STUDIES ON THE RISK OF *TAENIA SOLIUM* EXPOSURE FROM PORK PRODUCED IN PUNJAB, INDIA

Thesis

Submitted to Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
in
VETERINARY PUBLIC HEALTH AND EPIDEMIOLOGY
(Minor Subject: Veterinary Parasitology)

By

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CERTIFICATE – I

This is to certify that the thesis entitled, **"STUDIES ON THE RISK OF *TAENIA SOLIUM* EXPOSURE FROM PORK PRODUCED IN PUNJAB, INDIA"** submitted for the degree of **M.V.Sc.,** in the subject of **Veterinary Public Health and Epidemiology** (Minor subject: **Veterinary Parasitology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Megha G. K (L-2017-V-79-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that the thesis entitled, **"STUDIES ON THE RISK OF *TAENIA SOLIUM* EXPOSURE FROM PORK PRODUCED IN PUNJAB, INDIA"** submitted by **Megha G. K (L-2017-V-79-M)** to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.V.Sc** in the subject of **Veterinary Public Health and Epidemiology** (Minor subject: **Veterinary Parasitology**) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

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ABSTRACT

Taenia solium cysticercosis is an important parasitic disease with animal and human health significance. Studies have shown that *T. solium* is endemic in Punjab. Till date no studies were conducted in Punjab to know the risk of *T. solium* infection in pork produced in Punjab and to know the effectiveness of oxfendazole against *T. solium* in pigs reared in Punjab. A Quantitative risk assessment (QRA) model was constructed to understand the risk of human *T. solium* infection from pork produced in Punjab. The analysis was conducted in R statistical software. Missing data on pig slaughtering practices and pork consuming population were collected through surveillance. To determine the frequency of pork consumption, a total of 922 subjects were surveyed. Out of 117 pork consumers, 115 consumed cooked pork while only 2 persons showed their preference for consuming raw pork. A significant association of pork consumption with sex, education and residence was recorded. The results showed that that there is a probability of 0.0242 (95% CI, 0.0079-0.0783) that any one pork meal contains at least one cyst before cooking. The probability that any one pork meal containing at least one viable cyst in Punjab before cooking was found to be 0.0121 (95% CI, 0.0039- 0.0391). Probability of any one pork meal being infective after cooking at consumption was found to be is 0.0003 (95% CI, 0.000-0.0018). A randomized controlled trail was conducted to know the effect of oxfendazole in controlling *T. solium* cysticercosis in pigs in Punjab. We selected 60 pigs 4 months of age and pigs were randomly assigned in three groups: T1 group (Treatment 1; Treated with 30mg/kg b.wt of oxfendazole at 4months old age; n=20), T2 group (Treatment 2; Treated with 30mg/kg b.wt of oxfendazole at 9months old age; n=20) and the C group (Control; Treated with placebo; n=20). Serological status of these pigs against *T. solium* was tested using sandwich antigen ELISA at 4, 9 and 12 months of age. Three pigs from T1 (treatment 1) group were positive at 4 months but became negative in subsequent samplings. One pig from the control group turned seropositive during the course of the study. However, none of the pigs was found positive from the treatment groups. This study showed that oxfendazole is an effective control measure against *T. solium* infection in Punjab but further studies should be conducted to prove statistical significance of this study.

Keywords: *Taenia solium*, risk assessment, pork consumption, oxfendazole.

Signature of Major Advisor **Signature of the Student**

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LIST OF ABBREVIATIONS

%	:	Per cent
@	:	At the rate of
=	:	Equals, the sigh of equality
≤	:	Lesser-than or equal to
≥	:	Greater-than or equal to
°C	:	Degree Celsius
µl	:	Microliter
Ag	:	Antigen
b.wt	:	Body weight
BC	:	Before Christ
C	:	Control group
DALY	:	Disability-adjusted life year
ELISA	:	Enzyme linked immunosorbent assay
Eq	:	Equation
et al	:	And others
Fig.	:	Figure
g	:	Gram
i.e.	:	That is
m	:	Meter
mins	:	Minutes
ml	:	Millilitre
mm	:	Millimetre
no.		Number
OD	:	Optical density
OFZ	:	Oxfendazole
Pr	:	Probability
QMRA	:	Quantitative microbial risk assessment model
QRA	:	Quantitative Risk Assessment
rpm	:	Revolutions per minute
spp	:	Species
T1	:	Treatment group 1
T2	:	Treatment group 2
x	:	Sign of multiplication
ρ/rho- value	:	Spearman's rank correlation coefficient value

CHAPTER I

INTRODUCTION

The parasite *Taenia solium* belongs to the Class Cestoda, Order Cycliphyllidea, Family Taeniidae, and Genus Taenia. *Taeniidea* tapeworms are usually large and length of adult worm varies from 3-5 m or up to 8 m. These worms can survive up to 25 years. The gravid proglottids are 10-12 mm long by 5-6 mm wide, and irregularly alternating and single genital pore are present. The worm has large number of testis and the ovary is in the posterior part of the proglottid. They have 7-16 lateral branches in uterus. The gravid proglottids, each contains 40,000 eggs and are voided in the faeces frequently in chains.

Ebers papyrus in 1500BC described tapeworms, *Taenia* spp eggs and *Taenia solium* cysticerci which he found in the intestines and stomach of Egyptian mummies. In 1558 first case of human neurocysticercosis was recorded by Rumler during the autopsy of an epilepsy patient had a liquid filled vesicles adherent to meninges. Later in 1697, Malpighi documented the parasitic nature of these by describing the scolex of *T. solium* inside the vesicle. The term *Taenia cellulose* was coined by Gmelin for these vesicles. *T. solium* lifecycle was completely known by the experiments done in Belgium and Germany and was further confirmed by a seminal work done by Yoshino. He infected himself by cysticerci of *T. solium* to study the cestode lifecycle (Del Brutto *et al* 2015).

Human acts as definitive host by harbouring adult worms in the intestine, resulting in the occurrence of taeniasis. When a human subject with taeniasis defecate, this might result in releasing five parasite segments per day containing thousands of eggs to the environment. Pigs being the only intermediate host get the infection either due to the direct ingestion of human faeces having parasitic eggs or ingesting eggs through contaminated feed and water. After ingestion, oncosphere or embryo are released which primarily migrates to the muscles and mature to cysticercus (metacestode stage). In a span of 10-12 weeks of ingestion cysticerci will be established as a cyst. When undercooked infected pork meat is consumed human develops taeniasis. This cycle gets completed when eggs that are produced by worms are passed in feces which take about two months after ingestion of eggs (Carabin and

Traore 2014). After 2 months of infection, infected humans release proglottids containing eggs in the faeces.

In humans, *T. solium* causes two different clinical presentations: taeniasis and cysticercosis. Human cysticercosis occurs due to accidental ingestion of eggs through contaminated food/water or self-infection due to poor hygienic practices. Additionally, contaminated uncooked vegetables or other contaminated materials could also serve as a source of infection (Mahajan 1982). Cysticercosis can affect many organs such as subcutaneous tissues, muscles, eyes and central nervous system. When CNS gets affected with the formation of cyst it is called neurocysticercosis (NCC) (O'Neal *et al* 2014). Seizures, headache, intracranial hypertension, encephalitis, cognitive impairment and stroke are the manifestation of human NCC (Scharf 1988 and Shandera *et al* 1994). Neurocysticercosis attributes about 30% of seizure disorders in endemic areas around the world. In rural endemic villages, 10-20% of residents are found to have brain lesions consistent with NCC (O'Neal *et al* 2014).

Cysticercosis is a major public health problem, especially in the developing world (Prasad *et al* 2008). *Taenia solium* cysticercosis is a neglected zoonosis and is responsible for approximately 1/3rd of epilepsy cases in the developing world (Thomas *et al* 2017). Neurocysticercosis (NCC) caused by *Taenia solium* oocyst is considered to be the most common parasitic infestation of the central nervous system. Certain cultural, socio-economic and sanitary practices promote the perpetuation of taeniosis and cysticercosis in many endemic countries (Sarti and Rajshekar 2003).

Porcine cysticercosis is prevalent in the countries which lack adequate sanitary infrastructures and health education (Flores *et al* 2001). The prevalence of NCC as a cause of active epilepsy in India is one per 1000 population which is at least 1.2 million persons in India are suffering from active epilepsy due to NCC (Rajshekar *et al* 2016). A recent descriptive study showed an overall seropositivity in 32.5% cases for NCC in South Indian states (Thamilselvan *et al* 2016). Prevalence of taeniasis was 18.6% and epilepsy in 5.8% out of which 48.3% had NCC in rural pig-farming community of Uttar Pradesh, India (Prasad *et al* 2011). Prevalence of NCC was 4.5% in children presenting with acute onset seizure/focal neurological deficit (Kumar *et al* 2017). The prevalence in a north Indian study of epilepsy without NCC, epilepsy with

NCC and epilepsy with NCC along with other causes was found to be 6.53/1000 persons, 3.48/1000 persons and 10.0/1000 persons, respectively (Goel *et al* 2011).

In India, pigs are mostly reared in the backyards of rural households in small numbers for supplementing their meagre incomes; animals are usually housed close to human dwellings and let free to scavenge in garbage dumps. Kitchen waste and available feed are used for supplementation. People defecate in open dwellings due to lack of sanitary infrastructures, this perpetuates the occurrence of cysticercosis in pigs (Morales *et al* 2006). Although pigs do not show any overt symptoms of infection unless there is presence of thousands of cyst throughout the body however, human health hazard from pork of such pigs is high. The disease is prevalent in both northern and southern states of India although prevalence is higher in the North (Singh 2018).

Human neurocysticercosis is an important public health concern in India. NCC-associated active epilepsy caused a combined median loss of Rs. 12.03 billion in India (Singh *et al* 2016). The median DALYs per thousand persons per year in the country were estimated to be 1.73 (Singh *et al* 2016). Overall prevalence of epilepsy in India has been reported at 5.59/1000 population, with no statistically different rates between men and women or urban and rural residents (Sridharan and Murthy 1999).

Human activities have a major impact on the life cycle of this parasite. Taeniosis is exclusively a human disease, man is responsible for parasite’s eggs dispersion through outdoor defecation and indiscriminate disposal of faeces (Sarti and Rajshekar 2003). The precipitating factors in transmission of taeniosis are contact between pigs and human faeces, lack of inspection of pork, consumption of unclean vegetables, contaminated water and poor personal hygiene (Sarti *et al* 1988).

The consumption of raw or lightly cooked pork and the inability to recognise infected meat are two risk factors which have been significantly associated with human taeniosis. The other promoting factors for the transmission of the disease include contact between pigs and human faeces, lack of inspection of pork, consumption of unclean vegetables and water contaminated with infected human faeces, lack of potable water, insufficient latrines, low education and, finally, poor personal hygiene (e.g. inadequate washing of hands before eating and after defecating) (Sarti *et al* 2003 and Thomas *et al* 2017).

T. solium is vulnerable to control and have greater potential of eradication because of the following characteristics (1) they require human beings as the definitive host for the completion of life cycle; (2) *Taenia solium* taeniasis occurs in humans only after consumption of uncooked/undercooked pork (3) taeniosis is the only source of infection for the intermediate host; (4) pigs can be monitored, considering the short life span of the animal; (5) there are no wild reservoirs; and (6) safe and efficacious antiparasitic drugs against taeniosis are available (Prasad *et al* 2008 and Sarti *et al* 2003).

Reducing the volume of infected meat entering the food chain is therefore an important issue to be considered, by a public health practitioner. In addition to this, to estimate the probability of any one pork meal consumed containing at least one viable cyst, a process of risk analysis, whereby risks are identified, assessed, communicated and mitigated, can achieve understanding of the current risks posed by pork consumption in developing countries. A stochastic, quantitative risk assessment, as part of a risk analysis process, allows us to incorporate quantitative data and the uncertainty and variability that surround these data, in order to establish a quantitative estimate of risk and a probability interval around that estimate. As far as we are aware, no such studies have been conducted in Punjab state of India.

In view of this, the current study was focussed on the following objectives

1. To determine the risk of *Taenia solium* exposure from pork produced in Punjab, India.
2. To evaluate the effectiveness of oxfendazole/any other suitable anthelmintic in reducing the prevalence of porcine cysticercosis in India.

CHAPTER – II

REVIEW OF LITERATURE

2.1 History

Tape worms were recognized in mummies long back in 3000-1500 BC from ancient Egypt. Later in 448–385 BC it was mentioned in Aristophanes about the occurrence of swine cysticercosis. Ancient Greece considered pigs to be impure, which is a possible reason for the prohibition of pork at the time when the Koran was written by Muhammad (570–632 AD). Ebers papyrus in 1500BC described tapeworms, *Taenia* spp eggs and *Taenia solium* cysticerci which he found in the intestines and stomach of Egyptian mummies (Del Brutto *et al* 2015). In 1558 first case of human neurocysticercosis was recorded by Rumler during the autopsy of an epilepsy patient had a liquid filled vesicles adherent to meninges. Later in 1697, Malpighi documented the parasitic nature of these by describing the scolex of *T. solium* inside the vesicle. The term *Taenia cellulose* was coined by Gmelin for these vesicles.

T. solium lifecycle was completely known by the experiments done in Belgium and Germany which was confirmed by a seminal work done by Yoshino. He infected himself by cysticerci of *T. solium* to study the cestode lifecycle (Del Brutto *et al* 2015). The life history patterns for taeniids *Taenia saginata* by Goeze, 1782: *Cysticercus bovis* by Cobbold, 1866; *T. solium* Linnaeus, 1758; *T. multiceps* by Leske,1780; *Coenurus cerebralis* by Batsch, 1786; *T. taeniaeformis* by Batsch, 1786 ; *Hydatigera taeniaeformis* and *Strobilocercus fasciolaris* by Rudolphi, 1808 and more recently, a number of generic names have continued to be applied to putative species-groups of Taenia like *Multiceps* by Goeze (1782), *Hydatigera* by Lamarck (1816), *Fimbriotaenia* by Korniushin and Sharpilo (1986), based primarily on differences in larval morphology (Hoberg 2002).

Etiology, Morphology and Life-cycle

Table 1: Taxonomic Classification of *T. solium* (CABI 2019)

Kingdom	Animalia
Phylum	Platyhelminthes
Class	Cestoidea
Order	Cyclophyllidea
Family	Taeniidae
Genus	*Taenia*
Species	*Solium*

Cyclophyllidean tapeworms are among the crown groups of the eucestodes. Among this diverse conglomeration are the Taeniidae, highly characteristic tapeworms in the subfamilies Taeniinae and Echinococcinae that occur as adults in the intestine of carnivorous and omnivorous mammals, including humans. The genus *Taenia* been large and ungainly, with the actual number of valid species now appearing to be closer to 40 with 33 based on adults and metacestodes, five based only on adults and two species based only on metacestodes (Hoberg 2002).

