

**PREVALENCE AND RELATED RISK FACTORS OF PORCINE  
CYSTICERCOSIS AND AFRICAN SWINE FEVER IN SELECTED  
URBAN/PERI-URBAN AREAS OF MOROGORO, TANZANIA**

**BY**

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN  
APPLIED MICROBIOLOGY OF SOKOINE UNIVERSITY OF  
AGRICULTURE.  
MOROGORO, TANZANIA.**

**2012**

**ABSTRACT**

This study was carried out to estimate the prevalence and related risk factors of porcine cysticercosis (caused by *Taenia solium*) and African swine fever (ASF) in domestic pigs, and assesses the state of pork inspection in urban/peri-urban areas of Morogoro region, Tanzania, between November 2010 and January 2011. A two stage random sampling was employed. A total of 260 live pigs were tested serologically. Serum samples were tested for the presence of circulating parasite antigen using a monoclonal antibody-based sandwich enzyme-linked immunosorbent assay (Ag-ELISA) and indirect ELISA (Ab-ELISA) for porcine cysticercosis and ASF, respectively. In addition, a questionnaire survey to collect information on pig production, occurrence and awareness of porcine cysticercosis and African swine fever, risk factors for both diseases was conducted in the selected households from which pigs were sampled. A total of 18 pork traders were also interviewed to collect information on the status of pork inspection. Four pigs (1.54%: 95%CI=0.04–3.1) were found positive by the Ag-ELISA with no statistical significant differences by age group ( $P=0.57$ ), while ASF antibody titre detection revealed no specific ASF antibody response in all 260 pigs. This study recommends further extensive surveillance aiming at monitoring porcine cysticercosis dynamics in urban/peri-urban pig farming so that more baseline information can be available not only for research purposes but even for design and implementation of long term control strategies. It is recommended that surveillance and control of ASF outbreak in future should focus on the active monitoring, early detection and effective quarantine measures at the point of ASF occurrence.

**DECLARATION**

I, **Isaac Josred Makundi**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and has neither been nor concurrently being submitted for a degree award in any other University.

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Date

The above declaration is confirmed by:

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(Supervisor)

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Date

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## ACKNOWLEDGEMENTS

First of all, I am grateful to my Almighty God for keeping me alive and in a good state until the end of this work. Secondly, I acknowledge the financial support from DANIDA) extended to me via the Peri-Urban Livestock Farming Project without forgetting the assistance from the Belgian Technical Cooperation (BTC) for sponsoring my studies. My great and sincere gratitude is accorded to my supervisors: Prof. M. V. Johansen, Prof. J. P. Nielsen (from the University of Copenhagen, Denmark) and Dr. H. A. Ngowi of the Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture (SUA), for their tireless efforts, advice, constructive criticism and guidance. I extend my sincere thanks to pig keeping households in Morogoro Urban district for their willing participation in my research study and more so by allowing me to sample their pigs. Of great assistance were the Boma, Bigwa, Kingolwira and Mazimbu wards livestock field officers. I am also indebted to Mr. A. Manyesela, of the Department of Veterinary Microbiology and Parasitology, SUA, for his great cooperation and support during the sample collection in the field. I acknowledge with thanks the great cooperation and technical support received from the Department of Virology, Animal Disease Research Institute (ADRI) – Temeke, Dar-es-salaam during the sample analysis for African swine fever – Indirect ELISA. I am very grateful to Dr. C. S. Sikasunge and Mr. C. Mwelwa of the School of Veterinary Medicine, University of Zambia, for helping and guiding me through the analysis of pig serum samples using antigen–Enzyme Linked Immunosorbent Assay (Ag-ELISA). Lastly, I would like to recognize and appreciate the roles played in one way or another by all those who are not mentioned in this part.

**DEDICATION**

This Thesis is dedicated first and foremost to my wife Mrs. Eliakunda M. Makundi. Thank you for your special support, care and encouragement. Secondly, special dedication goes to my Parents Mr. and Mrs. J. E. Makundi for taking good care of me since the childhood and be able to take me to school up to the University level.

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**LIST OF ABBREVIATIONS AND SYMBOLS**

%	Percentage
°C	Degrees Celsius
µm	Micrometer
$\chi^2$	Chi-square
<i>df</i>	Degree of freedom
mm	Millimetre
nm	Nanometre
Ab	Antibody
Ag	Antigen
ADRI	Animal Disease Research Institute
ASF	African swine fever
BTC	Bilateral Technical Cooperation
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
cm	centimetre
CISA-INIA	Centro de Investigacion en Sanidad Animal-Instituto Nacional de Investigacion y tecnologia agraria y Alimentaria

CPE	Cytopathic effect
DANIDA	Danish International Development Agency
DNA	Deoxyribonucleic Acid
ELISA	Enzyme -Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FAT	Fluorescent Antibody Test
HA	Haemadsorption
IB	Immunoblotting
IFA	Indirect fluorescent Antibody
Kbp	Kilo base pair
Km	Kilometre
MWLD	Ministry of Water and Livestock Development
OIE	Office International des Epizooties
OPD	Ortho-phenylenediamine
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
TCA	Trichloroacetic acid

## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Background information

Peri-urban pig farming as used here refers to farm units close to cities and/or towns which operate intensive or semi intensive systems to raise pigs. Most of pigs in Tanzania are raised by parastatal organizations, seminary schools and colleges, as well as individuals. Although its contribution to the national economy is still rather low, the pig industry is becoming an increasingly important backyard activity for a number of Tanzanian families (Kambarage *et al.*, 1990). It is now realised that pig production is one of the main profitable means of household income and family subsistence. Major constraints to increased pig production include poor nutrition, limited market access, lack of efficient pig extension services, farmer's lack of knowledge of pig management and diseases such as African swine fever, mange mites and helminths.

This study focused mainly on two important pig diseases namely, porcine cysticercosis and African swine fever.

#### 1.2 Porcine cysticercosis

*Taenia solium* is an important helminth zoonosis as the pig is an intermediate host and man is a definitive host. Humans can also serve as intermediate hosts if they consume *T. solium* eggs (Mutua *et al.*, 2007). In addition to its public health importance *T. solium* causes great economic losses in the pig industry due to

condemnation of carcasses found to have cysts. The life cycle of *T. solium* occurs mostly in environments with low socio-economic or low hygiene levels, deficient sanitary facilities, or poor pig rearing practices where pigs can gain access to human faeces. In northern Tanzania, porcine cysticercosis has been reported in the northern highlands with prevalence ranging from 0.3 – 13.3% in slaughter pigs (Nsengwa and Mbise, 1995; Boa *et al.*, 1995) and 3.2 – 46.7% (average 17.4%) in smallholder pig-farming villages based on lingual examination (Ngowi *et al.*, 2004a). In southern regions of the country, prevalence ranging from 5.5 – 16.9% has been reported based on the lingual examination (Boa *et al.*, 2001; Boa *et al.*, 2006).

The sensitivity of the lingual palpation method to detect porcine cysticercosis has continuously been questioned. Even though this method requires technical expertise; it is of low sensitivity, approximately 21% (Dorny *et al.*, 2004) and is capable of detecting *T. solium* cysts only in heavily infected pigs. Few cases have been reported in pigs during routine meat examination.

However, the assessment of the true burden of the disease has been hindered by the high cost of more sensitive and specific diagnostic tools necessary for collection of reliable epidemiological data (Mutua *et al.*, 2007). Therefore, there is a strong need for development of a simple and cheap pen-side test with high sensitivity and specificity for diagnosis of porcine cysticercosis.

### 1.3 African swine fever

African swine fever (ASF) is a highly contagious disease of domestic pigs with a wide range of clinical forms varying from hyper/acute to chronic or in-apparent form. African swine fever virus (ASFV) is an icosahedral cytoplasmic DNA virus that infects pigs and soft ticks of the *Ornithodoros* genus. ASFV has variable pathogenicity in domestic pigs, with infections ranging from being highly lethal to subclinical. Currently, there is no vaccine for ASF (Gallardo *et al.*, 2009). In northern Tanzania a cross-sectional serological survey conducted on domestic pigs in seven geographical and administrative districts of northern highlands using indirect enzyme-linked immunosorbent assay (ELISA) reported that 14.9% of the 94 farms surveyed had pigs with antibodies against ASF virus. The overall sero-prevalence to ASF antibodies in pigs was found to be 7 % among 313 pigs. The level of sero-positive farms ranged from 0-80% and prevalence between districts ranged from 0-17.4% (Swai *et al.*, 2005).

