quantities of *C. bovis* may cause hepatic dysfunction (Scandrett et al. 2009). The decrease in the activity of AST in infected cattle may be due to the larger quantity of cysts and subsequent chronic destruction of hepatic parenchyma (Pinzani and Rombonts, 2004; Otto et al. 2010). Similarly, urea levels significantly decreased (P<0.05) and those for creatinine significantly increased (P<0.05) in diseased animals compared to healthy ones. Similar findings were observed by Kandil et al (2012) who reported significantly (P<0.05) increased creatinine level in serum of highly infested cattle compared to non-infested ones. Decreased levels of serum urea are possibly due to hepatic damage in animals, hence a decreased capacity of the liver to convert ammonia. The cholesterol level in cysticercosis infested cattle and buffaloes was significantly decreased (P<0.05) compared to healthy ones. Decreases in cholesterol could be due to the role that cholesterol plays in pathogenesis by enabling the larvae
to stay alive in host tissues or due to disruptions in hepatic function and alterations in the hormonal secretion provoked by the presence of parasites. Cholesterol augmented the survival of ascariasis larval growth when added to RPMI-1640 culture medium and there may be some factors or enzymes, which allow the parasite to breakup and consume lipids/cholesterol (Urban et al. 1984, Wiedermann et al. 1991).

5.3. Histopathological changes induced in Bovine Cysticercosis affected organs

Histopathological changes induced by bovine cysticercosis in different organs of infected animals were studied in detail. Affected organs were collected during postmortem to study these changes. For this purpose slides were prepared to detect histopathological changes induced by bovine cysticercosis. These slides were studied in pathological lab by using microscope (Olympus-B.X-40) under the supervision of a member of the American College of Veterinary Pathologists in the Veterinary Diagnostic Laboratory in the Department of Veterinary Population Medicine, University of Minnesota, USA.

Infested livers displayed prominent portal fibrosis. Affected triads contain aggregates of lymphocytes (and small numbers of macrophages containing tan pigment) along with a mild degree of biliary hyperplasia. Several large and irregular hyper plastic bile ducts were also observed. The hepatic parenchyma is subdivided into islands of irregular hepatocytes, bordered by fibrous connective tissue and in various stages of vacuolar degeneration. Hepatocytes were somewhat distorted by fibrous connective tissue. In several areas of prominent fibrosis hepatocytes were absent or lost.

Tongue tissue myofibers contained sarcocysts of varying diameter. The sarcocysts had a thick, hyperseosinophilic wall surrounding hundreds of crescent-shaped organisms. In esophagus, within the tunica muscularis were sixteen intracellular sarcocysts of varying size, similar to those described in the tongue.
Similar study conducted by Costa et al. (2012) uncovered 253 dead cysts out of 416; these differentiated by nodular firm whitish lesions containing a yellowish substance. The cysts were characterized by caseous and calcareous matter, multinucleate giant cells and histiocytes in palisade and penetrated composed mainly by lymphoid cells, covered up by fibrosis. Infrequently the lesion margins displayed granulation. Fibrous nodules, loaded with lymphoid or mixed infiltrates, were commonly observed.

5.4. Economic Losses

Bovine Cysticercosis is food safety issue and imposes heavy economic losses in food production. This issue is of significant importance for the export meat business in Punjab. In Pakistan meat trade is rising at this time. The export income from the meat trade was 123.61 million dollars in the year of 2012-13 (Economic Survey of Pakistan 2013-14). In Pakistan demand of meat can only be fulfilled by producing disease free meat. Controlling bovine cysticercosis will enable the bovine agronomy to satisfy both domestic and international demands. Animals used for milk production or breeding may at the end of their useful life, can be used for meat consumption, underscroing the need to control cysticercosis in these populations as well. Pakistan is the 4th largest producer of Halal meat in the world. Exporting meat that is certifiably disease free will insure that Pakistan’s bovine agronomy will continue to occupy a commanding position in the world (Mughal, 2013).

In this study, economic losses due to bovine cysticercosis were estimated according to the method of Mellau et al. (2010). Unfortunately, in Pakistan, estimates on the economic losses due to bovine cysticercosis are very difficult to generate due to insufficient information. Records of processed animals from November, 2012 to October, 2013 were compiled and surveyed in this study. Out of total 64862 beef animals slaughtered 46310 and 18552 were cattle and buffalo, respectively. 1352 cattle and 588 buffalo carcasses were rejected, mostly due to bovine
cysticercosis. The livers, tongues, heart and lungs of cattle and buffalo were condemned most often. Exact records of condemnation, refrigerating and downgrading of carcasses, however, were not available. Total economic losses due to bovine cysticercosis have been estimated to be, in Punjab Province, $274,992 or US$0.274 million to meat industry. The costs include treating human taeniasis, processing of infected cattle carcasses (costs of freezing, boiling, and condemnation), as well as the costs involved in the examination procedures (Mann, 1984). An annual losses due to treatment in USA was USD 100,000 (Robert, 1995), in South Africa USD 428 million (Abdusslam, 1975). In two developing countries bovine cysticercosis resulted annual losses of USD 4 million (Kenya) and USD 2 million in Botswana (Grindle, 1978). Again, costs included treatment and condemnation of infected animal carcasses, which are unsuitable for export-this creates losses for the bovine agronomies of these countries (Gracey, 1981). In feedlot cattle, the incidence may be as high as 40% or as low as 3% (Reinecke, 1983).