T. solium exhibits a globular structure at one end, called a scolex (Figure 1), which narrows down on one side forming a region of proliferative tissues from which proglottids are produced in a chain, forming the strobila. Suckers are four in total and the large rostellum with two rows of 11–14 hooklets 100–160 µm long. Proglottids (Figure 2) near the neck are immature and undifferentiated, whilst the distant once are progressively mature, accommodating a large number of eggs. The strobila length is usually between 2 and 3 m with 800–1000 segments. Gravid proglottid of *T. solium* consists of characteristic branching of the uterus in the mature segments serves to distinguish between *Taenia* species. Gravid proglottides measure 12 X 6 mm and possess a uterus with seven to ten lateral branches containing both testes and ovary (Sciutto *et al* 2000). Eggs (Figure 3) measure 30-45 µm and contain an oncosphere (hexacanth larvae) having six hooks. Gravid proglottids or eggs are passed out in the

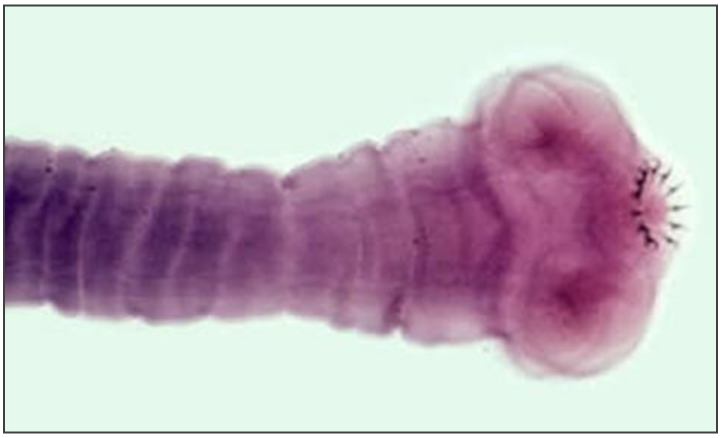

Figure 1: Scolex of *T. solium* (CDC, 2017)

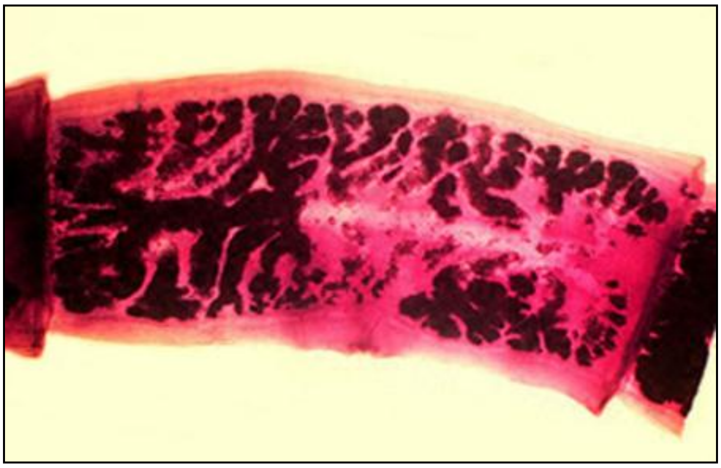

Figure 2: Mature proglottid of *T. solium* (CDC, 2017)

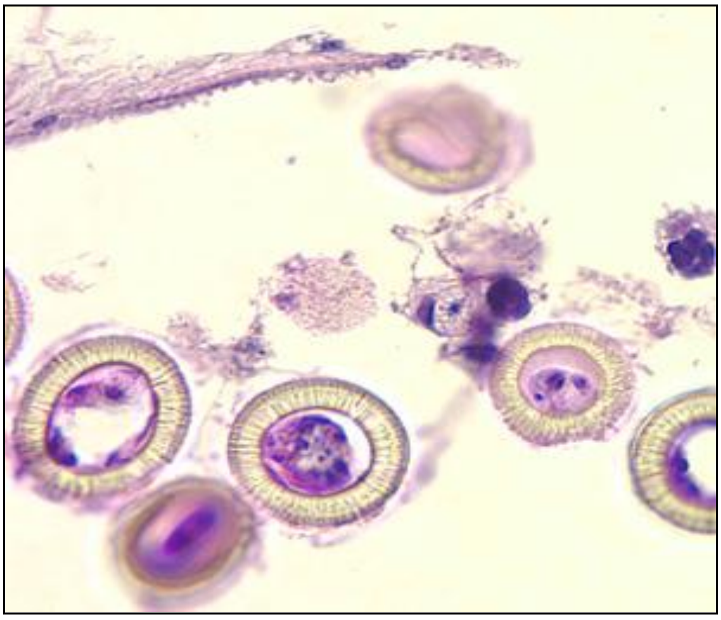

Figure 3: *T. solium* eggs under light microscope (CDC, 2017)

faeces of humans infected with the adult worm. Each gravid segment contains 30,000–50,000 eggs.

The *T. solium* life cycle (Figure 5) mainly involves humans as the only definitive host (bearing the tapeworm) and intermediate host (bearing cysticerci) as pig. Even though cysticerci can infect humans but unless cannibalistic, are not intermediary hosts for transmission. Occasionally dogs, sheep and wild boar have been found to naturally harbor *T. solium* cysticerci (Sciutto *et al* 2000). Pigs being intermediate hosts become infected after the ingestion of food or water contaminated with eggs or gravid proglottids. Whereas humans get taeniosis as a result of eating raw or undercooked pork infected with the larval form of *T. solium* ('measly pork'-Figure 4). Whilst, cysticercosis in humans occurs after the ingestion of drinking water, uncooked vegetables or other material contaminated with parasite eggs.

In human body cysticercosis can occur anywhere, but only when the cysticercus is in nervous system (NCC) or the eye humans exclusively exhibits symptoms (Nash *et al* 1984). In the normal cycle of transmission, gravid proglottids containing infective eggs gets detached from the distal end of the adult worm and are passed with faeces of definitive host with taeniosis. This is most commonly observed in rural areas of endemic countries due to the presence of favouring conditions for the completion of the life cycle of *Taenia solium such as* illiteracy, poverty, open-air defecation, warm climate, allowance of free roaming pigs, and consumption of improperly cooked pork under poor hygienic conditions.

2.2 Prevalence of porcine cysticercosis in India

Singh *et al* (2018) conducted a study to estimate the prevalence and distribution of *T. solium* porcine cysticercosis in the Punjab, India. The authors compared the disease prevalence in pigs reared within and outside Punjab. They inspected 642 pigs reared in Punjab and 450 imported from other states at slaughter shops. Overall, 3.69% and 8.77% true prevalence was found in pigs reared in Punjab and outside the state respectively.

Kakoty *et al* (2017) carried out meat inspection of 316 pig carcasses in different market places of Sivasagar district of Assam for a period of one year. Out of these, 4 carcasses were found positive for porcine cysticercosis containing viable cysts. The highest density of *cysticercus cellulosae* was found in skeletal muscles and lowest in tongue. The genomic DNA from positive carcasses was subjected for PCR assay targeting large subunit rRNA gene with a molecular sizes of 286 bp. All DNA samples extracted from 4 cysticercosis positive pigs, identified as viable cysts at meat inspection were also confirmed as *cysticercus cellulosae* by PCR assay.

Chawhan *et al* (2015) did molecular characterisation of *Taenia solium* cysticercosis from naturally infected pigs. They examined a total of 519 pigs slaughtered in small slaughter shops in the urban slums of Punjab state in northern India. They found 4.23% overall prevalence but the proportion of positive cases were significantly higher stray/ scavenging pigs (10.41%) than in farm pigs (0.61%) positive for *T. solium*. They sequenced ten representative samples targeting cytochrome oxidase 1 gene amplified products in both directions for phylogenetic analysis. Sequencing demonstrated that all cysticerci were of the Asian genotype of *T. solium* and not of the African/Latin American genotype or *T. asiatica*. Thus, confirming the presence of *T. solium* porcine cysticercosis in Punjab.

Saravanan *et al* (2014) investigated the prevalence of *Taenia solium* cysticercosis in pigs slaughtered at local makeshift slaughter houses in Bareilly, Northern India and examined 175 pigs for cysticercosis. They found an overall prevalence of 5.14%.

Borkataki *et al* (2012) studied prevalence of cysticercosis according to age, sex, breed of carcasses and seasonal occurrence in a period of 1year from March 2002 to February 2003. A total of 978 pigs were examined, out of which 93 pigs (9.50%) were found to be positive for infection. District wise highest (13.70%) prevalence was found in Karbianglong and lowest (7.55%) in Nagaon district. Age wise prevalence study showed highest (11.41%) in the age group of 7-12 months and lowest (7.60%) in the age group 19-24 months. Sex wise prevalence was found to be 9.15 and 10.39 percent in male and female respectively. Breed wise prevalence was found more in cross bred (12.53%) than in local breed (7.49%). Seasonal prevalence was highest during pre-monsoon (10.93%) and lowest (7.82%) during monsoon season. Though there were variation in the rate of prevalence of porcine cysticercosis, disease was

Figure 4: Pork containing cysticerci

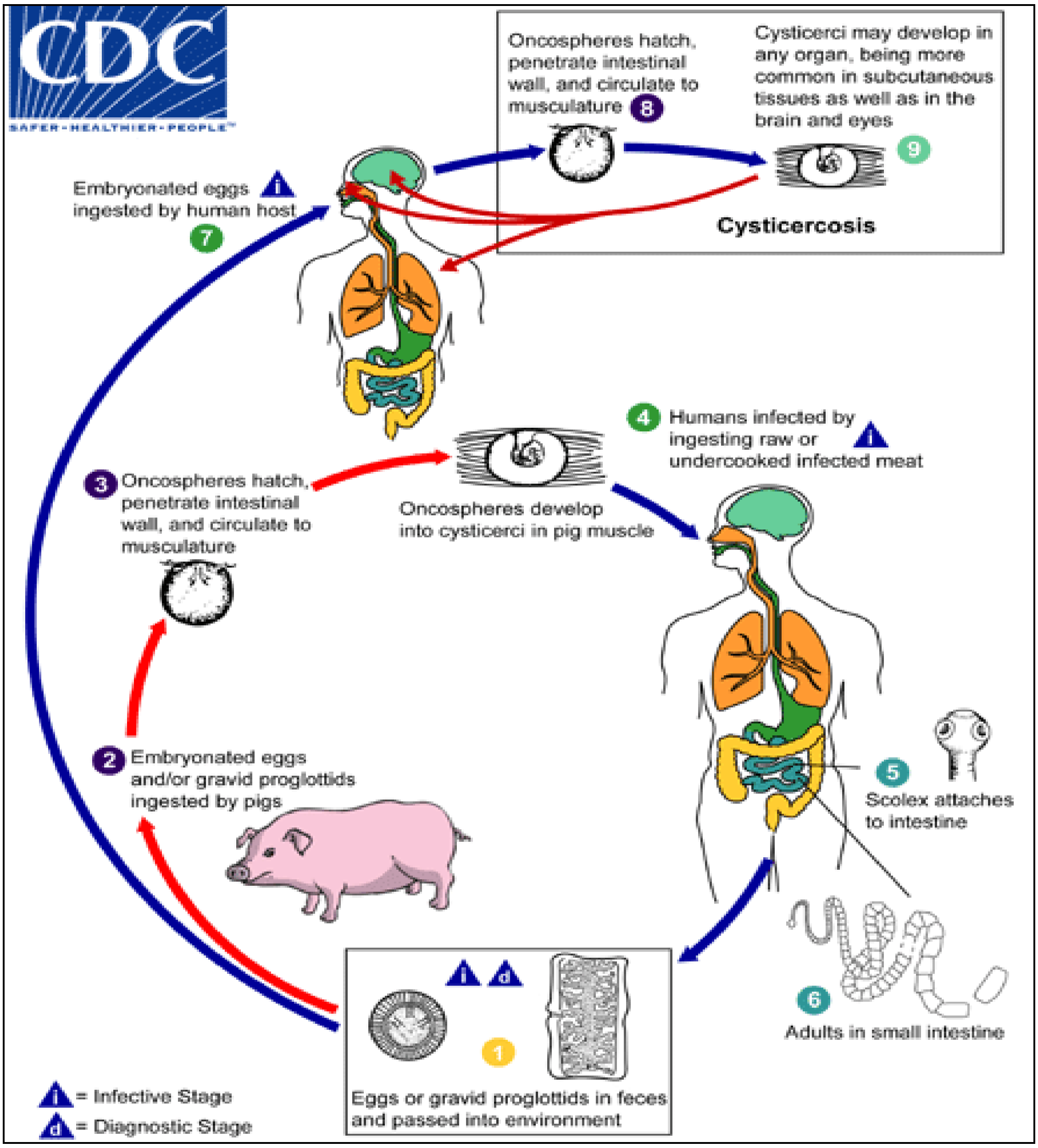

Figure 5 Life cycle of *T. solium* (CDC, 2014)

found to exist in the study area throughout the year that ranged from 5.71 to 14.06 percent.

Mohan *et al (*2013) assessed prevalence of porcine cysticercosis in rural and urban areas of southern India by an antigen ELISA and enzyme linked immune electro transfer blot (EITB) for antibodies. They performed these tests on 112 porcine blood samples, 13 (11.6%) were positive for cysticercal antigens and 67 (59.8 %) tested positive for serum antibodies indicating high exposure to *T. solium* eggs. Thus, indicating a high prevalence of porcine cysticercosis in rural and urban areas of southern India.

Prakash *et al (*2007) investigated the prevalence of neurocysticercosis among free ranging pigs and studied pathomorphological lesions of affected brains. A total of 200 brains were collected from pigs slaughtered at a local abattoir situated in Bareilly (U.P). Gross and histopathological examination revealed 3 % (6/200) occurrence of neurocysticercosis in pigs. This study indicated that the prevalence of the disease is very high among free ranging pigs and the area can be categorized as highly endemic zone.

Hafeez *et al (*2004) conducted a systematic study for a period of three years (from July, 2000 to August, 2003) to determine the prevalence of *Cysticercus cellulosae* from pigs in Southern states of India. Conventional meat inspection as well as immunodiagnostic tests were used for the detection of porcine cysticercosis. By classical meat inspection, per cent positivities of 3.52, 5.50, 5.73 and 5.38 were recorded in Andhra Pradesh, Tamilnadu, Karnataka and Kerala respectively. By Countercurrent Immuno electrophoresis (CIEP) 6.16, 5.83, 6.04 and 5.69 percent of sera samples were found positive in Andhra Pradesh, Tamilnadu, Karnataka and Kerala respectively. By ELISA 6.50, 6.22, 6.40 and 6.50 per cent of sera samples were found positive in Andhra Pradesh, Tamilnadu, Karnataka and Kerala respectively. Sensitivity of CIEP and ELISA was higher than conventional meat inspection in all these states of South India.