Antibodies are usually not detected in pigs infected with highly virulent ASFV as the pigs die before the antibodies are produced. Antibodies are produced in pigs infected with low or moderately virulent ASF viruses, but these have been observed not to be fully neutralising antibodies (OIE, 2008). In Tanzania, ASF has become enzootic in domestic pigs and therefore sero-surveillance could be the method of choice in detecting surviving carriers. Otherwise, due to the fact that pig has a short life span (farrowing to slaughter) the detection of passive transfer of specific anti-ASF antibodies and its persistence in exposed and subsequent generations is of paramount significance. Currently, the Office International des Epizooties (OIE)-approved

assays for ASFV-specific antibody determination, consisting of an initial screening of sera by enzyme-linked immunosorbent assay (OIE-ELISA), followed by an Immunoblotting assay or indirect fluorescent antibody test to confirm the results for samples with doubtful and false positive results.

Antiabong *et al.*, (2007) observed that in domestic pigs, vertical transmission plays a central role in the epizootics of the disease specifically in cases when the antigens are presented to the foetus before development of the immune responses and in this regards immune responses to these particular antigens would therefore be minimal. This might enable the antigen to persist indefinitely in the animal.

Therefore, in view of the above facts estimation of specific ASFV antibodies is of significant importance not only to provide the baseline information about the disease sero-prevalence but also the risk factors related to the domestic pig cycle of the disease.

#### **1.4 Problem statement and justification**

In Tanzania, there is very little information so far about the status of pig diseases in various areas of the country even though the pig industry is growing tremendously. For example, the national pig population in 1980 was estimated at 205 000 pigs, in 2 000 was 370 000 and currently more than 1 200 000 pigs (FAO 2002; MWLD, 2006). Usually ASF occurs as an outbreak and since the disease is endemic in the country most of pig keepers and traders have continuously incur great loss whenever the disease occurs. Little information is currently known about the role of domestic

pig and soft tick cycle in the epidemiology of the disease, and this has been due to the very little serological surveillance which needs to be routinely done. In fact, much emphasis has been focused to combat the disease only during the outbreaks.

Porcine cysticercosis has been extensively studied in rural settings where there is big number of pig populations. There is little information about the prevalence of the disease in urban/peri-urban areas in spite of an increase in the number of pigs and pig keepers in both urban and peri-urban areas, and transportation of pigs from rural to urban areas which could introduce infections in these areas.

## **1.5 Objectives:**

### **1.5.1 Main objective**

Assess the prevalence of porcine cysticercosis and African swine fever in selected urban/peri-urban areas of Morogoro, Tanzania.

### **1.5.2 Specific objectives**

- i) Estimate the prevalence of cysticercosis among domestic pigs by using antigen ELISA;
- ii) Estimate specific ASF viral antibodies by using antibody ELISA;
- iii) Identify risk factors associated with the occurrence of porcine cysticercosis and ASF in urban/peri-urban pig farming.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Porcine cysticercosis

Porcine cysticercosis, OIE list B disease is an important parasitic zoonosis infecting both pigs and human beings. The disease cause few symptoms in the pigs except in massive infection. Sarti *et al.*, (1992) observed that massive infection in pigs is facilitated by their coprophagic habits. In the life cycle of this parasite, humans are the definitive hosts harbouring the adult tapeworm in the small intestine, whereas pigs are the common intermediate hosts (Fig. 1). However, humans can also harbour the cystic stage after inadvertently swallowing *T. solium* eggs (Soulsby, 1982; Flisser and Gyorkos, 2007).

##### 2.1.1 Aetiological agent

Porcine cysticercosis is a disease in pigs caused by the larval form of a tapeworm, *Taenia solium*. Pigs usually get infected by eating contaminated food containing human faeces with *T. solium* eggs and human get infected by eating undercooked pork and /or accidental swallowing of *T. solium* eggs (Soulsby, 1982).

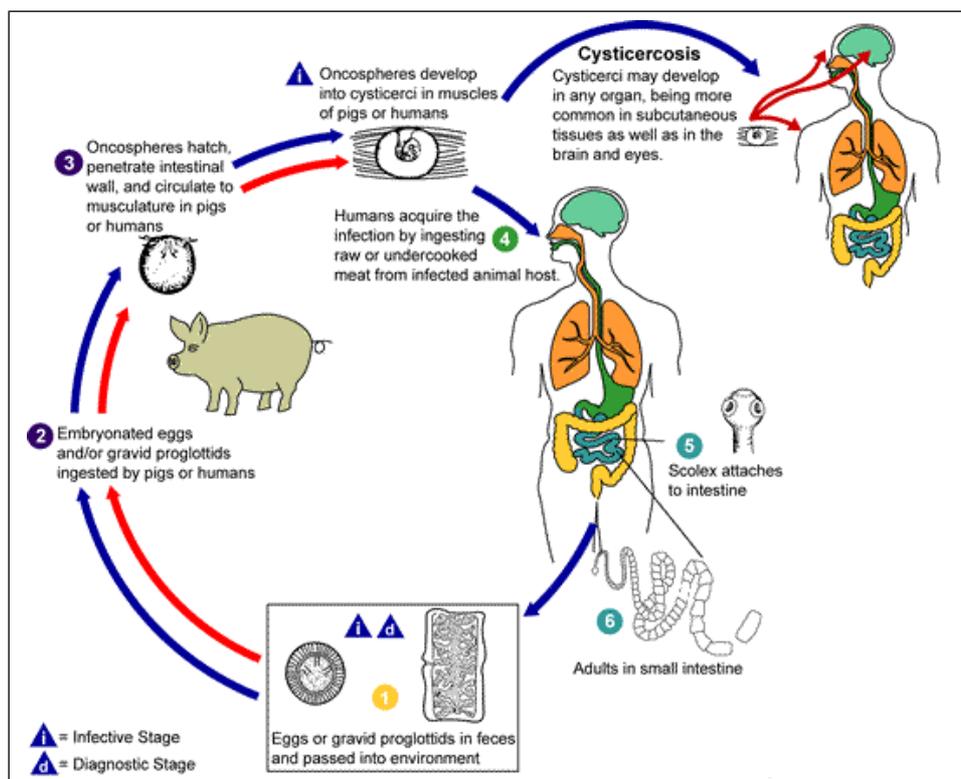


Figure 1: Life cycle of *Taenia solium*

Source: Centers for Disease Control and Prevention

[[www.dpd.cdc.gov/Cysticercosis](http://www.dpd.cdc.gov/Cysticercosis)]

## 2.1.2 Diagnosis of porcine cysticercosis:

### 2.1.2.1 Lingual and post mortem (meat inspection) examination:

In many African countries porcine cysticercosis has routinely been diagnosed by lingual and post-mortem examination.

With lingual examination pig need to be snared and a mouth should be opened and mouth gag inserted, then the pig's tongue should be extended by holding it with a cotton cloth to enable palpation. A cyst could be defined as any palpable whitish vesicular mass, measuring approximately 1 to 2 mm, on the underside of the tongue (Mutua *et al.*, 2007). Although the method is very specific, it has low sensitivity

(Gonzalez *et al.*, 1990). Dorny *et al.* (2004) has observed that tongue examination has a sensitivity of 21% and specificity of 100%.

Meat inspection is the only diagnostic method carried out on large scale in slaughterhouses for the post-mortem detection of pig cysticercosis. The method is more sensitive to detect dead, degenerated, or calcified cysticerci; but is most likely to miss quite a number of viable cysticerci, as they have the same pinkish-red colour as the meat (Wanzala *et al.*, 2003). The procedure is based on the partial incision and careful observation in the “predilection” sites (sites with higher density of cysts than elsewhere in the carcass): heart, masseters, tongue, and *triceps brachii* (Boa *et al.*, 2002). The technique has demonstrated low sensitivity. In Zambia, meat inspection detected 38.7% (n=31) of total carcass dissection positive pigs (Phiri *et al.*, 2006). Boa *et al.* (2002) showed that routine meat inspection involving visual inspection of incised and intact surfaces of heart, tongue, external and internal masseter muscles, and *triceps brachii* muscles can only reveal 10.6% of the total carcass cysts.

#### **2.1.2.2 Antibody detection methods**

Several methods have been employed to detect antibodies to *T. solium* infections in man and pigs, such as the complement fixation test, hemagglutination, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), dipstick-ELISA, latex agglutination and immunoblot techniques (Ferreira *et al.*, 1997; Garcia and Sotelo, 1991; Ito *et al.*, 1998; Miller *et al.*, 1984; Rocha *et al.*, 2002; Tsang *et al.*, 1989). In developing countries, ELISA is preferred because of its better availability, simplicity and lower cost compared with immunoblot (Rosas *et al.*, 1986). With this

ELISA, antibody responses were detectable in experimentally infected pigs harbouring 16 or more cysts (confirmed at necropsy) from 30 days 80 after experimental infection. The assay appears to be species-specific since pigs naturally infected with the metacestode stages of *T. hydatigena* were negative in ELISA (Sato *et al.*, 2003).