**Conclusion**

- Bovine cysticercosis is quite prevalent in cattle and buffalo being slaughtered in Punjab. The buffalo were more susceptible to cysticercosis than cattle. Moreover, various risk factors like species, sex, age and type of organ are associated with the development of cysticercosis in cattle and buffalo.

- Cysts of variable size were present in different organs, with the most effected organ being the liver.

- ELISA test is sensitive and specific for the diagnosis of bovine cysticercosis in live animals (cattle and buffalo) than conventional method of postmortem examination.

- Bovine cysticercosis induces pathological changes in liver, tongue and esophagus as well as deleterious effects on various blood and biochemical parameters.
• Bovine cysticercosis imposes heavy economic losses to the meat industry in terms of rejection of meat and reduced exports.

**Recommendations/Suggestions**

The method which is used for the inspection of meat/beef is a routine inspection protocol performed postmortem. Vaccination and proper chemotherapy against this infection are not available. Usual meat inspection techniques are less susceptible (pick only 7.5% of infected cases) and are time consuming. The spread of infection is maintained between humans and cattle/buffalo because carcasses with low level infestations can be easily missed and approved for human consumption. Thus taeniasis/cysticercosis remains prevalent zoonoses that affects human health and economy through condemnation, quality degradation of frozen beef, cost of refrigeration, cost of human therapy, lowering productivity of infected workers. Therefore following recommendations/suggestions are proposed:

• Comprehensive meat inspection should be enforced in every abattoir.

• Slaughtering protocols should be modified to enhance meat quality for export.

• Meat containing cysts should be condemned at abattoirs and destroyed.

• Immuno-diagnostics (ELISA) should be adopted to increase reliability of meat inspection procedure.