Sharma *et al* (2004) recorded the prevalence of swine cysticercosis and the correlation between the disease and various epidemiological factors in Punjab. Two hundred and thirty-six pig carcasses were examined out of which age-wise prevalence was more (6.48%) in younger pigs of <1 year of age than those aged >1 year (6.25%). Prevalence rate was found to be 8.82% in males and 4.48% in females. The overall prevalence rate was found to be 6.35%.

Prasad *et al* (2002) examined 50 slaughtered pigs in Uttar Pradesh, India between November 2000 and June 2001 for *Taenia solium* infection. Thirteen pigs were positive for *T. solium* cysticercosis giving a high overall prevalence of 26%. Such high prevalence indicates the need for detailed assessment of the disease burden in this community.

Deka *et al* (1990) investigated 2980 pigs slaughtered in the western parts of Uttar Pradesh to know the epidemiology of *T. solium* cysticercosis. Overall infection in these carcasses was 15.5%. The highest rate of infection was observed at the Central Dairy Farm, Aligarh (17.4%). The intensity of infection was highest in the skeletal muscles of the thigh, tongue and neck. They found infection to be greatest in older animals and except for slight higher infection rate in autumn and summer, there was no such seasonal variation.

Pathak and Gaur (1989) screened 3550 pigs, 530 cattle, 370 buffalo, 1850 sheep and 2100 goat of different breeds, age and sex from different parts of Uttar Pradesh State (India) from 1980 to 1985 for the presence of cysticerci. They calculated economic losses due to condemnation of affected meat. The overall incidence in pigs was 9.3%, there was no statistical difference in the rate of infection with regards to age and sex. Sheep, goat, cattle and buffalo were found negative for *T. solium* cysticercosis.

2.3 Prevalence of porcine cysticercosis in other countries

Ng-nuguyen *et al* (2018) conducted a cross-sectional study of household pigs in three districts of Dak Lak Province, Central Highlands of Vietnam and visited 408 households in six villages between June and October 2015. A questionnaire was administered to the head of each household, and within each household, serum samples were collected from three pigs. Serum samples were analyzed using the recombinant T24H antigen in enzyme-linked immuno-electro transfer blot assay and lentil lectin purified glycoprotein in EITB assay. The prevalence of porcine *T. solium* cysticercosis in this study was low at 0.94 cases per 100 pigs at risk, in agreement with other studies conducted throughout Vietnam.

Mkupasi and Shonyela (2017) conducted a cross-sectional survey to establish the prevalence of porcine cysticercosis involving 698 pigs by tongue examination and 330 pigs by Ag-ELISA test. This study results indicated that porcine cysticercosis is endemic in Nyasa District (Tanzania) with prevalence of 6.3% and 33.3% during lingual examination and for Ag-ELISA respectively

The apparent and corrected prevalences of *T. solium* cysticercosis were investigated in pork carcasses slaughtered and retailed in Antananarivo (Madagascar) as 4.6 % and 21.03 % respectively (Porphyre *et al* 2015). They underline the need for improved surveillance and control programmes to limit *T. solium* cysticercosis in carcasses by introducing a risk-based meat inspection procedure that accounts for the origin and breed of the pigs, and the season.

An active abattoir survey was conducted from January to May, 2015 by Atawalna *et al* (2015) at two pig slaughter points in the Kumasi metropolis of Ghana. Ante-mortem examination of 4121 randomly selected pigs was conducted and their sex and breed were recorded. Overall prevalence of PCC was 2.31% and was based on post-mortem examination. Female pigs (4.59%) were more affected than males (1.44%). The Ashanti Black pig (8%) was more affected than cross breeds (0.5%).

Prevalence and associated risk factors of *Taenia solium* cysticercosis in pigs within Nay Pyi Taw area, Myanmar was determined by conducting cross-sectional surveys (Khaing *et al* 2015). Three hundred pigs were inspected in slaughter houses and 364 pigs were randomly selected and examined from 203 households from three town ships in Nay Pyi Taw area. The prevalence of porcine cysticercosis in meat inspection was 23.67%. Seroprevalence of *T. solium* cysticercosis in pigs in the study area was 15.93%. Significant associated risk factors with *T. solium* cysticercosis were gender, increased age, husbandry system, feed type, not using anthelmintic in pigs, not using anthelmintic in owner, no hand-washing before feeding, and pork consumption owner in the study area. This is the first report of porcine cysticercosis in Myanmar.

Pondja *et al* (2010) carried out a cross-sectional survey between September and November, 2007 in 11 villages in Mozambique. A total of 661 pigs were tested serologically for the presence of circulating parasite antigen and examined by tongue inspection. Two hundred thirty-one samples (34.9%) were found positive by the Ag-ELISA, while by tongue inspection on the same animals cysticerci were detected in eighty pigs (12.7%). Increasing age and free-range pig husbandry system were important risk factors for porcine cysticercosis in the district.

Kagira *et al* (2010) did a cross-sectional study to investigate the disease in free-range pigs on 182 smallholder farms in Busia District, Kenya. Sero-prevalence was found to be 4% (11/182) using enzyme-linked immunosorbent assay (ELISA) while 9% of the total farms were found sero-positive. All pigs examined in the slaughter slab survey were seronegative. The only significant risk factor associated with the occurrence of cysticercosis was lack of latrines at household level. The study shows that porcine cysticercosis is prevalent in free-range pigs in Busia District, Kenya.

A community-based study of pigs owned by resource- poor, emerging pig producers from 21 villages in the Eastern Cape Province was conducted (Krecek *et al* 2008). Lingual examination (tongue palpation) in live pigs, two enzyme-linked immunosorbent assays (ELISAs), which detect parasite antigen and an enzyme immunotransfer blot (EITB) assay, which detects antiparasite antibody, were used to verify endemicity and estimate apparent prevalence. Results indicate that the parasite is indeed present in the study villages and that true prevalence was 64.6%. The apparent prevalence as measured by each of the four tests was: 11.9% for lingual examination, 54.8% for B158/B60 Ag-ELISA, 40.6% for HP10 Ag-ELISA and 33.3% for EITB.

A total of 1691 free-range pigs in selected districts of Eastern, Southern and Western provinces of Zambia were examined out of which 183 (10.8%) were positive on tongue examination. Ag-ELISA gave a seroprevalence of 23.3% (Sikasunge *et al* 2008). When considering the factors in a logistic regression analysis, only breed type was significantly associated with porcine cysticercosis. The crossbred pigs were 72%

more likely to have had cysticercosis than the Nsenga (dwarf local) breed as determined by Ag-ELISA. The result that crossbred pigs had a higher prevalence of *T. solium* cysticercosis suggests that pig breeds may display different susceptibility to cysticercosis.

Boa *et al* (2006) examined lingually a total of 722, 808 and 302 live pigs in Chunya and Iringa Rural Districts and Ruvuma Region respectively in Songea and Mbinga Districts to estimate prevalence rates and risk factors for porcine cysticercosis. Structured questionnaire interviews identified factors associated with the disease prevalence in both Chunya and Iringa Rural Districts were free-ranging of pigs, home slaughtering of pigs and pork not being inspected. While in Chunya and Iringa Rural Districts lack of latrine and barbecuing were found a risk factor, respectively.

Nagowi *et al* (2004) examined seventy carcasses of pigs of 1-2 years age in a survey conducted on extra-intestinal porcine helminths at three slaughter slabs that receive pigs from Mbulu, a district in northern Tanzania during a period between December 1997 and March 1998. They did ante-mortem lingual examination for *Taenia solium* cysticercosis followed by post-mortem inspection. The cases of porcine cysticercosis were less in this study compared to previous studies suggests that pig traders are conducting their own ante-mortem lingual examinations before purchasing pigs in the rural communities where the parasite is still highly prevalent. Hence they concluded that improved meat inspection could prove useful in reducing the local population's risk of infection with parasites.

To determine the prevalence and etiologic factors of *Taenia solium* cysticercosis in pigs in the state of Bahia, north eastern Brazil; Sakai *et al* (2001) performed serological surveys in Salvador. Serum samples of randomly selected free-roaming pigs in the study areas were obtained and examined by the enzyme-linked immunoelectrotransfer blot assay (EITB). The prevalence of antibodies to *T. solium* was 4.4% (2 of 45) in Salvador, 3.2% (3 of 93) in Santo Amaro, and 23.5% (24 of 102) in Jequie. Poor sanitary conditions, such as an open sewer system and no inspection process of pork before marketing was associated for the significant rise in seroprevalence of Jequie.

Boa *et al* (1995) examined eighty three carcasses of pigs at three abattoirs northern Tanzania. Overall prevalence of 13.3% *Taenia solium* metacestodes were found in all the three abattoirs. During routine meat inspection in Kiboroloni, Moshi 6.2-6.9% of the pigs were found to harbour *T. solium* metacestodes.

Gonzalez *et al* (1990) examined the utility of the tongue test as a diagnostic tool for porcine cysticercosis. The tongue test results were compared with 2 serologic methods for the detection of cysticercosis, the enzyme-linked immunosorbent assay (ELISA) and the enzyme-linked immuno-electrotransfer blot assay (EITB), and also with necropsy results. They examined 11 animals from an endemic area and 42 animals from an area free of cysticercosis. They found sensitivity of 70% and a specificity of 100% for tongue test. Prevalence of 23.4% by tongue examination, 31.2% by necropsy, 37.7% by ELISA, and 51.9% by EITB of porcine cysticercosis in Huancayo was found.

2.4 Risk Assessment

Meester *et al* (2019) developed a quantitative microbial risk assessment model (QMRA) to quantify the risk of human *T. solium* exposure from meat of home slaughtered pigs, in comparison to controlled slaughtered pigs, in European countries. The researcher found 13.83 times higher prevalence of contaminated pork portions from home slaughtered pigs than controlled slaughtered pigs. This difference is brought about by the higher prevalence of cysticercosis in pigs that are home raised and slaughtered. Meat inspection did not affect the higher exposure from pork that is home slaughtered. Cooking meat effectively lowered the risk of exposure to *T. solium* infected pork.

A Quantitative Risk Assessment (QRA) model was adapted by Kiermeier *et al* *(*2019) and updated to quantify the risk of human *T. saginata* infection from consumption of Australian beef domestically and in key export markets. They used the model to investigate the effect of reducing current post-mortem inspection (PMI) protocols by removing the need to incise the masseters, or by removing all incisions, for low risk cattle. The results of the QRA indicate that the risk of human *T. saginata* infection from consumption of Australian beef is very low.

Thomas *et al* (2017) estimated a risk of any one pork meal being infected with *T. solium* cysticercus at the point of consumption in western Kenya by building a quantitative food chain risk assessment model. This model indicated that any one pork meal consumed in western Kenya has a 0.006 probability of containing at least one viable *T. solium* cysticercus at the point of consumption and therefore being potentially infectious to humans. This equates to 22,282 potentially infective pork meals consumed in the course of one year within Busia District alone. The authors observed a high risk of *T. solium* infection associated with pork consumption in western Kenya.

2.5 Therapeutic Control of *T. solium* cysticercosis

A randomized controlled field trial was conducted by Pondja *et al* (2012) where two hundred and sixteen piglets aged 4 months were selected and assigned randomly to oxfendazole treatment or control groups. Fifty-four piglets were treated at 4 months of age (T1), while another 54 piglets were treated at 9 months of age (T2) and these were matched with 108 control pigs from the same litters and raised under the same conditions. At the end of the study, 66.7% of the controls were found positive, whereas 21.4% of the T1 and 9.1% of the T2 pigs were positive, respectively. There was a significant reduction in the risk of *T. solium* cysticercosis if pigs were treated with OFZ either at 4 months or at 9 months of age.

Mkupasi *et al* (2013) tested different drugs on porcine cysticercosis with varying efficacies such as flubendazole, fenbendazole, albendazole sulphoxide, oxfendazole, praziquantel and nitazoxanide. Oxfendazole was found to be effective for the control of porcine cysticercosis; however, it needs to be integrated with other control approaches. There is a need for standardised guidelines for evaluating the efficacy of anthelmintics against porcine cysticercosis, and more efficacy studies are needed since the conclusions so far are based on a limited number of studies using few infected pigs.

Hernández-Bello *et al* (2013) studied the effect of 16α-bromoepiandrosterone (EpiBr), a dehydroepiandrosterone (DHEA) analogue on the cysticerci of *Taenia solium*. EpiBr reduced scolex evagination, growth, motility, and viability in dose- and time-dependent fashions during in-vitro treatment of *T. solium* cultures. EpiBr administration prior to infection with *T. solium* cysticerci in hamsters reduced the number and size of developed taenias in the intestine, when compared to controls. Thus authors concluded that the results leave open the possibility of assessing the potential of this hormonal analogue as a possible antiparasite drug for cysticercosis and taeniosis.

Pondja *et al* (2012) carried out a randomized controlled field trial to evaluate the effectiveness of a single oral dose of 30 mg/kg of oxfendazole (OFZ) treatment for control of porcine cysticercosis in 4 rural villages of Angonia district, north-western Mozambique. Results showed that there was a significant reduction in the risk of *T. solium* cysticercosis if pigs were treated with OFZ either at 4 months or at 9 months of age. Strategic treatment of pigs in endemic areas should be further explored as a means to control *T. solium* cysticercosis/taeniosis.

Gonzalez *et al* (2012) tested seven antiparasitic regimens in 42 naturally infected pigs with cysticercosis, and compared with prednisone alone (n = 6) or no treatment (n = 6). The numbers of viable cysts in muscles and in the brain were examined after necropsy and were significantly decreased in pigs receiving combined albendazole plus praziquantel, albendazole alone, or oxfendazole. Pigs receiving praziquantel alone and nitazoxanide had numerous surviving cysts. Control (untreated) pigs and prednisone-treated pigs had many more viable cysts, suggesting no effect. Combined albendazole plus praziquantel, and oxfendazole, showed a strong cysticidal effect.

Gonzalez *et al* (2001) evaluated the full potential of single-dose oxfendazole treatment for pigs as a control measure for porcine cysticercosis. In this study 20 cysticercosis positive pigs were treated with oxfendazole and later matched with 41 naive pigs and exposed to a natural challenge in a hyperendemic area. New infections were found by serologic testing in 15 of the 32 controls (47%), and by the presence of cysts at necropsy in 12 of them (37%). Only minute residual scars were detected in the carcasses of oxfendazole-treated pigs as the authors concluded that pigs with cysticercosis, once treated with oxfendazole, are protected from new infections for at least three months.

Gonzales (1996) conducted a randomized, no treatment-controlled study. They evaluated the efficacy and safety of oxfendazole and praziquantel for the treatment of porcine cysticercosis in 16 naturally infected pigs. Four groups of four pigs were treated with oxfendazole, praziquantel, oxfendazole plus praziquantel, or untreated. Twelve weeks post-treatment the pigs were humanely killed, the number of cyst was counted, and parasite viability was assessed by cyst evagination. They found that praziquantel treatment reduced the number of cysts, but did not decrease the viability of the remaining parasites. Whereas, treatment with oxfendazole alone or oxfendazole plus praziquantel killed all of the parasites, left only microcalcifications in the meat. They concluded that oxfendazole provides a practical, effective, inexpensive, and single-dose therapy for porcine cysticercosis.