### **2.1.2.3 Antigen detection methods**

Antibody detection has two important drawbacks in clinical settings: (i) it may indicate exposure to infection and not necessarily the presence of an established, viable infection, resulting in transient antibodies (Garcia *et al.*, 2001) and (ii) antibody may persist long after the parasite has been eliminated by immune mechanisms and/or drug therapy (Harrison *et al.*, 1989; Garcia *et al.*, 1997).

Several assays have been developed to detect parasite antigens, but only the monoclonal antibody-based tests directed at defined parasite antigens may ensure reproducibility and specificity (Correa *et al.*, 1989; Harrison *et al.*, 1989; Brandt *et al.*, 1992; Choromanski *et al.*, 1990; Wang *et al.*, 1992; Erhart *et al.*, 2002).

Recently there has been development of new serological technique for detection of *C. cellulosae* called parasite antigen detecting ELISA and the method has already been adapted and used in several regions of Africa including Tanzania (Ngowi *et al.*, 2008), Uganda (Waiswa *et al.*, 2009), Zambia (Sikasunge *et al.*, 2008) and South Africa (Krecek *et al.*, 2008). The method is useful because of its high sensitivity and specificity, 86.7% and 94.7% respectively (Dorny *et al.*, 2004).

### **2.1.3 Cross-reactivity of Ag – ELISA tests**

Sero-epidemiological surveys of porcine cysticercosis that make use of monoclonal antibody – based sandwich ELISAs (Ag – ELISA) pose great challenge in diagnosis of the disease in the field due to cross-reaction with other species in the same genus *Taenia*. Several reports (Dorny *et al.*, 2003; Sato *et al.*, 2003) pointed out that the tests are not species specific and cross-react with *T. hydatigena* and or *T. asiatica* cysticerci in pigs.

## **2.2 African swine fever**

African swine fever (ASF), OIE list A disease is a highly contagious viral disease of domestic pigs responsible for a wide variety of clinical symptoms. In the typical and acute form, the lymphoreticular endothelial cells are affected resulting in widespread haemorrhages. Morbidity and mortality classical approach 100%. In sub acute cases pigs lose condition and die of pneumonia. Chronically survivors are characterized by emaciation, stunted growth, haemorrhagic necrosis of skin overlying bony protuberances, followed by abscessation and deep ulceration (Lubisi, 2005).

### **2.2.1 Aetiological agent**

African swine fever virus (ASFV) presently constitutes the only member of the genus *Asfivirus* within the family *Asfarviridae* (Dixon *et al.*, 2000). ASFV is a large cytoplasmic replicating icosahedral virus that contains a linear double-stranded DNA genome. The genome varies in length between 170 and 190 kbp (Blasco *et al.* 1989). ASFV has similarities with two other groups of cytoplasmic DNA viruses, the Poxviruses and Iridoviruses, but is sufficiently different from these families to be

classified as the only member of a separate virus family and genus (Dixon *et al.*, 2000 and Salas, 1999). It infects vertebrate hosts of the Suidae family and soft argasid ticks of the genus *Ornithodoros* and it is the only known DNA arbovirus.

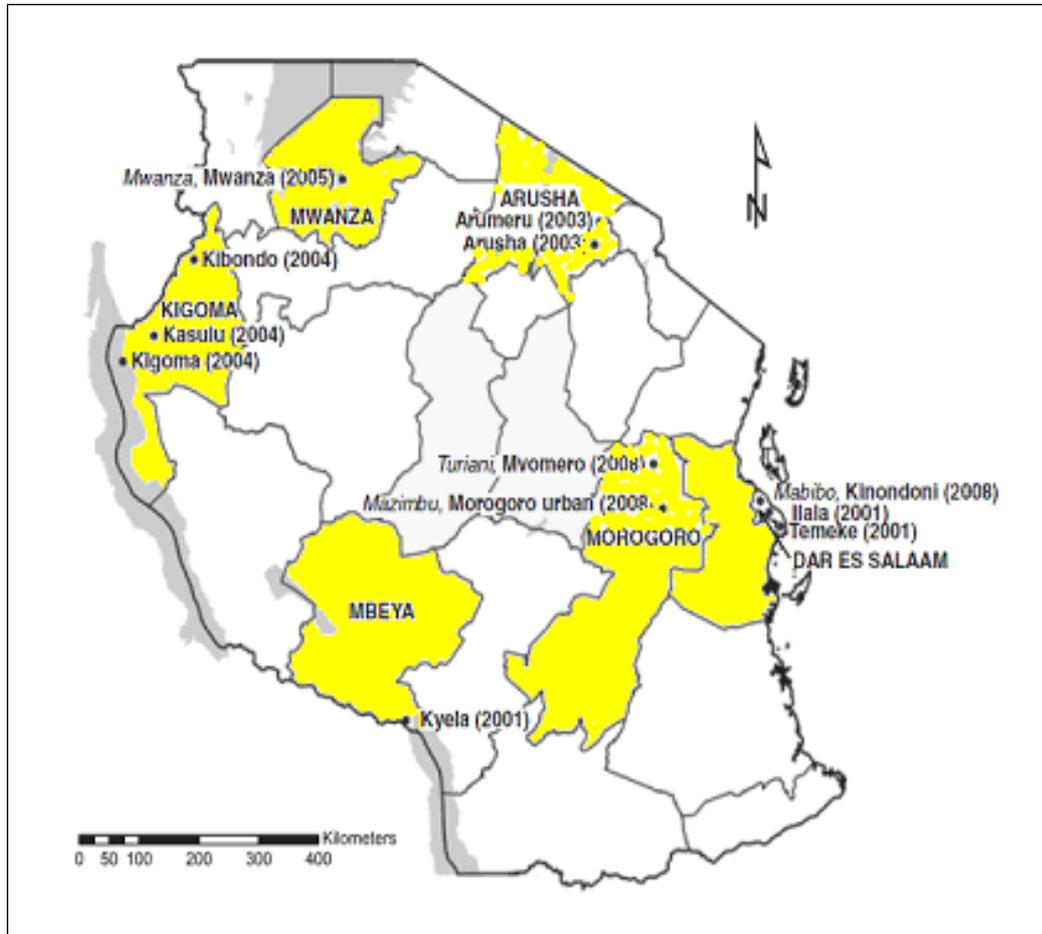


Figure 2: Map of Tanzania showing the regions (indicated by capital letters and yellow colour) where ASF outbreaks have occurred since 2000. ASF outbreaks occurred in Dar-es-salaam (Ilala and Temeke districts) and Mbeya (Kyela district) regions in 2001, Arusha region (Arusha and Arumeru districts) in 2003, Kigoma region (Kasulu, Kigoma and Kibondo districts) in 2004, Mwanza region (Mwanza City) in 2005 and Morogoro (Turiani in Mvomero district and Mazimbu in Morogoro Urban district) and Dar-es-salaam (Mabibo in Kinondoni district) region in 2008.

Source: Misinzo *et al.*, (2011).

## **2.2.2 Laboratory diagnosis of ASF**

Field diagnosis of ASF should be confirmed in the laboratory since the clinico-pathological signs are not pathognomonic for ASF (Lubisi, 2005). Laboratory diagnostic procedures for ASF fall into two groups: the first contains the tests for virus isolation and the detection of virus antigens and genomic DNA, while the second contains the tests for antibody detection. The selection of the tests to be carried out depends on the disease situation and laboratory diagnostic capacity in the area or country.

### **2.2.2.1 Virus isolation**

ASFV is usually isolated by inoculating blood or tissue samples from suspect pigs into pig leukocyte or bone marrow cultures (CFSPH, 2006). The haemadsorption (HA) test is based on the fact that pig erythrocytes will adhere to the surface of pig monocyte or macrophage cells infected with ASFV and that most virus isolates produce this phenomenon of haemadsorption. A positive result in the HA test is definitive for ASF diagnosis. A very small number of 'nonhaemadsorbing' viruses have been isolated, most of which are avirulent, but some do produce typical acute ASF (OIE, 2008). Other tests to detect the ASF virus include animal inoculation, leukocyte culture for the autorosette test and electron microscopic examination of tissues.

### **2.2.2.2 Detection of genome nucleic acids**

Nucleic acids can be detected with a polymerase chain reaction (PCR) assay or by the hybridization of nucleic acid probes to tissue sections. PCR techniques have been

developed, using primers from a highly conserved region of the genome, to detect and identify a wide range of isolates belonging to all the known virus genotypes, including both nonhaemadsorbing viruses and isolates of low virulence. PCR is particularly useful in putrefied samples that cannot be used for virus isolation and antigen detection. A rapid, real time PCR technique using tonsil scraping samples has recently been published. This test can detect the virus a few days before the onset of symptoms (CFSPH, 2006). Restriction fragment length polymorphism (RFLP) can also be used for detecting genome nucleic acids.