• Awareness to the public / students at school level can play an important role in efforts to control this disease.
Bovine Cysticercosis is infection of cattle muscles which occurs when the intermediate host ingests eggs of *Taenia saginata*, which develop into the larval stage. *T. saginata* is a cestode, the adult stage of which is found in human intestine. The distribution of this parasite is international, but with very high prevalences in developing countries. The adult Taenia infection in human is known as taeniasis and the condition caused by larval stage infestation in bovines is called cysticercosis. The occurrence of *T. saginata* infestation is high in developing countries where hygienic measures are poor and where the residents habitually consume rare or inadequately cooked or sun-cured meat. The disease is also a problem in developed countries where considerable undercooked beefsteak (a beef dish) is eaten as meal. It is important to note that parasitic eggs have been known to stay viable though all stages of sewage treatment. It is significant; too, that even the stringent levels of meat product inspection regimens of highly developed countries have not succeeded in eliminating this parasite. Keeping in view the importance of this disease to the bovine agronomy, the present study was designed with the objective to 1) determine the prevalence of bovine cysticercosis through postmortem inspection in cattle and buffaloes slaughtered at abattoirs in Pakistan 2) Diagnosis of bovine cysticercosis from the serum of animals by using ELISA 3) Determine the effect of *Bovine Cysticercosis* on hemogram and serum biochemistry as an additional approach for diagnosis 4) Study the histopathological changes induced by Bovine Cysticercosis in affected organs 5) estimate the
economic losses due to this disease. A total of 2400 animals (n=1200 cattle; n=1200 buffaloes) were randomly selected, tagged and included in this study. Data on each animal was recorded in a “data capture form” where entries included species, sex, age, breed, zone etc. Each animal included in this study was subjected to ante-mortem inspection. During ante-mortem inspection, body temperature, respiration rate, heart rate, apparent anomalies, blemishes, other clinical abnormalities and body condition scoring were recorded. Once the ante-mortem inspection was finished, the animals were slaughtered and postmortem examination of each cattle and buffalo was conducted. During postmortem examination liver, kidneys, lungs, heart, tongue, esophagus, skeletal muscles and masseter (external and internal) muscles were examined for the presence of larvae and cysts of *Bovine Cysticercosis*. For hematological and biochemical studies, blood and serum samples from 60 animals (30 cows and 30 buffaloes) which were positive for Cysticercosis through ELISA were collected. Furthermore, blood samples from 60 animals (30 cows and 30 buffaloes) negative for Cysticercosis through ELISA were also collected and used as control negative group. These blood samples were processed using automated hematology analyzer and biochemistry analyzer. At the time of postmortem, affected organs (Liver, Lung, heart, tongue, esophagus and skeleton muscle, masseter muscles) were also collected from the infected cattle and buffaloes for histopathological examination. Tissue samples of liver, heart, esophagus and tongue were fixed in 10% buffered formalin and were processed for histopathological examination. Economic losses due to bovine cysticercosis were also estimated. Overall prevalence (through postmortem inspection procedure) of Bovine Cysticercosis in cattle and buffaloes was 2.92 and 3.17 percent, respectively. Statistically the difference in prevalence of Bovine Cysticercosis between cattle and buffaloes was not significant (P>0.05). The prevalence of *Bovine Cysticercosis* through ELISA was 13.33 and 14.44 percent in cattle and buffaloes, respectively. The difference was non-significant between cattle and buffaloes. When
compared, significantly higher (P<0.05) prevalence was observed by ELISA than through postmortem inspection. The infestation rates of cysticercosis were higher in public abattoirs (5%) than private abattoirs (1.67%). The disease was more prevalent in females (3.83%) than males (2.8%). Furthermore, this study revealed that highest rates of bovine cysticercosis were observed in cattle (3.75%) and buffaloes (3.5%) of North Punjab, followed by South Punjab where prevalence of bovine cysticercosis was 2.75% and 3.25% in cattle and buffaloes, respectively. On the other hand, lowest prevalence of bovine cysticercosis was found in cattle (2.25%) and buffaloes (2.75%) in central Punjab. Prevalence of bovine cysticercosis was higher (6.8%) in 3-5 years age group of cattle and buffaloes than 1-3 years age group (1.98%) and 6 month-1 year (0.81%). The results of present study revealed that there was no effect of cysticercosis on blood parameters except decrease in the hemoglobin and platelets values of infected animals. The values of hemoglobin and platelets count were significantly different (P<0.05) in healthy vs. bovine cysticercosis infected cattle and buffaloes. On the other hand, WBCs, PCV, MCV, MCH and MCHC values were not significantly different (P>0.05) between healthy and bovine cysticercosis infected cattle and buffaloes. Infestation was found to significantly decrease in total serum proteins (P<0.05) of infested animals (buffaloes/cattle). Decreased levels of albumin in infested cattle and buffaloes were observed when compared to non-infested animals. Results of present study described significant decrease (P<0.05) in AST while ALT levels were increased. Similarly, urea levels were significantly decreased (P<0.05) while creatinine were significantly increased (P<0.05) in diseased animals compared to healthy animals. The cholesterol level in cysticercosis infested cattle and buffaloes was significantly lower (P<0.05) compared to non-infested animals. Histopathological changes induced by bovine cysticercosis in different organs of infected animals were studied in detail. In liver, there was prominent portal fibrosis with bridging between triads. Affected triads contained aggregates of lymphocytes (and small
numbers of macrophages containing tan pigment) along with a mild degree of biliary hyperplasia. Several large and irregular hyperplastic bile ducts were also observed. The hepatic parenchyma was subdivided into islands of irregular hepatocytes, bordered by fibrous connective tissue and in various stages of vacuolar degeneration. Hepatocytes were somewhat distorted by fibrous connective tissue. In several areas of prominent fibrosis hepatocytes were absent or lost. Within tongue myofibers of the deep muscle layer were five intracellular sarcocysts of varying diameter. The sarcocysts had a thick, hyperseosinophilic wall surrounding hundreds of crescent-shaped organisms. In esophagus, within the tunica muscularis were sixteen intracellular sarcocysts of varying size, similar to those described in the tongue. Bovine cysticercosis is food safety issue and imposes heavy economical losses in food production. This issue has significant importance for the export industries of meat in Punjab. To find out the causes of carcass and organ rejection, a total 64862 slaughtered beef animals were studied. Among them 46310 and 18552 were cattle and buffaloes, respectively. From these totals, 1352 cattle and 588 buffaloes were rejected. The main cause for carcass rejection and condemnations was bovine cysticercosis. The livers, tongues, heart and lungs of cattle and buffaloes were condemned the most often. The annual economic losses to meat industry due to bovine cysticercosis were estimated to be $274992 or US$ 0.274 million in abattoirs of Punjab Province alone. In conclusion, 1) Bovine cysticercosis is quiet prevalent in cattle and buffaloes being slaughtered at public and private abattoirs in Punjab. The buffaloes were more susceptible to cysticercosis than cattle. Moreover various risk factors like species, sex, age, breed, zone and type of organ are associated with the development of Cysticercosis in cattle and buffalos 2) Cysts of variable size were present in different organs as diagnosed in postmortem examination. Liver tissue was found to have the highest *T. Saginata* larvae loads 3) ELISA test is sensitive and specific for diagnosis of bovine cysticercosis in live animals (cattle and buffaloes) and is more sensitive than conventional
methods of postmortem examination 4) Bovine cysticercosis induces pathological changes in liver, tongue and esophagus and it has deleterious effects on various blood and biochemical parameters 5) Bovine Cysticercosis imposes heavy economic losses to the meat industry in due to carcass/tissue rejection, costs related to treatment of meat and reduced export volume of bovine products.


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