CHAPTER III

MATERIALS AND METHODS

3.1 Experiment 1

To determine the risk of *Taenia solium* exposure from pork produced in Punjab, India

3.1.1 Ethics approval

The necessary ethics approval for the collection of information related with the frequency and distribution of pork consumers through surveillance were granted by the Institutional Ethics Committee (IEC), Dayanand Medical College & Hospital, Ludhiana (Approval number: DMCH/R&D/2018/761).

The necessary ethics permission for the inspection of pig carcasses was granted by the Institutional Ethics Committee, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana (Approval number IAEC/2015/97-129).

3.1.2 Risk question

What is the annual probability risk that any one pork meal consumed in Punjab state of India contains at least one viable cyst of *Taenia solium* during the year 2018- 2019.

3.1.3 Risk assessment model

A stochastic quantitative risk assessment model was developed using R statistical program (R statistical package version 3.4.0, R Development Core Team [2015], http://www.r-project.org).

3.1.4 Model specifications

The decision/event tree depicting the chain of events has been presented in Fig. 6.

We used decision tree method and included all the possible ways through which pork meal could reach a pork consumer. Using this decision tree, we defined 15 possible situations based on the field scenario of pig slaughter and pork consumption in Punjab (Table 2, Eq. 1-15).

After this, we estimated probability of each of the above-mentioned situation (Eq. 16) followed by risk of any one pork meal being potentially infective (Eq. 17). Finally, we estimated the total number of people consuming pork (Eq. 18) and number of pork meals consumed per year in the state (Eq. 19) followed by calculation of total number of potentially infected pork meals consumed in one year in Punjab (Eq. 20).

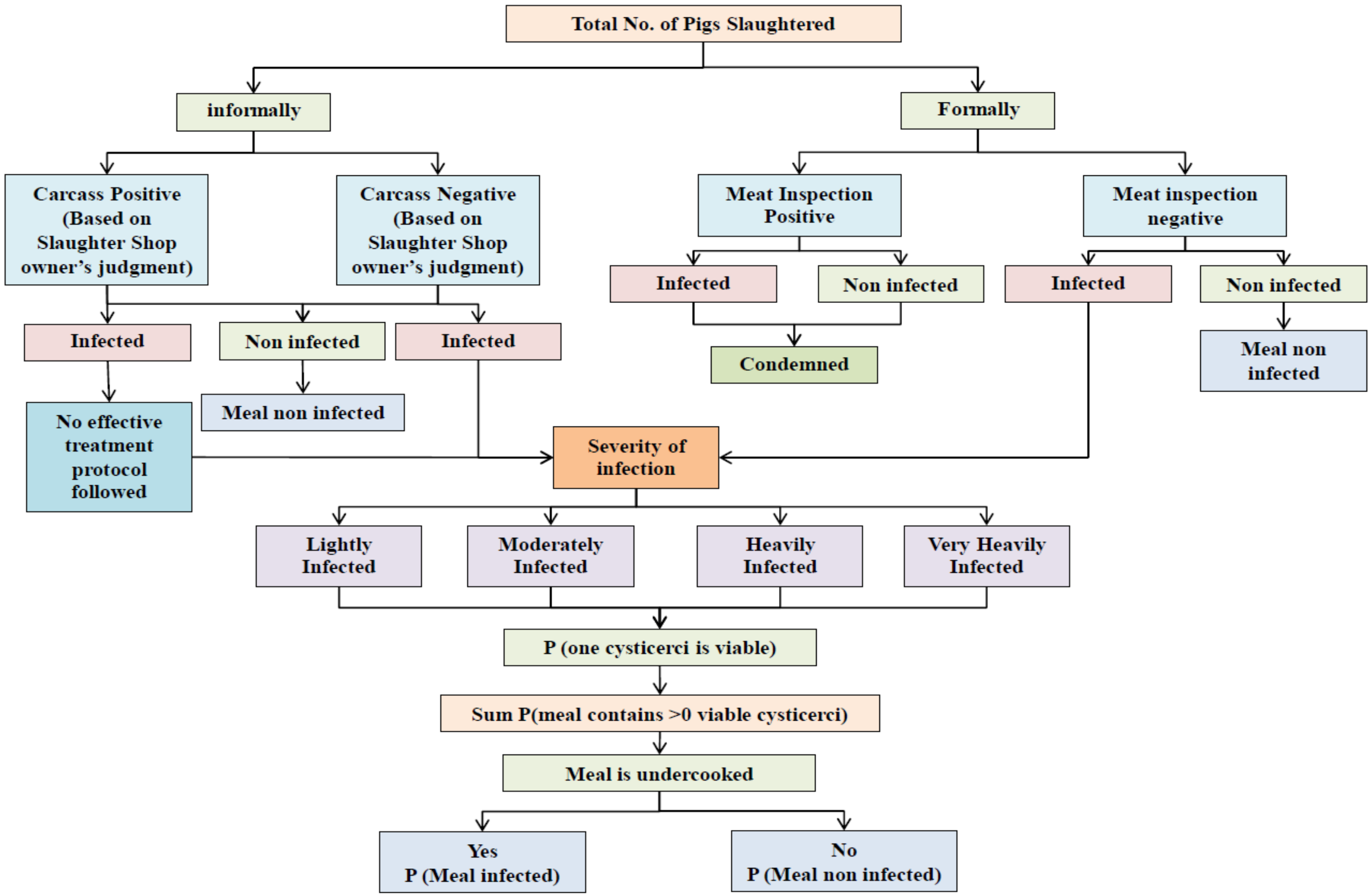

Fig. 6: **Structure of risk assessment model showing situations that any one pork meal consumed in Punjab contains at least one viable cyst of *Taenia solium***

Table 2: Situations used for estimating the annual probability risk that any one pork meal consumed in Punjab state of India contains at least one viable cyst of *Taenia solium* during the year 2018-19

Situations depicting field scenario of pig slaughter and infection status in Punjab			
Situation 1	=	Pig being lightly infected |* Pig is not detected at meat inspection| Pig is formally slaughtered.	... (1)
Situation 2	=	Pig being lightly infected | Pig is detected and condemned at meat inspection | Pig is formally slaughtered.	... (2)
Situation 3	=	Pig being moderately infected | Pig is not detected at meat inspection | Pig is formally slaughtered.	... (3)
Situation 4	=	Pig being moderately infected | Pig is detected and condemned at meat inspection | Pig is formally slaughtered.	... (4)
Situation 5	=	Pig being heavily infected |Pig is not detected at meat inspection | heavily infected |Pig is formally slaughtered.	... (5)
Situation 6	=	Pig being heavily infected |Pig is detected and condemned at meat inspection | Pig is formally slaughtered.	... (6)
Situation 7	=	Pig being very heavily infected |Pig is not detected at meat inspection | Pig is formally slaughtered.	... (7)
Situation 8	=	Pig being very heavily infected |Pig is detected at meat inspection and condemned | Pig is formally slaughtered.	... (8)
Situation 9	=	Pig being not infected |Pig is not detected at meat inspection | Pig is formally slaughtered.	. (9)
Situation 10	=	Pig being not infected |Pig is detected and condemned at meat inspection (false positive) | Pig is formally slaughtered.	. (10)
Situation 11	=	Pig is lightly infected | sold at meat shop | Pig is informally slaughtered.	. (11)
Situation 12	=	Pig is moderately infected | sold at meat shop| Pig is informally slaughtered.	. (12)
Situation 13	=	Pig is heavily infected | sold at meat shop | Pig is informally slaughtered.	. (13)

Situation 14	=	Pig is very heavily infected | sold at meat shop | Pig is informally slaughtered.	... (14)
Situation 15	=	Pig is uninfected |Pig is informally slaughtered.	... (15)
Probability (Pr) of each situation (1-15)			
Pr (situation x)	=	(Pr (slaughter formally/informally) x Pr (infected/not infected) x Pr (lightly/moderately/heavy/ very heavy infected) x Pr (detected/not detected))	... (16)
Risk of any one pork meal being potentially infective			
Pr (any one pork meal is infective at consumption)	=	((Pr (pork meal contains a cyst |Situation x Pr (Situation 1) +……… +Pr (pork meal contains a cyst | Situation 15) x Pr (Situation 15)) x Pr (any one cyst is viable prior to cooking)) x Pr (Meal is undercooked)	... (17)
Number of people consuming pork			
Number of people consuming pork (daily/ weekly/ monthly/ yearly/ special occasions)	=	Population of Punjab x proportion of population consuming pork (daily /weekly /monthly / yearly or on special occasions).	... (18)
Number of pork meals consumed per year			
Number of pork meals consumed per year	=	(number of people consuming pork daily x 365) + (number of people consuming pork weekly x 52) + (number of people consuming pork monthly x 12) + (number of people consuming pork yearly x 1) + (number of people consuming pork on special occasions x 0.5)	... (19)
Number of potentially infected pork meals consumed in one year in Punjab			
Total number of potentially infective pork meals consumed in a period of one year by Punjab population	=	Pr (meal Infective) x (no. of pork meals consumed per year)	... (20)

3.1.4 Model parameters

All the input parameters have been mentioned in Table 3. Beta distributions were applied to account for uncertainty and variability in the pig slaughter probability, prevalence of infection, and probability that infected pig is detected at meat inspection. Uniform distribution was applied for the severity of infection viz. number of cysts per infected pig carcass.

Table 3: Information on the input parameters used to feed the model in the current study

Parameter	Definition	Source	Probability	Distribution (α, ß)	References
P1	Probability pig was slaughtered formally	Total pigs slaughtered in Punjab = 17120 Pigs slaughtered formally = 6923 at Slaughter House, Municipal Corporation Chandigarh*	0.4043 (0.323-0.411)	Beta (6924, 10198)	BAHFS (2017)
P2	Probability pig was slaughtered informally	Pigs slaughtered informally = 10277	0.5956 (0.589-0.602)	Beta (10198, 6924)	BAHFS (2017)
P3	Probability pig is infected (formally slaughter)	Number of pig carcasses inspected=113 Number of carcasses positive for cysts=5	0.0521 (0.023-0.090)	Beta (6, 109)	This study
P4	Probability of pig is not infected (formally slaughter)		1 – P3		
P5	Probability pig is lightly infected (<50cyst) (formally slaughter)	0/5 (see text)	0.1428 (0.009-0.393)	Beta (1,6)	This study
P6	Probability pig is moderately infected (50>100 cysts) (formally slaughter)	0/5 (see text)	0.1428 (0.009-0.393)	Beta (1,6)	This study
P7	Probability pig is heavily infected (100> 500cysts) (formally slaughter)	1/5 (see text)	0.2857 (0.063-0.582)	Beta (2,5)	This study

Parameter	Definition	Source	Probability	Distribution (α, ß)	References
P8	Probability pig is very heavily infected (>500 cysts) (formally slaughter)	4/5 (see text)	0.7142 (0.418-0.937)	Beta (5,2)	This study
P9	Probability pig is infected (informally slaughter)	Number of pig carcasses inspected=1132 Number of carcasses positive for cysts=24 Number of pigs inspected to know the severity of the infection=19	0.0220 (0.015-0.030)	Beta (25, 1109)	Singh *et al* (2018)
P10	Probability of pig is not infected (informally slaughter)		1-P9		
P11	Probability pig is lightly infected (<50cyst) (informally slaughter)	0/19 (19 positive carcasses inspected)	0.0476 (0.003-0.139)	Beta (1,20)	Singh *et al* (2018)
P12	Probability pig is moderately infected (50>100 cysts) (informally slaughter)	0/19 (19 positive carcasses inspected)	0.0476 (0.003-0.139)	Beta (1,20)	Singh *et al* (2018)
P13	Probability pig is heavily infected (100> 500cysts) (informally slaughter)	0/19 (19 positive carcasses inspected)	0.0476 (0.003-0.139)	Beta (1,20)	Singh *et al* (2018)
P14	Probability pig is very heavily infected (>500 cysts) (informally slaughter)	19/19 (19 positive carcasses inspected)	0.9523 (0.861-0.997)	Beta (20,1)	Singh *et al* (2018)

Parameter	Definition	Source	Probability	Distribution (α, ß)	References
P15	Probability infected pig (slaughtered formally) is detected at meat inspection (as currently performed in the study area)	38% (diagnostic sensitivity 38%) 12 out of 31	0.3939 (0.260-0.536)	Beta (13,20)	Singh *et al* (2018), Phiri *et al* (2006)
P16	Probability infected pig (slaughtered formally) is not detected at meat inspection		1-P15		
P17	Probability uninfected pig (slaughtered formally) is detected at meat inspection (false positive)	0% (diagnostic specificity 100%)	0	-	Singh *et al* (2018), Phiri *et al* (2006)
P18	Probability uninfected pig (slaughtered formally) is not detected at meat inspection		1-P17		
P19	Probability of infected pig carcasses sold (formal slaughter)		0		
P20	Probability of infected pig carcasses sold (informal slaughter)	Number of pigs found positive for cysts= 61 Number of infected pig carcasses sold at meat shop including those with ineffective treatment in Punjab= 61	0.9841 (0.953-0.999)	Beta (62,1)	Singh (2018)

Parameter	**Definition**	**Source**	**Probability**	**Distribution (α, ß)**	**References**
P21	Probability anyone meal contains at least one cyst (lightly infected)	number of cysts per pig carcass (lightly infected) = 25.5 (Uniform (1,50)) mean number of meals per pig= 560.747 Mean dressed weight per pig (960000 kg/17120 pigs=56.07 kg) (BAHFS 2017) Assumption of 100gm per person mean portion size	0.0454		BAHFS (2017), Thomas *et al* (2017)
P22	probability anyone meal contains at least one cyst (moderately infected)	number of cysts per pig carcass (moderately infected) = 75.5 (Uniform (51,100))	0.1346	-	BAHFS (2017), Thomas *et al* (2017)
P23	Probability any one meal contains at least one cyst (heavily infected pig)	number of cysts per pig carcass (heavily infected) = 300.5 (Uniform (101,500))	0.5358		BAHFS (2017), Thomas *et al* (2017)
P24	Probability any one meal contains at least one cyst (very heavily infected pig)	number of cysts per pig carcass (very heavily infected) = 1245 (Uniform (526,1964))	2.2202		BAHFS (2017), Thomas *et al* (2017)
P25	Probability any one cysticercus is viable	Total cysts counted= 12151 Number of viable cysts= 6581	0.5415 (0.534-0.549)	Beta (6582, 5571)	Singh *et al* (2018)

Parameter	Definition	Source	Probability	Distribution (α, ß)	References
P26	Proportion of population consuming daily (among pork consumers)	Total number of people surveyed= 900 Total number of pork eaters= 117 Population consuming pork daily=6	0.0588 (0.028-0.098)	Beta (7,112)	This study
P28	Proportion of population consuming weekly (among pork consumers)	Population consuming pork weekly= 25	0.2184 (0.159-0.283)	Beta (26,93)	This study
P29	Proportion of population consuming monthly (among pork consumers)	Population consuming pork monthly= 39	0.3361 (0.267-0.409)	Beta (40,79)	This study
P30	Proportion of population consuming yearly (among pork consumers)	Population consuming pork yearly= 39	0.3361 (0.267-0.409)	Beta (40,79)	This study
P31	Proportion of Punjab population consuming pork on special occasions (among pork consumers)	Population consuming pork on special occasion= 8	0.0756 (0.040-0.119)	Beta (9,110)	This study
P32	Probability pork eaten undercooked	Population consuming undercooked= 2	0.0252 (0.007-0.052)	Beta (3,116)	This study
P33	Estimated human population of Punjab	2,77,43,338	Fixed value	-	Census of India (2011)

3.1.5 Sources of data

The data on number of pigs slaughtered, mean dressed weight per pig and total human population were taken from official records (see table 2 for details). Data on prevalence and intensity of *T. solium* cysticercosis in informally slaughtered pigs, diagnostic sensitivity and specificity of meat inspection, probability that any cysticercus is viable were collected through review of national and international published literature (see table 2 for details).