### **2.2.2.3 Antigen detection**

ASFV antigens can be found in tissue smears or cryostat sections, as well as in the buffy coat, with the fluorescent antibody test (FAT). The World Organization for Animal Health (OIE) does not consider this test alone to be sufficient for diagnosis, although it is useful in conjunction with other assays (CFSPH, 2006). Therefore The FAT can be used as an additional method to detect antigen in tissues of suspect pigs in the field or those inoculated at the laboratory. Positive FAT plus clinical signs and appropriate lesions can provide a presumptive diagnosis of ASF. It can also be used to detect ASFV antigen in leukocyte cultures in which no HA is observed and can thus identify nonhaemadsorbing strains of virus. It also distinguishes between the CPE produced by ASFV and that produced by other viruses, such as Aujeszky's disease virus or a cytotoxic inoculum. However, it is important to note that in subacute and chronic disease, FAT has a significantly decreased sensitivity. This reduction in sensitivity may be related to the formation of antigen-antibody complexes in the tissues of infected pigs which block the interaction between the

ASFV antigen and ASF conjugate (OIE, 2008). The method is very useful in acute cases of ASF as at this stage the virus is present in large amounts in the blood and in any tissue with a rich blood supply (Lubisi, 2005). Detecting antigens in tissues or blood indicates the evidence that the virus itself is or was present.

#### **2.2.2.4 Antibody detection**

Serology is also useful for diagnosis, particularly in endemic regions. The presence of virus strains with reduced virulence and the resulting presence of asymptomatic infected animals make the serological diagnosis a valuable tool for the control of the disease in affected countries. As a general rule, pigs that survive natural infection develop antibodies against ASFV from 7 to 10 days after infection. These antibodies persist for long periods of time, sometimes for life, perhaps due to continuous antigenic stimulation by the frequent occurrence of persistent infection. Thus, antibody detection is a rational approach to the detection of the subacute and chronic forms of the disease (Gallardo *et al.*, 2009).

Many serologic tests have been developed for the diagnosis of African swine fever, but only a few have been standardized for routine use in diagnostic laboratories. These tests include the enzyme-linked immunosorbent assay (ELISA), immunoblotting, indirect fluorescent antibody (IFA) and counter immunoelectrophoresis (immuno-electro-osmophoresis) tests. The ELISA is prescribed for international trade (CFSPH, 2006). It is also the most sensitive test to detect singular perhaps chronically infected animals. Presence of antibodies in blood

is an indication of animal body reaction to the virus which might either be present or eliminated.

### **2.2.3 ELISA OIE for serological diagnosis for African swine fever**

The enzyme immunoassay ELISA test is a very useful technique widely used for serological diagnosis of ASF. The ELISA method is highly sensitive, specificity indexes high speed and low cost. The technique is based in the use of antibodies or antigens labelled with an enzyme, so the resulting conjugates have enzymatic and immunologic activity. Being one of the components (antigens or antibody) labelled with an enzyme and insolubilized, the antibody-antigen reaction will be immobilized and it is easy develop by addition of specific substrate that could be read in a spectrophotometer.

There are a widely types of ELISA. In case of ASF, it is used indirect ELISA. The antigen is fixed in the plate. Samples with antibodies against ASF will recognise antigen so an antigen-antibody complex will be formed. After that, the conjugate is added and fix the complex. With several washing steps, all material not fixed is removed. Adding substrate the result of the technique can be obtained: develop of colour in wells, indicates ASF antibody presence (CISA – INIA, 2008).

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Study Area

Morogoro region is located on the eastern part of Tanzania Mainland (Fig. 3) and lies between latitudes 5°58' and 10°00' south of the Equator and between longitudes 35°25' and 38°30' east of Greenwich meridian. It is bordered by seven regions. In the north are Tanga and Manyara while in the eastern side are the Coast Region and Lindi regions. On the western there are Dodoma and Iringa Regions while Ruvuma is located in the southern side of the Region. Morogoro Region has a total area of 73 039 km<sup>2</sup> out of which 2 240 km<sup>2</sup> is covered by water. This area makes it the second largest region in Tanzania (the largest is Tabora). The Region covers about 7.7 percent of the total area of Tanzania. Administratively, Morogoro Region is divided into six (6) districts, namely Kilosa, Kilombero, Ulanga, Mvomero, Morogoro Urban and Rural districts. The Districts are subdivided into divisions, wards, villages and streets (for urban wards)/vitongoji (for rural wards). All districts of the Region except Morogoro Urban in 2002 had more population living in rural areas. Morogoro Urban district is different from others in terms of the number of people living in urban areas due to its urban nature, and harbouring the headquarter of the Region.

Morogoro Urban district was the study site (Fig. 3). The Morogoro Urban district is highly urbanised as the district forms the Morogoro Municipality which is one of the major urban localities in the country. The district is the Regional Headquarters of Morogoro region and is located about 195 km to the west of Dar es Salaam City. Its

altitude is around 488 metres above the sea level. Morogoro Urban has a total land area of 531 sq. Km. and lies in a surface enclave formed by two major mountains of Uluguru mountains (south east) and Mindu mountains (on the west), both being part of the Eastern Arc mountains. The highest temperature occurs in November and December, during which the mean temperature ranges between 28 °C and 30 °C. The minimum temperatures are in June and August where the temperature ranges between 14 °C and 16 °C. There are two rainfall seasons in the area. Long rains season occur between March and May (821 – 1 505mm average rainfall) and short rains season (400mm average rainfall) occur between October and December each year. According to the population and Housing Census of 2002, the population of the district was 227 921 people of which 49.61% were women (113 022) and 50.39% were men (114 839) and the growth rate was 4.7% per annum. The population of pigs in the district was estimated to be 2 316 pigs (district livestock records, 2002). Administratively, Morogoro Urban district has only one division, 19 wards and 274 local streets (Fig. 4).

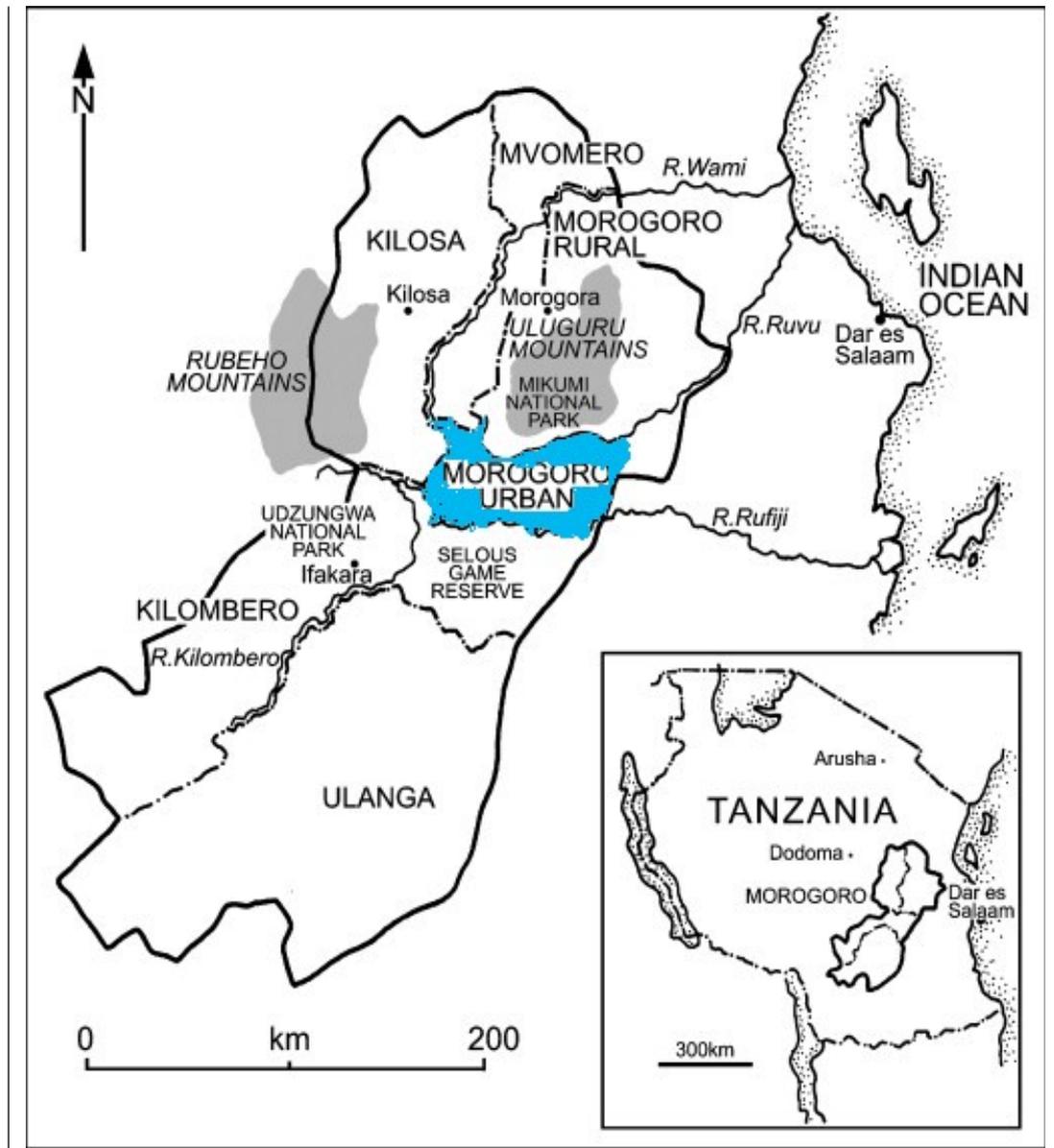


Figure 3: Map of Morogoro Region: Morogoro Urban (coloured blue) was the study site.

Source: Paavola, J. (2008).

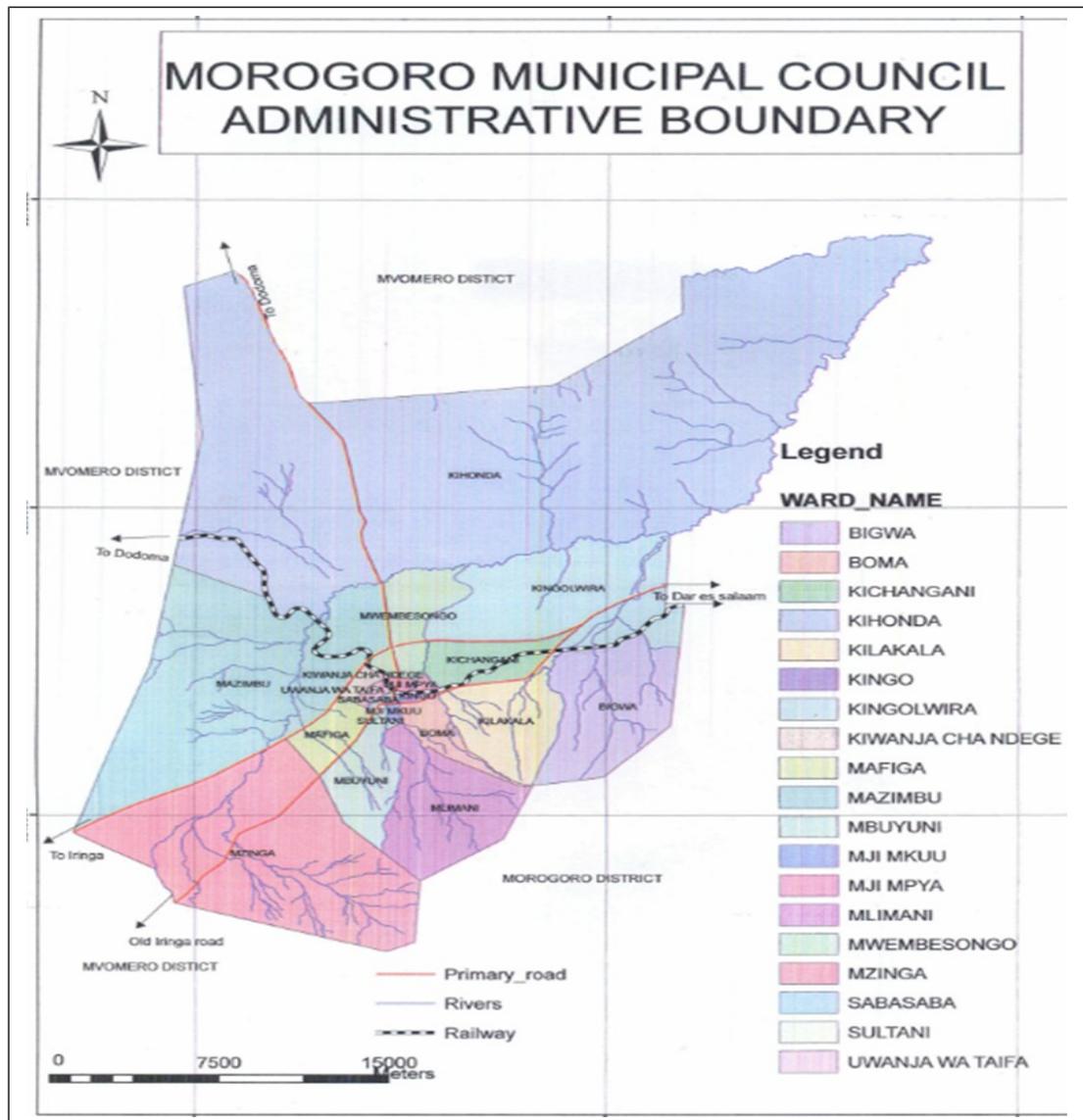


Figure 4: Morogoro municipal council and its local administrative wards.

Source: Morogoro municipal council.

### 3.2 Study Design

A Cross-sectional study design was used in this study whereby sero-prevalence of porcine cysticercosis and African swine fever, risk factors for transmission of the two infections and the related knowledge and practices were all assessed at a single point

in time. The field study was carried out in Morogoro Urban district between November 2010 and January 2011.

### 3.3 Sample Size

Sample size for determination of sero-prevalence was calculated according to Daniel, W. W. (1999):  $n = Z^2 P (1 - P)/d^2$ , where:  $n$  = sample size,  $Z$ = $Z$  statistic for a level of confidence; i.e. 1.96 at 95% confidence level,  $P$ =estimated prevalence and  $d$ =precision (in proportion of one; if 5%,  $d = 0.05$ ).

Previous studies elsewhere in East African region have found a prevalence of porcine cysticercosis to be 20.6 - 56.6% (Phiri *et al.*, 2002). No study has been carried out in urban and or peri-urban areas of Tanzania. The sample size for a cross-sectional survey to estimate prevalence was calculated based on random sampling of pigs. Therefore;  $n = (1.96^2 \times 0.206 \times 0.794) / 0.05^2 = 252$ , the minimum number to sample was 252 pigs and this figure was rounded to 260 pigs. The similar sample size was also used for African swine fever virus antibody detection using the same paired sera samples for cysticercosis. The reason for choosing the low range prevalence is due to the fact that urban/peri-urban pig farming practices total confinement and hence possibility of having lower prevalence as compared to rural pigs.

### 3.4 Sample Selection

A two stage random sampling (primary sampling units being farms and pigs within farms being secondary units) was employed and focus was given to the households with two or more pigs per herd. The description for the specific class of pig sampled

was as follows: weaners (4 months), growers (5 – 8 months) and adults (8 months and above). Sow with litter was counted as one unit of sampling. However, the number of pigs to be sampled within the given herd was determined by the total number of pigs based on age category, thus sampling pig numbers proportional to herd size. The information about the number of pigs in each herd was collected prior to the actual visit.

Out of 19 municipal wards, in Morogoro Urban four wards namely Bigwa, Boma, Kingolwira and Mazimbu were selected based on large number of pigs found and many households keeping the pigs (district livestock population reports). Fifteen local administrative streets were randomly selected from a total of 60 streets among the four wards. A total of 42 out of 152 pig keeping households were randomly selected from a list of farms obtained from the ward livestock reports (unpublished data). In addition to that 18 out of 38 pork traders were conveniently selected based on the status of their activity known to the livestock field officers and easily accessibility.

### **3.5 Data Collection**

In this study data collection involved two stages; firstly, the administration of questionnaire to both pork traders and pig keepers and secondly blood sample collection from pigs.

### 3.5.1 Questionnaire Survey

During the farm visits, blood sample collection from the pigs and interviews with pig keepers was both done at the same time. The structured questionnaire was administered by the investigator and livestock field officers. The administration was face-to-face questionnaire interview and whenever possible confirmed by direct observation. Only one person in each study household was interviewed and priority was given to the household head and the person who takes care of pigs. However, if both of them were not available any person in the household who was able to respond to the questionnaire was interviewed. The questionnaire was pilot – tested in the field by the investigator in one Non-study Street and then revised accordingly to ensure relevance of the questions. A total of 42 questionnaires were administered to all pig keeping households. The questionnaire presented to the farmers focused on information related to: rearing system, knowledge of ASF and porcine cysticercosis, types and methods of feeding, latrine use, age and sex of pigs. The questionnaire for pig keeping households is presented in Appendix 1.