The missing data for the prevalence of *T. solium* cysticercosis in formally slaughtered pigs was generated by inspecting 113 pig carcasses at a formal slaughterhouse (MC slaughter Hall Chandigarh) in Punjab followed by counting of the number of cysts in the positive carcases.

Some small slaughter shops reported to store infected pork at -4 or -20 C for 24 hours in a deep freezer/refrigerator (see table 2 for details). However, the meat was only considered non-infected if it was stored for 4 days at -5 C, three days at -15 C, one day at -24 C (Sotelo *et al* 1986).

The missing data on frequency and intensity of pork consumption in Punjab were generated by collecting information through a structured questionnaire (see Appendix 1). Information included food consumption habits (veg/non-veg), pork consumption (y/n), cooking preferences of pork and frequency of pork consumption.

3.1.6 Sensitivity analysis

Sensitivity analyses were carried out to assess the impact of the input parameters on the main output probability that any one pork meal is infective at consumption. Spearman rank order correlation coefficients (rho/ρ-value) were calculated using R statistical program (R statistical package version 3.4.0, R Development Core Team [2015], http://www.r-project.org). This ρ-value illustrates the relationship between the input parameters and the output of interest. The correlation illustrated as the ρ-value near 0 the input has little effect on the output whereas at -1 or +1 the input fully influences the output.

3.1.7 Frequency and intensity of pork consumption in Punjab

3.1.7.1 Sample size estimation

Assuming that 50% of the subjects in the population have the factor of interest, for estimating the expected proportion with 5% absolute precision and 95% confidence, the sample size was estimated to be of 385 participants.

3.1.7.2 Data collection

In brief, detailed information from 922 (rural and urban participants) human subjects was collected. Rural subjects were surveyed in a public gathering (Pashu Palan Melas) at GADVASU, Ludhiana. There was a huge diversity of people belonging to different rural regions of Punjab, different economic class and also their education status. Urban subjects were surveyed in different marketplaces of Ludhiana for the collection of unbiased information. During the survey, subjects were randomly selected by selecting a person after an interval of 10 mins at a fixed location.

Data was compiled in an excel spreadsheet (Microsoft office Excel 2010). Continuous variables were assessed for normality in the data. Descriptive analysis was performed for all variables by keeping pork consumption as a response variable (Khatkar and Dhand 2014). The continuous variable age was not normally distributed and hence was log transformed.

Univariable logistic regression was performed using two logistic regression models (a) association of sex, age, education and residence (Rural/Urban) with the Pork consumption habits of the individuals (b) association of sex, age, education and residence (Rural/Urban) with the non-vegetarian/ vegetarian.

Multivariable logistic regression models were constructed to further understand the association between predictor and response variables.

The analysis was facilitated by “The R language and environment (R Development Core Team, 2006) using Rcmdr (R commander) package”.

3.2 Experiment 2

To evaluate the effectiveness of oxfendazole in reducing the prevalence of porcine cysticercosis in India

This study was conducted to understand the effectiveness of oxfendazole in reducing porcine cysticercosis.

3.2.1 Ethics approval

This study was ethically approved by Committee for the Purpose of Control and Supervision of Experiments on Animals, Animal Welfare Division, Ministry of Environment, Forest and Climate Change, Government of India through Institutional Ethics Committee, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana.

3.2.2 Study area and population

The current study was performed in a slum area located in a peri-urban area Haibowal, Ludhiana (co-ordinates: Longitude 75.8156, Latitude 30.9233). Pig raising is common in this area and pigs are allowed to roam and scavenge to meet their dietary requirements. There is a nullah that flows through this area and it is likely to be contaminated with human excreta. In addition, all the inhabitants residing in that area do not have access to toilets and pigs consuming human excreta is not uncommon. The study population consisted of 4-month-old pigs raised in this area that are allowed to roam/scavenge at least some parts of the day.

3.2.3 Sample size estimation

Assuming a prevalence of 10.41% in stray pigs (Chawhan *et al* 2015), and a 5 times reduction in prevalence to 2.0%, a sample size of 20 pigs per group was estimated, using 95% level of confidence and a power of 0.29 (https://www.stat.ubc .ca/~rollin /stats/ ssize/b2.html). The experiment was designed at a lower power as a preliminary experiment and investigations revealed that most of the scavenging pig owners are illiterate and their response rate to participate (for a period of 8 months) in this study is very low. Note that a sample size of 101 pigs per group is required at a power of 0.80 (https://www.stat.ubc.ca/~rollin/stats/ssize/b2.html).

3.2.4 Experimental study design

We conducted a three-armed randomized block control trail starting with zero day and follow-up assessments carried out for piglets at the age of 4 months, 9 months and 12 months in all the three groups – treatment 1 (T1), treatment 2 (T2) and control (C) group. In brief, we used pig litters/farms as blocks to control for others external factors and pigs were randomly assigned into different treatment (s) and control groups in different blocks. Overall, 60 pigs were selected belonging to 10 pig litters/farms. We assigned 20 piglets into all the three groups. These pigs were monitored for a period of 8 months. The inclusion criteria included 4-month-old pigs reared in the study area and allowed to scavenge for some part of the day. The exclusion criteria included pigs dewormed with any anthelmintic before the start of this study. Pigs belonging to a particular farm/litter were allocated in different groups randomly using a random number generator.

The Group 1 piglets (n=20) were treated with oxfendazole @ 30mg/kg body weight at the age of 4 months (T1), Group 2 piglets were treated at the age of 9 months (T2) whereas the third group (C) received placebo (empty capsules filled with glucose) at the age of 4 months. In brief, Oxfendazole 2200mg tablet was crushed and the drug was given based on the weight of the pig. Pigs were properly restrained, the tablet was dissolved in water and drenched into the animal's mouth by a 20ml syringe and ensured for the complete delivery of the drug (Figure 10).

Epidemiologic information related to number of animals in the herd, duration pigs are allowed to scavenge, sex and age of the animal and group to which animal was classified (T1, T2 or C) were recorded. Farmer experience (years) in pig rearing was also recorded.

The flow diagram of the study is shown in Figure 7.

3.2.5 Blood Collection

For identification of piglets, ear tags were applied to all the pigs involved in this study (Figure 8). Blood collection was carried out in all the three groups in three sampling rounds at 4, 9 and 12 months of age. Blood was collected from cephalic vein (Figure 9) into a vacutainer tubes and allowed to clot at room temperature. Later the serum samples were obtained by centrifugation, collected in to eppendorf tubes and stored at -20°c until use.

3.2.6 Sandwich antigen ELISA

The serum samples were analysed using commercially available Cysticercosis Antigen ELISA kit (Bio-X Diagnostic's, Rochefort, Belgium). It is an immunoenzyme test for detecting the viable cysticerci of various *Taenia spp.* and the test is done for antigenic diagnosis of cysticercosis from blood serum.

The detailed protocol of Antigen ELISA is as below:

- ✓ Preparation of samples
    - Serum samples, positive control and negative control were diluted to half-strength in the TCA solution (150 µl of serum sample and 150 µl TCA solution were taken in an eppendorf tubes and were mixed well on a vertex) (Figure 4).
    - Incubate tubes for 20mins at room temperature, Vortex again.
    - Tubes were centrifuged at 12000g for 10mins.
    - 150 µl of the supernatant obtained after centrifugation was transferred to a new set of labelled eppendorf tubes. Neutralizing buffer was added to each eppendorf to make the final dilution of 1:4.
- ✓ Took the microplate out of its packaging.
- ✓ Samples and controls were distributed the across the microplate at the rate of 100µl per well.
- ✓ Covered the lid and incubated the plate at 21+/- 3°C for 1 hour on a microplate shaker at 700-800rpm.
- ✓ Rinsed the plate with the washing solution prepared as instructed in the "composition of the kit". Repeated the washing procedure for two more times.
- ✓ Conjugate was added to each well at the rate of 100µl per well, covered the lid and incubated at 21+/- 3°C.
- ✓ After incubation is done plates were washed for three times using washing solution.

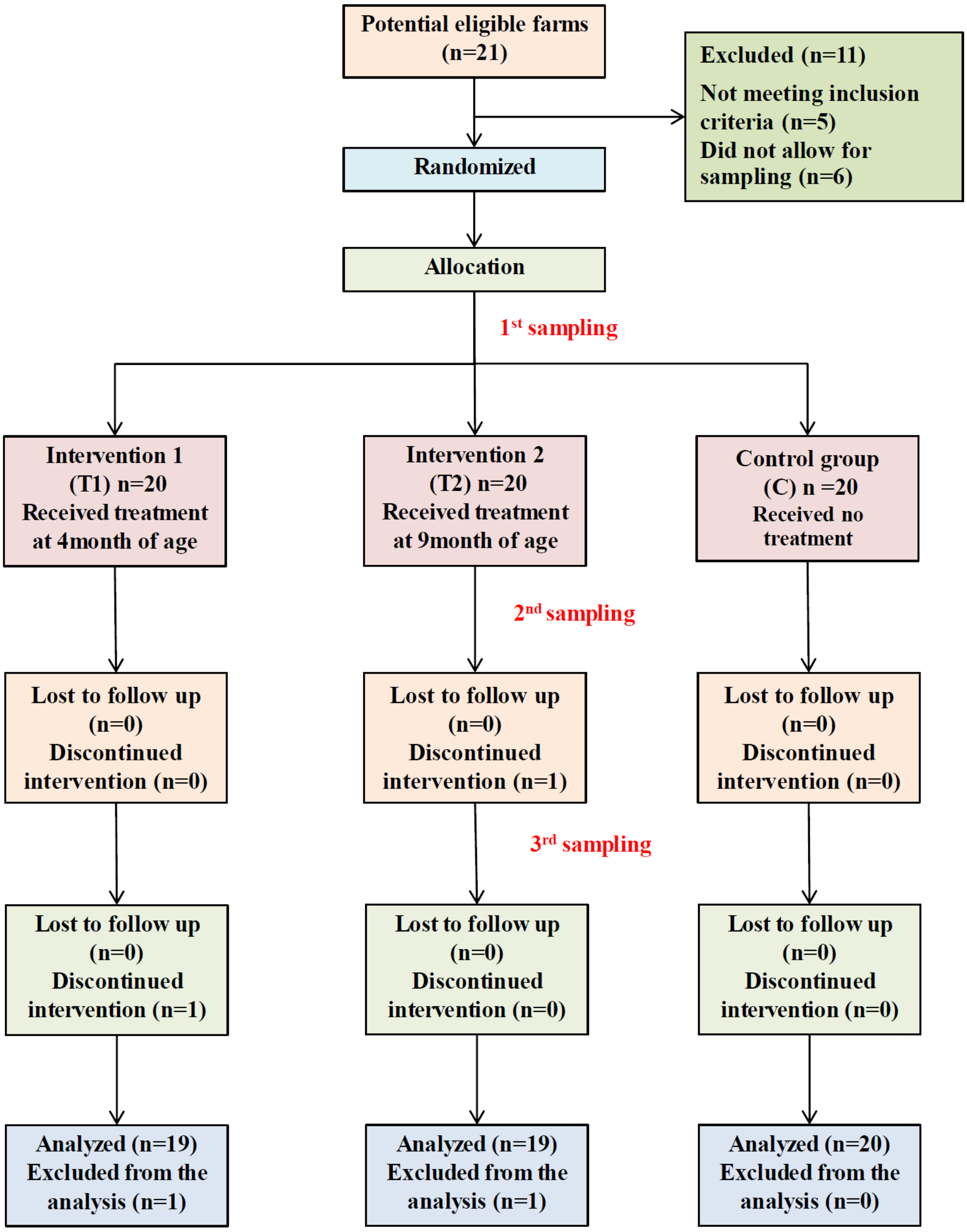

Figure 7: Study design to assess the effectiveness of oxfendazole in reducing the prevalence of porcine cysticercosis in India

Figure 8: Ear tagging for identification

Figure 9: Blood collection

Figure 10: Drenching diluted tablet suspension

- ✓ 100µl per well chromogen solution was added. Incubated at 21+/- 3°C, uncovered and in dark for 10mins.
- ✓ At last to stop the reaction stop solution 50µl per well was added.
- ✓ Optical densities were recorded using spectrophotometric plate reader and a 450nm filter.

3.2.7 Interpretation of Antigen ELISA

Net optical density (delta OD) of each sample and positive control was calculated by subtracting from each reading from the OD of the negative control. Then the delta OD of each sample is divided by delta OD of positive control and multiplied the results by 100 to express it in percentage.

$$\text{Net Optical density (delta OD)} = \text{Each reading} - \text{OD of negative control}$$

$$\text{Value (S/p)} = \frac{\text{Delta OD sample} \times 100}{\text{Delta OD positive control}}$$

The samples with value (S/P) (>11%) were considered positive, value (>7% and <= 11%) considered doubtful whereas value (<7%) considered negative.

3.2.8 Descriptive analysis

Data was compiled in an excel spreadsheet (Microsoft office Excel 2010). Continuous variables were assessed for normality in the data. The variables such as farmer experience in pig rearing, herd size, and total hours pigs are allowed to roam/scavenge in a day (24 hours) were not normally distributed and were transformed into categorical variables. Descriptive analysis was performed for all variables (Khatkar and Dhand, 2014).

3.2.9 Conditional logistic regression

The conditional logistic regression was performed, facilitated by R statistical program (R statistical package version 3.4.0, R Development Core Team (2015). The association between each explanatory variable with the outcome variables of all the three groups were assessed in conditional logistic regression. The association was considered significant if the p-value is <0.05 (based on likelihood ratio test). The outcome variable was test status (seropositive or negative) of a pig during the third sampling (at 12 months of age) tested using antigenic sandwich ELISA. Explanatory variables included number of animals in the herd, duration pigs are allowed to scavenge, sex and age of the animal, farming experience (in years) and the group to which animal was classified (T1, T2 or C). The matching was indicated by the strata variable farm/herd ID.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Experiment 1

To determine the risk of *Taenia solium* exposure from pork produced in Punjab, India

The probability of each of the 15 situations described within the risk assessment model is presented in Table 4.