A total of 18 pork traders were visited and interviewed in various pork centres, home slaughter and slaughtering slabs in five Morogoro Urban wards namely; Bigwa, Boma, Kihonda, Kilakala and Mazimbu. For the purpose of this study, a pork trader was defined as a person who either slaughtered and sold pork or purchased pork and prepared it for human consumption. Similarly, a pork centre was a place where pork was prepared (usually by frying) for human consumption. The questionnaire for pork traders is presented in Appendix 2.

### 3.5.2 Blood Sample Collection and Processing

Pigs were restrained by using a pig snare and 5 ml blood was collected from the jugular vein into plain vacutainer tubes. In the field, the blood sample was temporary stored in a cool box with ice packs. In the laboratory, the blood sample was centrifuged (4 000g for 10 minutes) within eight hours and the aliquots of sera were split into two halves and stored in vials for analyses. Serum samples were deep frozen at  $-20^{\circ}\text{C}$  until use.

### 3.6 Porcine Cysticercosis Antigen – ELISA

The circulating antigens of *Taenia solium* cysticercosis was detected by the use of the monoclonal antibody – based sandwich ELISA as described by Brandt *et al.*, 1992 and Dorny *et al.*, 2004. Two monoclonal antibodies denoted by B158C11A10 as the first – capturing antibody and B60H8A4 as the second – detecting antibody was used. The serum samples were first treated with trichloroacetic acid (TCA) to break the antigen-antibody complexes and then tested at a final dilution of 1: 4. Briefly the sandwich assay consisted of coating the plates with capturing antibody (B158C11A10), blocking, addition of TCA treated sera, after which the second biotin labelled antibody (B60H8A4), streptavidin labelled peroxidase and ortho-phenylenediamine (OPD) substrate were added consecutively. Washings were carried out in between the various steps. The reaction was stopped using sulphuric acid and the plates were read in a spectrophotometer at a wavelength of 492 nm. The cut-off was calculated using a modified Student *t*-test (Sokal *et al.*, 1981) programmed in MS Excel sheet, by comparing the optical density of each serum sample with a series of 8 negative reference serum samples at a probability level of *P*

< .001. A serum sample was considered as positive when the ratio (optical density of test sample/optical density cut-off) was  $\geq 1.0$ . The detailed procedure is described in Appendix 3.

### **3.7 ASF Antibody – ELISA**

The Ab – ELISA for African swine fever was carried out according to OIE Manual of Standards for Diagnostic Tests and Vaccines (2008) – Standard operating procedure for ASF antibody detection by Indirect ELISA. This particular test was carried out in collaboration with EU and FAO reference laboratory (CISA – INIA) for ASF upon receiving similar paired sera samples. The biological reagents required and supplied by CISA – INIA included: Semipurified ASFV antigen, ASF positive control reference serum, ASF negative control reference serum, ASF limit control reference serum and Protein-A horseradish peroxidase conjugate. The detailed procedure is described in Appendix 4.

### **3.8 Data Entry and Analysis**

Data were entered into Microsoft Office Excel 2007 and exported in Epi Info™, version 3. 5. 3 (CDC. Gov/epininfo/) for statistical analysis. Descriptive statistics were computed to determine the prevalence of porcine cysticercosis and African swine fever and their 95% confidence intervals. The farm prevalence of porcine cysticercosis was computed by dividing the number of farms that had at least one pig that tested positive by the total number of farms visited. Prevalence in pigs was calculated as the number of pigs tested positive divided by the total number of pigs

tested. Chi-square test ( $\chi^2$ ) was used to determine whether there is a statistically significant association between prevalence and pig level variables such as age.

Analysis of potential risk factors for seropositivity was determined by using a logistic regression model with seroconversion status as dependent variable and farm and pig level variables as among the independent variables. Risk factors with  $P < 0.05$  were considered significant.

### **3.8.1 Estimation of maximum number of diseased animals in a population when the sample size tested negative**

It is relevant to determine the number of diseased animals in this population if all pigs in a sample were tested negative. The following formula (Pfeiffer, 2010) allows the estimation of maximum number of positive cases ( $D$ ) in a source population size ( $N$ ) at 95% confidence level, given that all animals in a sample of size ( $n$ ) were negative:

$$D = \left( 1 - (1 - P)^{\frac{1}{n}} \right) \times \left( N - \frac{(n-1)}{2} \right)$$

Where:  $D$ =maximum number of positive animals,  $P$ =probability of finding at least one case in the sample; i.e. 0.95,  $N$ =population size and  $n$ =sample size.

Since,  $N=2\ 316$ ,  $n=260$ ,  $P=0.95$ ; Then, the maximum number of positive cases ( $D$ ) that might be present is 25 pigs at the maximum prevalence ( $p$ ) of 0.01.

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 General Results

A total of 15 local administrative streets of Morogoro Urban district were included in the study. A total of 42 pig keeping households and 18 pork traders were visited and interviewed in the district and all participants cooperated. On average, the area of land for most pig keeping households ranged from 450 and 4 900 m<sup>2</sup>. About 30 – 50% of all pig keeping households in the study area practiced mixed farming (raised pigs and cultivate crops) within their premises and therefore manures from the pigs were used to fertilise the crops. In this study, blood sample was collected from 263 pigs. Of the pigs sampled 57.8% were females and 42.2% were males. Of the male pigs 54.9% were not castrated and 45.1% were castrates. The age range of the sampled pigs was from 4 months to 3 years. The aging was based on farmers records/history and memory. Of them 57 pigs (21.6%) were weaners, 98 (37.3%) growers and 108 (41.1%) adults. The pigs were of exotic and crossed breeds. The number of pigs owned by the farmers ranged from 2 to 43 pigs (12 pigs per farmer).

#### 4.2 Prevalence of Porcine Cysticercosis

Of the 263 samples, 259 were screened with B158C11A10/B60H8A4 (inadequate volume of serum available in 4 samples) and four animals were positive to *T. solium* cysticercosis. The overall prevalence of porcine cysticercosis in the Morogoro

municipal division of Morogoro Urban district was 1.54% (95% CI = 0.04 – 3.1%) based on Ag – ELISA (see Table 1) with no statistical significant differences by age group ( $P = 0.57$ ). Four farms out of 42 farms had at least one positive case of cysticercosis therefore farm prevalence was estimated at 9.5% (95% CI = 0.7 – 18.3%).

Table 1: Prevalence of porcine cysticercosis by age category based on Ag – ELISA

Age category	No. screened	No. Seropositives	% seropositive (95% CI)
Weaners (4 months)	55	0	0 (0.0 – 0.0)
Growers (5 – 8 months)	96	2	2.1 (0 – 4.3)
Adults (>8 months)	108	2	1.9 (0 – 3.8)
Total	259	4	1.54 (0.04 – 3.1)

### 4.3 Questionnaire Survey and Direct Observations

#### 4.3.1 General characteristics of pig keeping households

The general pig husbandry practices, hygienic and sanitary conditions were inquired about and responses confirmed by direct observations. A total of 42 pig keeping households were interviewed and out of these, 40.5% were women and 59.5% were men with age range from 19 – 65 years old. About 2.4% of the respondents had adult education aimed at capacity to read and write, 54.8% had primary school education standard class 1 – 7, 40.5% had secondary school education "O" and "A" levels and 14.3% had College/University education.

About 55% of households in the study area were farmers and their main activity was agriculture (cultivate crops and keep domestic animals). Most of the farmers raised

more than one class of domestic animals (cattle, goats, local chickens, etc) and out of them approximately 7.4 % kept pigs.

#### **4.3.2 Pig husbandry practices in Morogoro Urban district**

The pig husbandry practices in the study area are summarized in Table 2. All pig keeping households in this region practiced total pig confinement even though some of them had seasonal pig pen breakdown that allowed few pigs to scavenge. The common feedstuffs used by the farmers were locally available in their places and these included maize bran, rice bran, green pastures and kitchen left over's i.e. the scraps of tomatoes, Irish and sweet potatoes, etc. Few respondents supplemented their pigs with sunflower seed cakes when they had money (usually after selling of some pigs or crops). Natural breeding was the only method of breeding practised in this region and about 50% of all pig keeping households kept their own boars. Boars were also used as the source of capital gain. Usually a farmer who needs to serve his/her sow on heat had to borrow a boar from a nearby farm and on return this farmer either deliver one piglet once his/her sow gave birth or pay 5 000/= Tanzanian shillings (equivalent to 3.2 USD).

About 43% of all farmers acquired pigs for keeping from their neighbours farmers within the street and nearly all of the respondents raised their pigs until slaughter even though some of them usually sell their piglets (within 2 – 4 months old) when the sow get farrowed. Slaughtering of pigs was usually carried out in slaughtering

slabs/home slaughters and the farmer had to contact a pork trader, i.e. butcher. In this study area, most of the pig houses were built of concreted/cemented brick (69.0%) and timber cuts off (21.4%) as material for the floor, galvanised iron sheets (71.4%) and thatched grass (11.9%) as roofing materials and timber cuts-off (69.0%) and tree/bamboo poles (14.3%) as material for the walls. Plates 1 and 2 illustrate various pig houses built by using timber cuts-off and tree poles. These were the common simple building structures that were used by about 83% of all pig keeping households in the study area.

In this study area, many pig keeping households kept few numbers of pigs and therefore these may generally fall under small scale pig production units. Among the visited farms, the minimum number of pigs found in the farms was 2 pigs and 43 pigs was the highest number of pigs raised. The number of pigs in the farms varied with the season and the investigator has found that many farmers sell their pigs during the dry/off season when there are no crops in the cultivated lands.

Table 2: Pig husbandry practices as assessed by questionnaire survey in Morogoro Urban district – Tanzania, 2010/2011.

Factor	Positive responses (%)
Reasons for keeping pigs	
<ul style="list-style-type: none"> <li>• Income generation</li> </ul>	<ul style="list-style-type: none"> <li>• 99</li> </ul>
<ul style="list-style-type: none"> <li>• Home consumption and manure</li> </ul>	<ul style="list-style-type: none"> <li>• 1</li> </ul>
Respondents having specific pig shelter	100
Herd size (total number of pigs per herd)	
<ul style="list-style-type: none"> <li>• 1 – 10 pigs</li> </ul>	<ul style="list-style-type: none"> <li>• 45.2</li> </ul>
<ul style="list-style-type: none"> <li>• 11 – 20 pigs</li> </ul>	<ul style="list-style-type: none"> <li>• 28.6</li> </ul>
<ul style="list-style-type: none"> <li>• 21 pigs and above</li> </ul>	<ul style="list-style-type: none"> <li>• 26.2</li> </ul>
Location of purchasing pig for keeping	
<ul style="list-style-type: none"> <li>• Within the street</li> </ul>	<ul style="list-style-type: none"> <li>• 42.9</li> </ul>
<ul style="list-style-type: none"> <li>• Neighbouring streets</li> </ul>	<ul style="list-style-type: none"> <li>• 26.2</li> </ul>
<ul style="list-style-type: none"> <li>• Far streets</li> </ul>	<ul style="list-style-type: none"> <li>• 19.0</li> </ul>
<ul style="list-style-type: none"> <li>• Other districts within the region</li> </ul>	<ul style="list-style-type: none"> <li>• 11.9</li> </ul>
Factors considered when purchasing pigs	
<ul style="list-style-type: none"> <li>• Examination for cysts</li> </ul>	<ul style="list-style-type: none"> <li>• 0.0</li> </ul>
<ul style="list-style-type: none"> <li>• Body condition characteristics</li> </ul>	<ul style="list-style-type: none"> <li>• 28.6</li> </ul>
<ul style="list-style-type: none"> <li>• Back ground records/history</li> </ul>	<ul style="list-style-type: none"> <li>• 4.8</li> </ul>
<ul style="list-style-type: none"> <li>• Both body condition and background records</li> </ul>	<ul style="list-style-type: none"> <li>• 50.0</li> </ul>
<ul style="list-style-type: none"> <li>• Never considered any factor</li> </ul>	<ul style="list-style-type: none"> <li>• 16.7</li> </ul>



Plate 1: One of pig housing designs; Morogoro Urban, Tanzania 2010/2011



Plate 2: Eighty three percent of pig houses in the study area were built of timber cut-off and tree poles, 2010/2011.

Based on questionnaire survey (Table 2), 50% of all respondents considered body condition characteristics and back ground records/history before purchasing pigs. Length, size and colour of the body were the basic criteria used under the body condition characteristics and most of the respondents were interested on long body, large body size and white in colour pigs or piglets.

Background records or history were also the selection criteria and the respondents were interested on reproductive and productive performance of the pig's or piglet's parents. About 16% of all respondents never considered any factor before purchasing their pigs. The investigator has observed that most of these respondents were buying pigs from their nearby pig keeping households and they had no background experience in pig farming.

#### **4.3.3 Practices related to the occurrence and transmission of porcine cysticercosis**

The results of questionnaire survey to assess the practices related to the occurrence and transmission of porcine cysticercosis are summarized in Table 3. In addition to the interview, the responses were confirmed by direct observations. Even though all farmers confined their pigs, the fact that some pigs in few farms were found outside demonstrated pig pens broken down and therefore the risk of being infected in the surroundings becomes high. Nearly all pig keeping households (97.6%) in the district had and were using latrines.

According to the Morogoro Urban water and sewerage supply authority (MORUWASA), the Morogoro Urban district was estimated to have a total number of 54 000 households (as per June 2008 annual report). Out of this, only 18 891 and 680 households had active connections of clean water and sewerage services respectively. The total percentage coverage of sewerage was only 3.3%. This shows

that many households waste water generated were not in a sewerage connection system and therefore the individual households were to find their alternative ways to take care of the waste water. Based on the above fact, the sewerage service had become a business and it is therefore under the private concern whereby the individual household had to pay for the service. In this case, specialized sewerage carriage trucks which were individually owned have been commonly used to carry waste water from household latrines. The individual household were to pay for the cost of service, approximately 40 000 Tanzania shillings per trip at the moment (equivalent to 25 USD). This cost of service was not affordable by most of the household occupants.

Table 3: Practices related to the occurrence and transmission of porcine cysticercosis in Morogoro Urban district, Tanzania.

Practices	Positive responses (%)
Pens allowing pigs to escape	76.2
Frequency of escape from pens which allows pigs to escape	
• High/always	• 14.3
• Only occasional	• 61.9
Lack of latrine	2.4
Latrines with absence of door	31.0
Faeces in the latrine surroundings	4.8

Only one respondent out of 42 had no latrine (Table 3). This respondent was considered to have no latrine even though his family was using the neighbour's latrine.

#### 4.3.4 Knowledge related to porcine cysticercosis

The results of questionnaire survey to assess the knowledge of pig keeping households on porcine cysticercosis are summarized in Table 4. These results suggested that many respondents in the study area were not informed about the porcine cysticercosis and therefore this might have negative impact in disease control. Plate 3 illustrates a poorly constructed latrine, built by using boxes without door and plate 4 illustrates a poorly constructed pig house which can easily allow pig in or out. Both practices were the risk factors predisposing pigs to the transmission of porcine cysticercosis in the study area.

Table 4: Knowledge on porcine cysticercosis among the pig keeping households in Morogoro Urban district – Tanzania, 2010/2011.

Respondents perception/knowledge	Positive responses (%)
Never experienced cysts in pigs	71.4
Unable to explain the disease in pigs clinically/post mortem	76.2
Not aware of how pig get infected	88.1
Capable to described the status of disease in the street	
• Non – existence	• 7.1
• Present but not serious	• 2.4
• Not aware of its existence	• 90.5
Never encountered cysticercosis among their pigs kept	97.6



Plate 3: A poorly constructed latrine in Morogoro Urban district – Tanzania, 2010/2011.



Plate 4: A poorly constructed pig house (pig can easily get out) in Morogoro Urban district – Tanzania, 2010/2011

#### **4.3.5 Questionnaire survey results for pork traders**

Of the eighteen interviewed pork traders, 6 were butchers who sold pork, 4 purchased pork and prepared it for human consumption and 8 were both butchers, vendors and processors of pork for consumption. Among these three categories of

pork traders, the two groups of butchers (14 in total) were aware and informed of pork inspection. The other category of pork traders who purchased and prepared pork for consumption were not aware of pork inspection and more often they were not sure of whether their purchased pork were inspected.

About 55.6% of all pigs slaughtered in either home slaughter or slaughter slabs came from the study area, i.e. Morogoro Urban district.

Table 5: The number of slaughtering facilities visited in the study area, 2010/2011.

Name of ward	Slaughtering facility
Bigwa	4
Boma	0
Kihonda	4
Kilakala	2
Mazimbu	4
Total	14

#### 4.3.6 Pork inspection

All slaughtering facilities visited in the area were private owned. Thirteen out of 14 slaughtering slabs were regarded as home slaughters since they were built within residential compound even though they were operated under business basis. Fourteen out of the 18 pork traders were aware of pork inspection as an important routine procedure that should be done to ensure the safety of pork for public consumption and some respondents pointed out that the livestock field officers were the responsible in-charge person. In this study area livestock field officers were also pork inspectors and they had formal qualification at diploma level in animal health and or production.