Using the QRA, we estimated that there is a probability of 0.0242 (95% CI, 0.0079-0.0783) that any one pork meal contains at least one cyst before cooking (Figure 11). The probability that any one pork meal containing at least one viable cyst in Punjab before cooking was found to be 0.0121 (95%, 0.0039- 0.0391) (Figure 12). Probability of any one pork meal being infective after cooking at consumption was found to be is 0.0003 (95%, 0.000-0.0018) (Figure 13).

We record that there are 13,51,56,823 pork meals consumed during the year 2018-2019 in Punjab. Overall, we found that there are 41,260 (95%, 1611-313369) potentially *T. solium* infective meals consumed during 2018-2019 in Punjab state of India (Figure 14).

We found a very low probability of any one pork meal being infective after cooking at consumption from pork produced in Punjab. On the other hand, Thomas *et al (*2017) found a probability of 0.006 of containing a viable cyst (after cooking) per pork meal consumed in Western Kenya. This comparatively higher probability of viable cyst in pork meals of Western Kenya is mostly due to the undercooked pork consuming practices in the Kenyan population (98/1386). The low probability in our study can be contributed to the fact that pork consumers of Punjab mostly consume cooked pork meals.

In Punjab, total number of infective pork meals consumed in a year from 13,51,56,823 total pork meals was very low when compared to 22,282 infective meals consumed by a smaller population 2,30,253 of Busia district, Western Kenya. This huge variation in the number of the infective meals consumed over a year in Busia district was addressed to the inefficient meat inspection which is avoiding only 1397 infective meals per year (Thomas *et al* 2017).

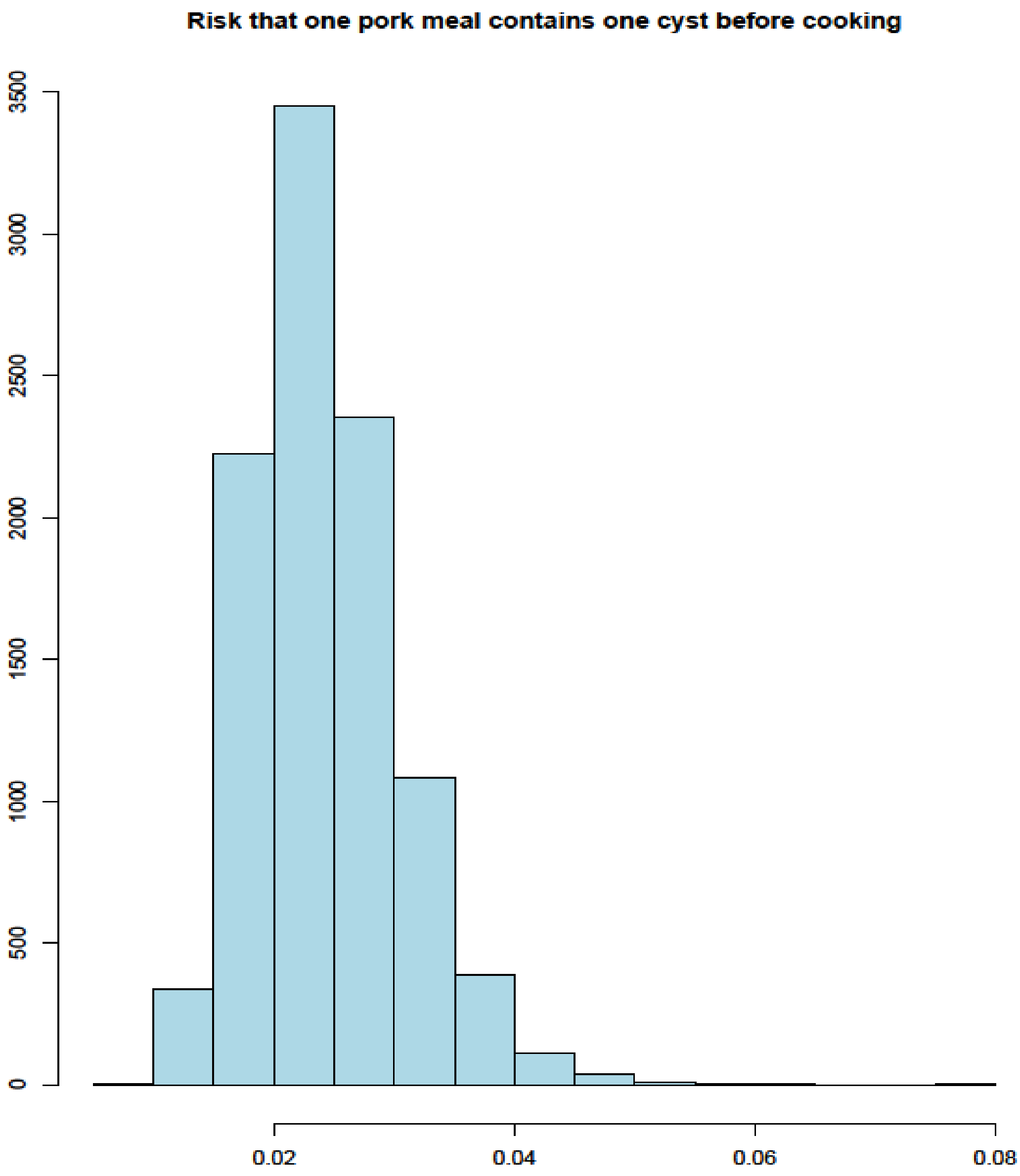

Figure 11: Histogram demonstrating the risk of containing at least one cyst before cooking

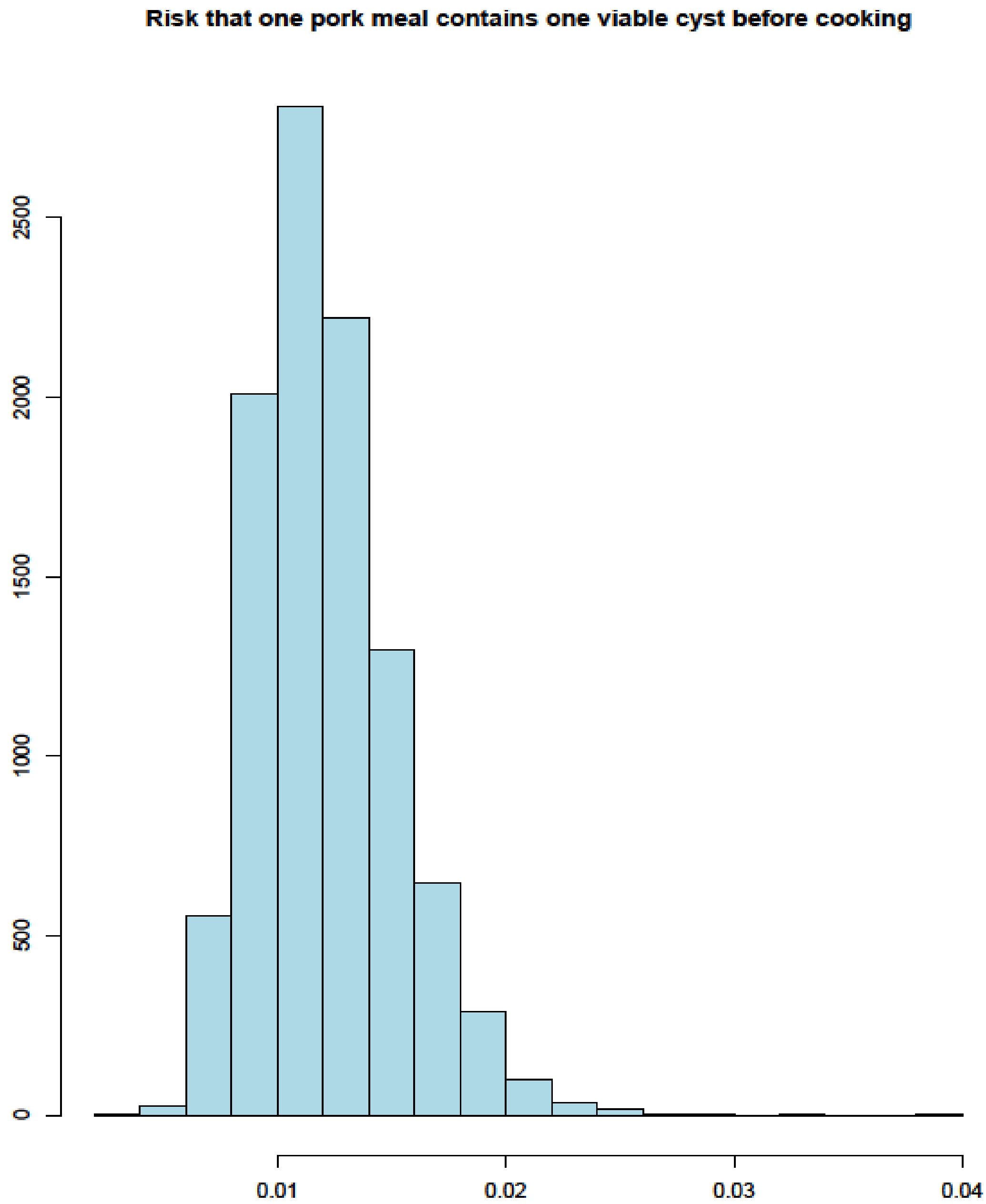

Figure 12: Histogram showing probability that one pork meal contains at least one viable cyst

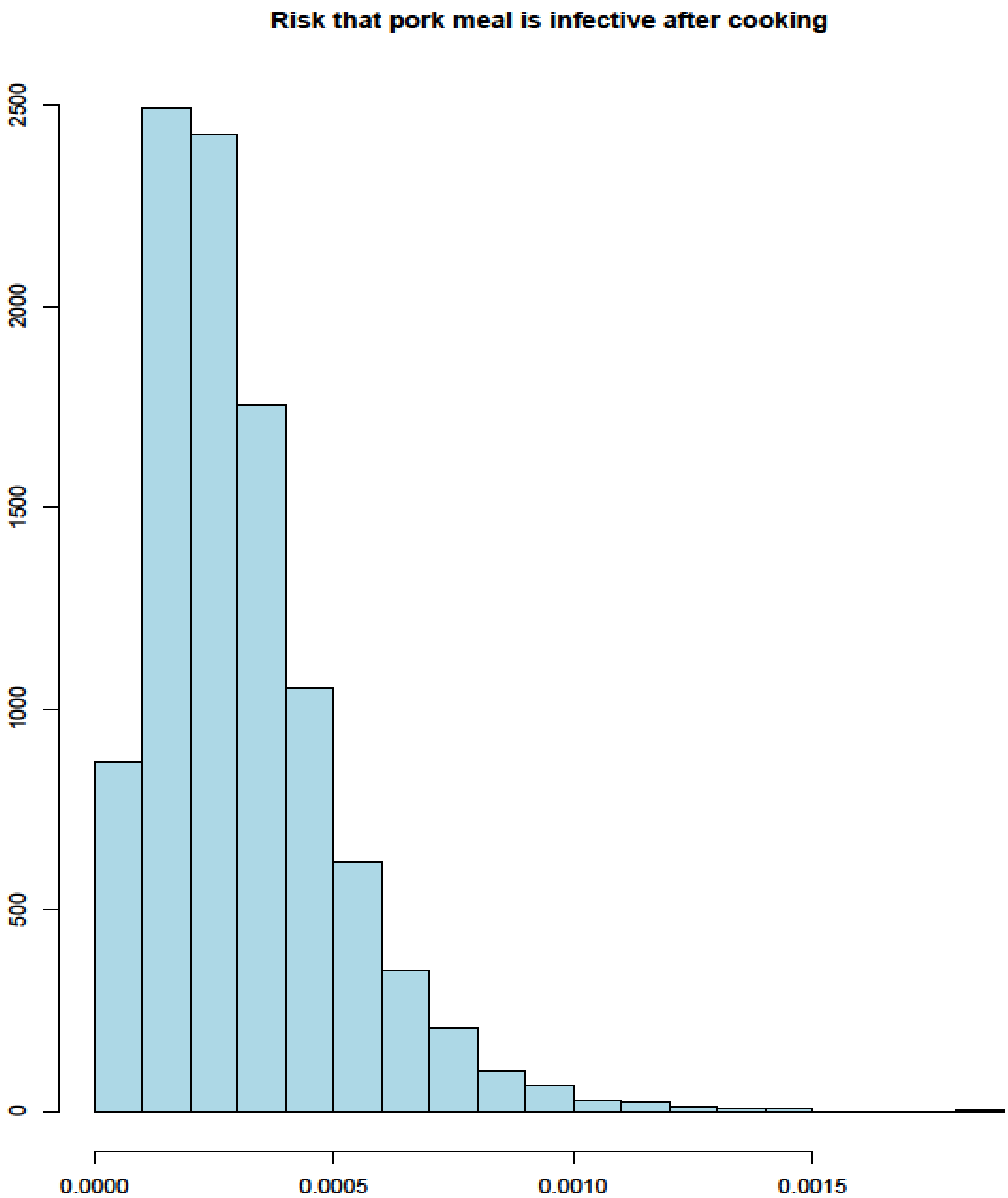

Figure 13: Histogram showing probability that pork is infective after meal cooking.

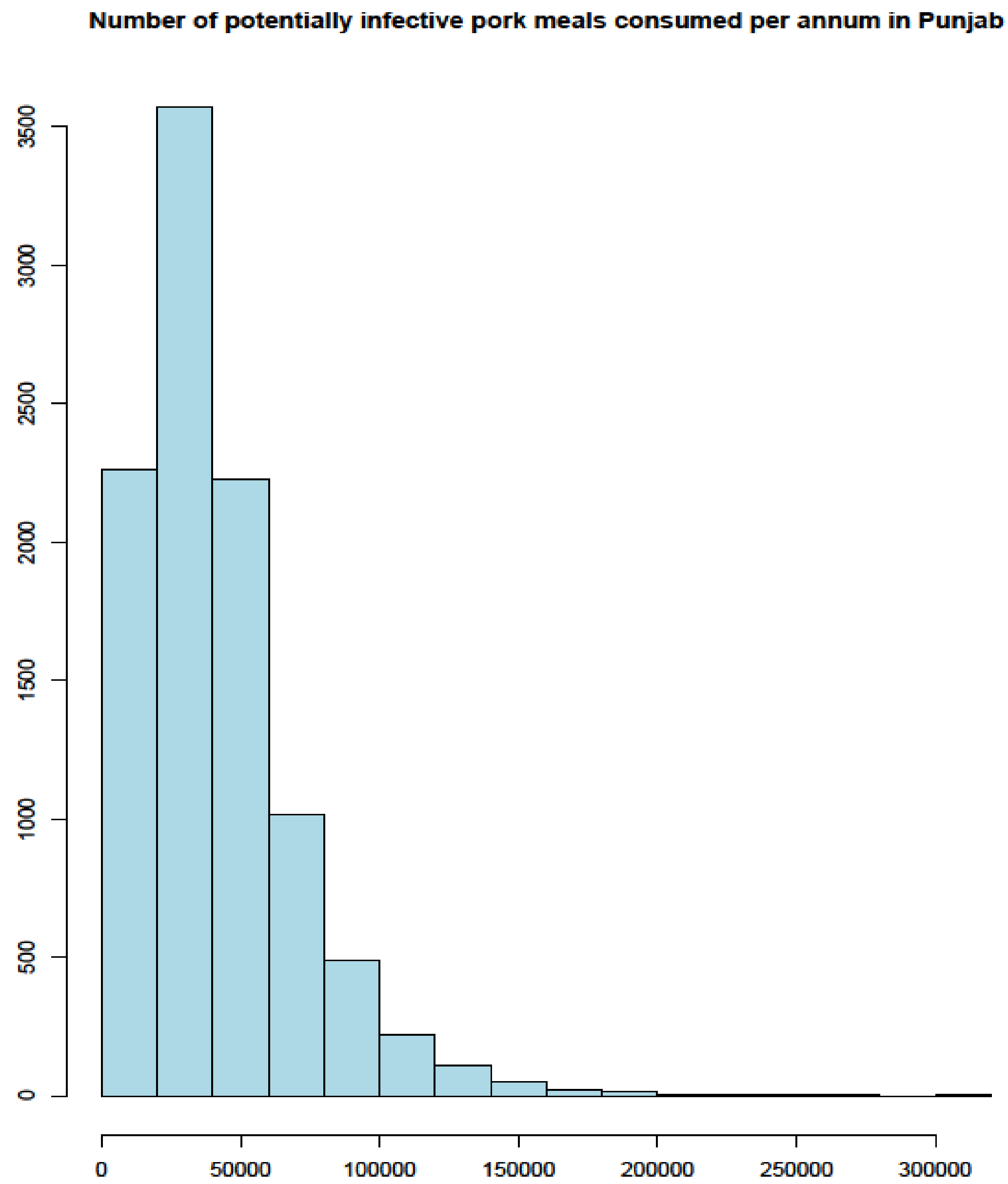

Figure 14: Histogram showing number of potentially infective meals consumed per annum in Punjab

Table 4: Probabilities of each situation described in the model

Situation	Probability	95% Uncertainty Interval
Situation 1 = Pig being lightly infected |* Pig is not detected at meat inspection| Pig is formally slaughtered.	0.00186	(0.0000-0.01785)
Situation 2= Pig being lightly infected | Pig is detected and condemned at meat inspection | Pig is formally slaughtered.	0.00120	(0.0000-0.01285)
Situation 3= Pig being moderately infected | Pig is not detected at meat inspection | Pig is formally slaughtered.	0.00185	(0.0000-0.01863)
Situation 4= Pig being moderately infected | Pig is detected and condemned at meat inspection | Pig is formally slaughtered.	0.0012	(0.0000-0.01223)
Situation 5= Pig being heavily infected |Pig is not detected at meat inspection | heavily infected |Pig is formally slaughtered.	0.00365	(0.00006-0.02655)
Situation 6= Pig being heavily infected |Pig is detected and condemned at meat inspection | Pig is formally slaughtered.	0.00236	(0.0000-0.01671)
Situation 7= Pig being very heavily infected |Pig is not detected at meat inspection | Pig is formally slaughtered.	0.00917	(0.00057-0.0343)
Situation 8= Pig being very heavily infected |Pig is detected at meat inspection and condemned | Pig is formally slaughtered.	0.00594	(0.00028-0.0250)
Situation 9= Pig being not infected |Pig is not detected at meat inspection | Pig is formally slaughtered.	0.3833	(0.3664-0.4094)
Situation 10= Pig being not infected |Pig is detected and condemned at meat inspection (false positive) | Pig is formally slaughtered.	0	0.000

Situation	Probability	95% Uncertainty Interval
Situation 11= Pig is lightly infected | sold at meat shop | Pig is informally slaughtered.	0.00061	0.0000-0.00188
Situation 12= Pig is moderately infected | sold at meat shop| Pig is informally slaughtered.	0.00061	0.0000-0.00502
Situation 13= Pig is heavily infected | sold at meat shop | Pig is informally slaughtered.	0.00062	0.0000-0.0052
Situation 14= Pig is very heavily infected | sold at meat shop | Pig is informally slaughtered.	0.01233	0.004694-0.0243
Situation 15= Pig is uninfected |Pig is informally slaughtered.	0.5731	0.4843-0.5958

4.1.1 Sensitivity Analysis

Spearman's rank correlation coefficient of different inputs is presented in Table 5. There were 4 inputs that had a ρ – values/rho- value (>0.1). These inputs were proportion of population consuming raw pork (ρ=0.9152), probability of pig being infected in formal slaughtered (ρ=0.2731), probability of pig being slaughtered informally (ρ=0.1538) and probability of pig being infected very heavily in formal slaughter (ρ=0.1223). In accordance to the fact that consumption of uncooked or undercooked pork meat is a promoting factor for disease transmission (Sarti and Rajshekar 2003), the sensitivity analysis of present model also showed a very strong positive correlation between raw pork consumption and probability that any one pork meal is infective at consumption. Thomas *et al* (2014) found less association (ρ=0.017) of this input i.e. risk of pork meal being undercooked but with the main output probability that pork meal contains atleast one viable cysticercus at consumption.

Table 5: Spearman's rank correlation of different inputs correlated with output probability of any one pork meal is infective at consumption (after cooking)

Input	rho / ρ – values	p-value	Standard Error
Probability of pig being infected in formal slaughter	0.2731	< 2.2e-16	1.2114e+11
Probability of pig being infected in informal slaughter	0.1538	< 2.2e-16	1.4102e+11
Probability of pig being infected and got detected and condemned during formal slaughter	-0.0694	3.679e-12	1.7824e+11
Probability of pig being very heavily infected in formal slaughter	0.1223	< 2.2e-16	1.4628e+11
Probability of pig being heavily infected in formal slaughter	0.0596	2.451e-09	1.5673e+11
Probability of pig being moderately infected in informal slaughter	-0.0032	0.7456	1.6721e+11
Probability of pig being very heavily infected in informal slaughter	0.0390	9.462e-05	1.6016e+11
Probability of pig being slaughtered informally	0.0036	0.718	1.6606e+11
Probability of pig being heavily infected in informal slaughter	0.0156	0.1179	1.6406e+11
Probability of selling infected pig in meat shop during informal slaughter	0.0189	0.05859	1.6351e+11
Number of cysts per pig which is heavily infected	0.0520	1.869e-07	1.5798e+11
Probability of pig being lightly infected in informal slaughter	-0.0180	0.07039	1.6968e+11
Number of cysts per pig which is moderately infected	0.0059	0.5495	1.6567e+11
Probability of pig being lightly infected in formal slaughter	-0.0040	0.6838	1.6735e+11
Probability of pig being slaughtered formally	0.0045	0.6493	1.6591e+11
Proportion of population consuming raw meal among pork consumers	0.9152	< 2.2e-16	1.4125e+10

4.1.2 Frequency distribution of pork consumption practices

We surveyed a total of 922 subjects. Out of which 383 subjects were vegetarians (41.54%), 539 subjects were non-vegetarians (58.46%). Among 539 non-vegetarians, 117 subjects were pork consumers which accounts for 12.68% of the total subjects. Nine per cent of the subjects were females, and majority were males (91%). Over 0.2169% of the total subjects (n=922) preferred raw meat while 12.47% of the subjects consume cooked pork.

Pork consumers preferences (among pork consumers) included pork consumption daily (5.1282%), weekly (21.36%), monthly (33.33%), yearly (33.33%) and on special occasions (6.83%). Univariate and bivariate descriptive analysis results are presented in Table 6 and 7 respectively.

Table 6: Summary statistics of univariate descriptive analysis of variables examined to understand the frequency distribution of the pork consumers in Punjab

Index	Variable Name	Category	Count	%
1	Sex	Female	83	9.00
		Male	839	91.00
		Total	922	100.00
2	Education	10+2	309	33.51
		10th	204	22.13
		<10	78	8.46
		Graduation	205	22.34
		PhD	1	0.11
		Post-graduation	82	8.9
		Uneducated	42	4.56
		Total	922	100.00
3	Non-Veg	Vegetarian	383	41.54
		Non-vegetarian	539	58.46
		Total	922	100.00
4	Pork consumers	Non-pork consumers	805	87.31
		Pork consumers	117	12.68
		Total	922	100.00

Index	Variable Name	Category	Count	%
5	Eating Preference (Among pork consumers)	Cooked pork consumers	115	98.29
		Raw pork consumers	2	0.017
		Total	117	100.00
6	Frequency of pork consumption (Total population)	Daily	6	0.6507
		Monthly	39	4.2299
		Weekly	25	2.711
		Yearly	39	4.2299
		On special occasions	8	0.8676
7	Frequency of pork consumption (among pork consumers) (Figure 15)	Daily	6	5.1282
		Monthly	39	33.33
		Weekly	25	21.36
		Yearly	39	33.33
		On special occasions	8	6.83
		Total	922	100.00
8	Rural/Urban	Rural	502	54.45
		Urban	420	45.55
		Total	922	100.00

Table 7: Bivariate descriptive analysis of variables sex and residence keeping Pork consumption as the response variable

Category of Sex		
	Non-Pork consumers	**Pork consumers**
Female	78	5
Male	727	112
Total	805	117
Category of Rural/Urban		
	Non-Pork consumers	**Pork consumers**
Rural	412	90
Urban	393	27
Total	805	117

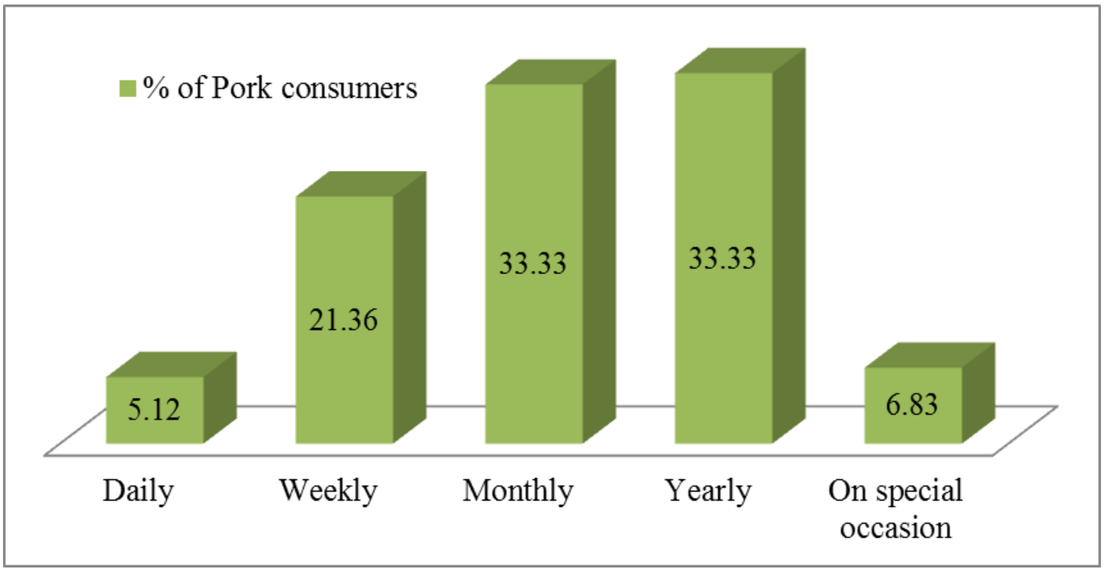

Figure 15: Bar graph showing the frequency of pork consumption among pork consumers in Punjab

There is scarcity of data on the number of pork consumers in India. Thomas *et al* (2017) mentioned that 7.07% (98/1386) pork eaters preferred undercooked pork consumption, 0.7088% (15/2116) of population consume pork daily, 16.304% (345/2116) consume pork weekly, 38.185% (808/2116) of the population consume pork monthly, 16.398% (347/2116) consume pork yearly whereas 0.4253% (9/2116) preferred pork on special occasions. Indian population meat consumption depends considerably on culture, tradition and urbanization. Pork consumption is more in North-eastern states of India. But total per-capita consumption of pork per month is as low as 0.006 kg (Devi *et al* 2014). When compared to other countries, even undercooked pork consumption is less in India. The frequency of consuming pork in population of Punjab was very less when compared to consumption pattern of population of Busia population (Thomas *et al* 2017).

Univariable results showed a significant association of pork consumption with sex, education and residence. While the univariable analysis of non-vegetarianism was showing significant association with sex, residence and age. Univariable analysis results of outcome variables as pork consumption and non-vegetarianism are presented in Table 8 and 9 respectively.

Multivariable logistic regression performed having non-vegetarianism as the outcome showed significant association between all the three variables sex, residence and age. Multivariable logistic regression results are presented in Table 10.

Table 8: Univarible results performed keeping pork consumption as the outcome variable

Parameter	Categories	Odds ratio	Standard Error	p-value (LR-Test)
Sex	Male	0.035	0.4723	0.035
	Female	Reference	0.4613	
Education	<10	Reference	0.338683	0.009
	10+2	1.31	0.372246	
	graduation	0.81	0.406934	
	Post-graduation	0.93	0.477917	
	10th	0.9933	0.398479	
	Uneducated	0	610.451972	
Residence	Rural	Reference	0.1164	<0.001
	Urban	0.33	0.2276	
Age		1.01	0.700118	0.989

LR-Test: Likelihood Ratio Test; n=922

Table 9: Univarible results performed keeping non-vegetarianism as the outcome variable

Parameter	Categories	Odds ratio	Standard Error	p-value (LR-Test)
Sex	Female	Reference	0.2232	0.001
	Male	2.18	0.2341	
Education	<10	Reference	0.2313	0.713
	10+2	1.01	0.2590	
	graduation	0.94	0.2712	
	Post-graduation	0.78	0.3194	
	10th	0.8	0.2708	
	Uneducated	1.19	0.3965	
Residence	Rural	Reference	0.0894	<0.001
	Urban	1.56	0.1355	
Age		0.18	-3.325	<0.001

LR-Test: Likelihood Ratio Test; n=922

Table 10: Multivariable logistic regression results performed keeping non-vegetarianism as the outcome variable

	OR	Standard Error	P (LR-test)
Age	0.17	0.4936	< 0.001
Sex (Reference : male)	3.2	0.2507	< 0.001
Residence (Reference : Urban)	1.9	0.1462	< 0.001

4.2 Experiment 2

To evaluate the effectiveness of oxfendazole in reducing the prevalence of porcine cysticercosis in India

We conducted a randomised block controlled trial to evaluate the effectiveness of oxfendazole in reducing the prevalence of porcine cysticercosis.

A total of 60 pigs, consisting of 20 (33.33%) for each group were enrolled. Sixty per cent of the pigs were male, majority (58.33%) of the pigs belong to farms with small herd size (<13). Pigs selected were from 10 different farms, majority of the owners had pig rearing experience <12 years (68.33%). All pigs were sampled thrice (at the age of 4, 9 and 12 months) except 2 pigs that were lost to follow up after first sampling. Descriptive analyses of pigs involved in this study have been mentioned in Table 11.

The most stringent way of determining whether a cause-effect relation exists between the intervention and the outcome is randomised control trial (RCT) (Sibbald 1998). RCT evaluating an intervention can provide a strong evidence of a cause-effect relation if one exists if the RCT is well designed and methodologically sound; it is therefore powerful in changing practice to improve patient outcome, this being the ultimate goal of research on therapeutic effectiveness (Kendall 2003).

4.2.1 Sandwich antigen ELISA

A total of 4 samples were found positive using antigen ELISA. Interestingly, all the 3 pigs found positive in treatment 1 (T1) group at 4 months of age (first sampling) became seronegative at 9 months of age (during second sampling).

However, one pig in the control group was found sero-positive at 9 months of age (during second sampling). Summary of ELISA results are presented in Table 12.

Our study showed a base-line prevalence of 5% at 4 months of age. A recent study conducted by Singh *et al* (2018) found *T. solium* prevalence of 3.69% in pigs produced in Punjab. Chawhan *et al (*2015) found prevalence of *T. solium* 4.23% in which stray/ scavenging pigs showed higher prevalence of 10.41% than farm pigs with a prevalence of 0.61%.

Table 11: Summary statistics for variables examined for pigs treated with oxfendazole followed by their *T. solium* seropositivity (Antigen ELISA test status) along with their controls

RBCT to see effectiveness of oxfendazole (n=20 pairs)						
Variable	**Treatment 1 (T1)**		**Treatment 2 (T2)**		**Control (C) group**	
	n	**%**	**n**	**%**	**n**	**%**
Experience of farmer in pig rearing (in years)						
Less than 12	12	60.00	16	80.00	17	65.00
More than 12	8	40.00	4	20.00	3	35.00
Total	20	100.00	20	100.00	20	100.00
Herd size						
Less than 13	10	50.00	14	70.00	14	55.00
More than 13	10	50.00	6	30.00	6	45.00
Total	20	100.00		100.00	20	100.00
Animal let out for roam/scavenging (in hours)						
Less than 2	13	65.00	18	90.00	18	80.00
More than 2	7	35.00	2	10.00	2	20.00
Total	20	100.00	20	100.00	20	100.00
Sex						
Female	9	45.00	6	30.00	6	45.00
Male	11	55.00	14	70.00	14	55.00
Total	20	100.00	20	100.00	20	100.00

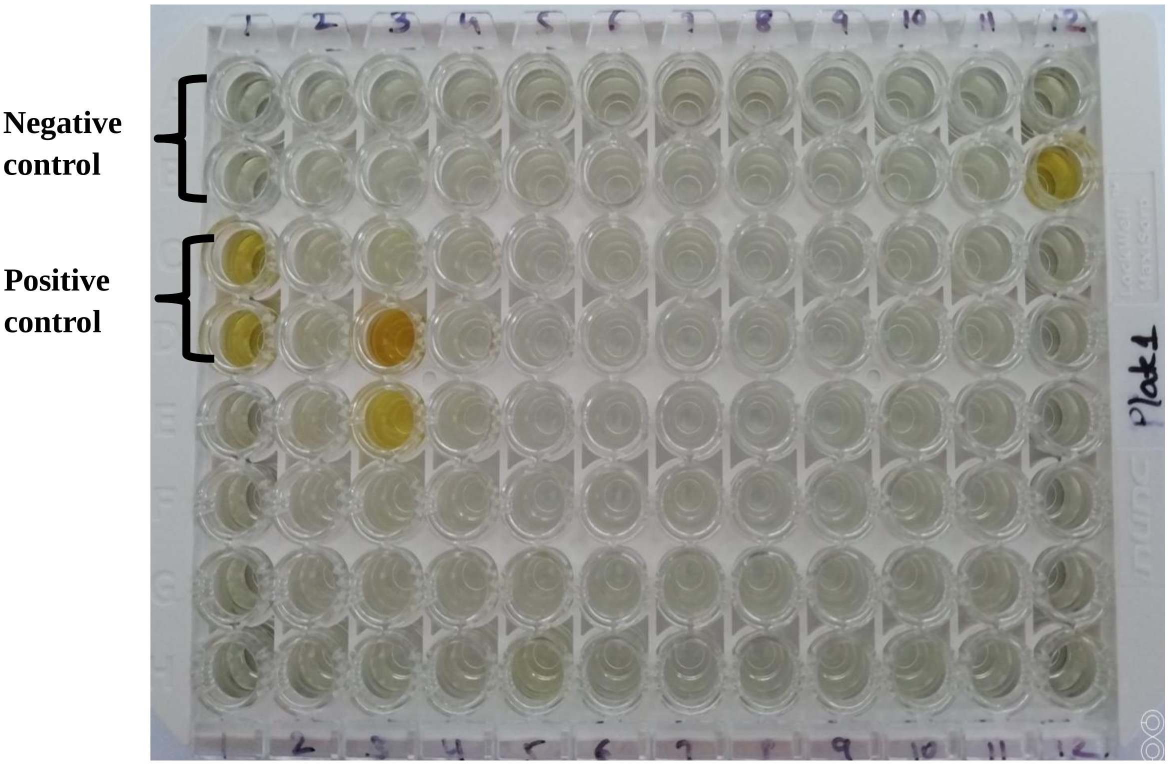

Figure 16: Sandwich antigen ELISA for the detection of viable cysticerci of *Taenia* species in pig serum samples (A1 and B1: Negative control; C1 and D1: Positive control)

Table 12: Seropositivity status of pigs enrolled in treatment and control groups in this study using sandwich antigen ELISA used for the detection of viable cysticerci of *Taenia* species

Group	Treatment 1 (T1)			Treatment 2 (T2)			Control (C)		
Sampling	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Positive	3	0	0	0	0	0	0	1	1
Negative	17	19	19	20	19	19	20	19	19
Lost to follow-up	0	1	1	0	1	1	0	0	0

4.2.2 Conditional logistic regression analysis

None of the pig was detected sero-positive in both the treatment groups at 12 months of age, whereas one pig was detected sero-positive in the Control group when tested at 12 months of age. This indicates effectiveness of oxfendazole in controlling *T. solium* cysticercosis in endemic areas. Results of conditional logistic regression have been presented in Table 8. However, no statistically significant associations were recorded between outcome variable (test status at the third outcome) and different risk factors as mentioned in Table 13. This might be due to smaller sample size used in the current study.

We also had certain limitations. Pigs could not be slaughtered at the end of study; therefore, accurate infection status could not be ascertained. We also had a lower sample size due to a low response from pig farmers.

Many other studies have also confirmed effectiveness of oxfendazole in controlling *T. solium* cysticercosis in pigs. For example, Pondja et al. (2012) found oxfendazole to be effective to control porcine cysticercosis. Gonzalez et al (1997) also confirmed the safety and efficacy of a single dose of 30 mg/kg oxfendazole for the treatment of porcine cysticercosis. However, it has been stated that oxfendazole cannot be a stand-alone approach because in high endemic areas a certain number of animals will get infected after treatment and before slaughter (Pondja *et al* 2012).

The current study indicates that oxfendazole is effective in controlling *T. solium* infection in pigs in Punjab. However, the effectiveness should be further explored by conducting studies with a larger sample size and post-mortem inspection at the end of the study.

Table 13: Conditional logistic-regression analysis of 20 oxfendazole treated/non-treated pig case-control pairs (Outcome: Pig test status (seropositive or negative) at 12 months of age; stratum variable: Farm/herd ID)

Factor	Beta	Standard error (Beta)	Odds ratio
Model 1 (Treatment status)			
Control	Referent	-	1.0
Group T1	-22.4	54800	$1.869 \times (10)^{-10}$
Group T2	-22.4	54800	$1.869 \times (10)^{-10}$
Likelihood ratio test=2.36, p=0.308			
Model 2 (Sex)			
Female	Referent	-	1.0
Male	20.8	41800	$1.079 \times (10)^{9}$
Likelihood ratio test=0.97, p=0.324			
Model 3 (time pig allowed to roam/scavenge in a day)			
Less than 2 hours	Referent	-	1.0
More than 2 hours	0.594	$1.13 \times (10)^{8}$	1.81
Likelihood ratio test=0, p=1			
Model 4 (Pig rearing experience)			
Less than 12 years	Referent	-	1.0
More than 12 years	1.06	$1.00 \times (10)^{8}$	2.886
Likelihood ratio test=0, p=1			

*95% CI: Inestimable

CHAPTER V

SUMMARY AND CONCLUSIONS

Taenia solium is an endemic disease in developing countries such as India and its persistence is increased by certain cultures, socio-economic and sanitary standards. Taeniosis is a neglected zoonotic disease causing huge monetary losses and disability-adjusted life years (DALYs) in India. *T. solium* has several characters which make it vulnerable for control but due to low economic status of developing countries it is being difficult to follow.

Disease control depends on the interruption in the transmission cycle especially in the definitive host humans. Humans acquire infection by consuming undercooked or raw infective meat. Hence meat inspection and proper cooking of pork before consumption are two important measures to break transmission to humans. Another approach is to control disease in pigs by treating them with effective anthelmintic and preventing the exposure of pigs to human faeces or contaminated feed and water. Addressing both the control strategies the present study was designed to know the risk of *T. solium* infection in pork produced in Punjab and to know the effectiveness of oxfendazole in controlling the disease in pigs reared in Punjab.

Quantitative risk assessment of *T. solium* in pork produced in Punjab was performed in R statistical program. Input parameters were taken from official records, through review of national and international published literature whilst the missing data was collected through surveillance at Muncipal slaughterhouse, Chandigarh.

The QRA model predicted that there is a probability of 0.0242 (95% CI, 0.0079-0.0783) that any one pork meal contains at least one cyst before cooking. The probability that any one pork meal containing at least one viable cyst in Punjab before cooking was found to be 0.0121 (95% CI, 0.0039- 0.0391). Probability of any one pork meal being infective after cooking at consumption was found to be is 0.0003 (95% CI, 0.000-0.0018).

We record that there are 13,51,56,823 pork meals consumed during the year 2018-2019 in Punjab. Overall, we found that there are 41,260 (95% CI, 1611-313369) potentially *T. solium* infective meals consumed during 2018-2019 in Punjab state of India.

A questionnaire based study was conducted to know the culinary practices and pork consumption frequencies of pork consumers in Punjab and to estimate the risk factors associated with the pork consumption in Punjab. A detailed information from 922 (rural and urban participants) human subjects was collected. Rural subjects were surveyed in a public gathering (Pashu Palan Melas) at GADVASU, Ludhiana. Urban subjects were surveyed in different marketplaces of Ludhiana for the collection of unbiased information. Out of 922 subjects surveyed 383 subjects were vegetarians (41.54%), 539 subjects were non-vegetarians (58.46%). Among 539 non-vegetarians, 117 subjects were pork consumers which accounts for 12.68% of the total subjects. 9% of the subjects were females, majority were males (91%). Out of 922 subjects, only 0.2169% of the subjects preferred raw meat while 12.47% of the subjects consume cooked pork.

Univariable results showed a significant association of pork consumption with sex, education and residence. The univariable analysis of non-vegetarianism was showing significant association with sex, residence and age. Multivariable logistic regression performed having non-vegetarianism as the outcome showed significant association between all the three variables sex, residence and age.

To evaluate the effectiveness of oxfendazole in pigs reared in Punjab a randomized control trail was conducted. Three groups consisting of two treatment groups and a control group were randomly allotted with 20 pigs each. The pigs allotted in treatment 1 (T1) group were treated with 30mg/kg b.wt oxfendazole at 4 months of age; treatment 2 (T2) group pigs were treated at 9 months of age with 30mg/kg b.wt oxfendazole whereas the pigs present in control (C) group were treated with placebo at 4months of age. All the three groups were sampled thrice at 4, 9 and 12 months of age and checked for seroprevalence.

None of the pig was detected sero-positive in both the treatment groups at 12 months of age, whereas one pig was detected sero-positve in the Control group when tested at 12 months of age. This indicates effectiveness of oxfendazole in controlling *T. solium* cysticercosis in endemic areas. However, no statistically significant associations were recorded between outcome variable (test status at the third outcome) and different risk factors. This might be due to smaller sample size used in the current study.

CONCLUSIONS

- There is a very low risk of getting exposed to potentially *T. solium* infective meals in Punjab, as the frequency of pork consumers that prefer eating undercooked/raw pork is very low.
- Absence of meat inspection is an important risk for the entry the infected meat in the food chain.
- Oxfendazole is an effective drug in controlling *T. solium* infection in piglets reared in Punjab, however further studies with a larger sample size are required to prove statistical significance of this study.

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ANNEXURE

School of Public Health and Zoonoses,
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All the information from this survey will be kept confidential. Your name will not be used in any published materials like reports and papers. Your participation is entirely voluntary and you can choose to stop answering questions at any time.
Do you want to participate in the survey?

Yes, I want to participate in this survey. Signature:

Date:

Sample ID:

DEMOGRAPHICS

1. Name:

2. Address: Postal Code:

3. Residence area: Urban ☐ Rural ☐

District ☐ Tehsil ☐

4. Contact no.

5. Age:years ☐

6. Sex: Male ☐ Female ☐

7. Education: Post Graduation ☐ Graduation ☐

10+2 ☐ Matriculation ☐

PERCEPTION AND PRACTICES

1. Are you: Vegetarian ☐ Non-vegetarian ☐

FORPORK CONSUMERS

1. If non-vegetarian, do you consume pork: Yes ☐ No ☐

2. If Yes, do you prefer eating: Cooked ☐ Lightly Cooked ☐ Raw ☐

3. How frequently you consume pork: Daily ☐ Weekly ☐

Monthly ☐ yearly ☐ On special occasion ☐

Thank you for filling this questionnaire.

VITA

Name of the student	:	Megha G. K
Father's name	:	Mr. Gopalakrishna G.K.
Mother's name	:	**Mrs. Annapoorna K.S.**
Nationality	:	**Indian**
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EDUCATIONAL QUALIFICATION

Bachelor degree	:	**B.V.Sc. & A.H.**
University	:	**KVAFSU, Bidar**
Year of Award	:	**2017**
OCPA	:	**7.54/10.00**
Master's degree	:	M.V.Sc.
OCPA	:	8.04/10.00
Awards/Distinctions/ Scholarships	:	National Talent Scholarship (ICAR)