Even though pork inspection was among of their routine daily activities, according to the pork traders they always had to pay for the transport and the cost of service in form of cash or one kg of pork per inspection. Eleven traders reported that pork inspection was usually carried out after slaughtering and seven traders had experienced condemnation of their pork due to several reasons. Five of them reported that presence of cysts was the reason for the condemnation and one pork trader pointed out that his pork was condemned due to abnormal pork discolouration which was later confirmed to be ASF by the livestock field officer. The other pork trader reported that his pork was condemned after being caught by the pork inspector selling pork without prior inspection.

Plates 5 to 7 illustrate the status of slaughter slabs and pork centre in Morogoro Urban district, 2010/2011. A sub-standard slaughter slab was characterised by poor design layout and sanitary measures, built within residential compound, unreliable water supply and lack of resting places (lairage). A well constructed slaughter slab was featured by good design layout and sanitary measures, fenced, presence of resting places and reliable water supply.



Plate 5: A poor pig slaughter slab 2010/2011.



Plate 6: A well constructed pig slaughter slab, 2010/2011



Plate 7: A pork centre in Morogoro Urban district – Tanzania

According to the trader, a positive case of cysticercosis (Plates 8 and 9) identified during the visit in the routine pork inspection was among of the pigs brought from neighbouring Dodoma region. The whole carcass of the pig with generalized cysts was poured with kerosene and then buried.



Plate 8: Multiple cysts (arrows) found on heart muscles, 2010/2011



Plate 9:  
Generalised  
cysts (white  
nodules  
found in the  
carcass),  
2010/2011

#### 4.4 Factors

##### **associated with porcine cysticercosis**

The factors that were considered in the analysis as risks associated with porcine cysticercosis at pig and farm level are presented in Table 6. The dependent variable is disease status. The logistic regression model was used to identify the risk factors which influence the occurrence of porcine cysticercosis in urban/peri-urban pig farming. Considering all risk factors included in the model none of the pig and farm variables were statistically significantly associated with the porcine cysticercosis ( $P > 0.05$ ).

Two out of the 4 positive farms were adjacently located in one street, and the other remaining farms were located in two different non neighbouring wards. Out of the four farms, the respondents from three farms declared that it was not unusual for the pig to get out of its house and scavenge even though the pig always came back through the same broken part of the house when the respondents brought foods to the other remaining pigs. This was confirmed by the investigator during the visit in such

a way that these respondents had to bring some foods to the other pigs in a house in order to attract the scavenging pig for blood sampling. These respondents pointed out that once the pig get out of its house for the first time to scavenge it usually became its behaviour and even if the broken part of house is repaired the pig would always find its alternative way out.

Based on the questionnaire survey and direct observation, the other remaining farm was built moderately strong and all pigs were properly confined. But the respondent declared that his pig used to scavenge while it was at the growing stage (5 – 8 months) and therefore he decided to repair the broken part and reinforce the whole building. Regular conflict due to religious differences from one of his neighbour was the reason for the pig house improvement. The sampled pig found positive in this farm was 18 months old. Therefore based on above findings, the investigator has found that these positive pigs in all four farms had a history of scavenging contrary to the other pigs within their respective farms which were found to have no infection. All pig keeping households in these four positive farms had pit latrines.

Table 6: Risk factors associated with porcine cysticercosis investigated during the study, 2010/2011.

Risk Factor	OR (95% CI)	p-value	LR
Pig level variables			

Sex	Female (F)	1.09 (0.61 – 1.98)	0.765	
	Male (M)	0.71 (0.35 – 1.46)	0.355	1.201
Age	<8 months	0.87 (0.59 – 1.27)	0.462	
	>8 months	0.94 (0.58 – 1.53)	0.804	0.736
Farm level variables				
	Introduction of pig into the herd	0.90 (0.11 – 7.06)	0.920	0.010
	Pens allowing pig to get out	0.93 (0.09 – 10.08)	0.953	0.003
	Knowledge of how pigs get infected	0.00 (0.00 - >1.0E1)	0.638	0.411
	Observation of cysts in pig	0.00 (0.00 - >1.0E1)	0.743	0.203
	Latrines without closing door	1.38 (0.13 – 14.72)	0.787	0.076

NB: OR=Odds Ratio, LR=Likelihood Ratio

#### 4.5 Prevalence of African swine fever (ASF) antibodies in sampled live pigs

The prevalence of ASF in sampled domestic pigs in Morogoro Urban district was estimated by the use of indirect enzyme-linked immunosorbent assay (ELISA). ASF antibody titre detection revealed no specific ASF antibody response was observed giving raised all samples negative result.

Table 7: The 2008 outbreak of ASF by ward among the visited farms in Morogoro Urban district based on questionnaire interviews, 2010/2011.

Name of ward	No. of farms visited	No. of farms with history of ASF occurrence	% of farms with history of ASF outbreak
Bigwa	15	3	20
Boma	8	0	0
Kingolwira	8	0	0
Mazimbu	11	10	90.9
Total	42	13	

Table 8: Knowledge of African swine fever in Morogoro Urban district – Tanzania based on questionnaire interviews, 2010/2011.

Factor/practice	Positive responses (%)
Respondents who never heard or experienced ASF	71.4
Respondents who lost pigs due to 2008 ASF outbreak	19.0
Respondents who were able to explain the disease clinically/post mortem	54.8
Respondents who didn't know how pig get infected	64.3
Respondents who ever receive and use for meal warthogs meat	14.3

The frequency of receive and use for meal warthogs meat (often/always)	7.1
Respondents who didn't know whether warthogs meat scraps can be source of ASF introduction	97.6

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## **CHAPTER FIVE**

### **5.0 DISCUSSION**

#### **5.1 Porcine Cysticercosis**

This study has achieved to estimate the prevalence of porcine cysticercosis in urban/peri-urban pig farming. There are no published reports of other similar studies

that were carried out in urban/peri-urban areas of Tanzania, making this study original in this context.

The present study has investigated the prevalence and the potential risk factors associated to *T. solium* cysticercosis in domestic pigs in Morogoro Urban district. The overall prevalence found was 1.54% based on detection of circulating antigens which indicates that the disease is present in the district. However, the available monoclonal antibody-based sandwiches ELISA (Ag-ELISA) are not species-specific and cross-react with *T. hydatigena* cysticerci in pigs. In the study area, data on the prevalence of *T. hydatigena* in pigs are not available. One study conducted in northern Tanzania found a prevalence of 1.4% (n = 70) in slaughtered pigs (Ngowi *et al.*, 2004b), indicating a low prevalence. Therefore, we cannot directly associated 1.54% prevalence with *T. solium* cysticercosis in this study area. Nevertheless, information from pork traders on the presence of porcine cysticercosis and the case detected by chance during a visit to a slaughter slab in this study suggests that the infection is present in the area, though it might be originating from neighbouring regions.

A recent study conducted in Dar es Salaam city to establish the prevalence of extra-intestinal porcine helminth infections in slaughtered pigs originated from the city and different areas of Tanzania (Mkupasi *et al.*, 2011) did not find any positive case of porcine cysticercosis among the pigs from Morogoro region even though the study didn't show the exact location of where the pigs came from. The study has examined a total of 731 pigs originated from different regions of Tanzania, with 14 pigs sourced from Morogoro region. The failure to detect any positive case in this study

might have been contributed by either the small sample size or differences in prevalence among the districts across this region.

Although all of the surveyed pig keeping households practised total confinement of their pigs, 76.2% of them reported seasonal pig escape from their confinement. As soon as pigs are free roaming the chances of getting access to human faeces from either the latrine or surroundings became increased. This apparent paradox could have two explanations. The tendency of human defecation at night particularly non-households residents in streets is a common practice in urban and sub-urban areas. Another explanation is that inefficient sewage system might have contributed to environmental contamination. This setback is even doubled during the long rains season since most of the households (74%) had pit latrines and therefore as usual the depth of underground water tends to raise leading to sewage overflow. In this study, farm visit for blood sampling and questionnaire survey were carried out during the short rains season. This form of environmental contamination as risk factors of porcine cysticercosis were also reported in a study conducted in Mexico (Sarti *et al.*, 1992) in a setting where the outlets of households' latrines pour into the surroundings of pig pens. Therefore, based on the above discussion the four (4) pigs could have been infected from either two sources since all of them had the similar history of scavenging.

This study however failed to associate statistically the pig and farm level variables with the occurrence of *T. solium* cysticercosis. These results agrees with the study conducted in eastern and southern provinces of Zambia which concluded that

statistical analysis failed to show associations between infection in pigs and various epidemiological factors which were considered possible risk factors (Phiri *et al.*, 2002). The low number of positive pigs and the small sample size may also have weakened the statistical significance of the relationship.

In Tanzania, porcine cysticercosis has been well studied in rural pig's husbandry setting. The study conducted in northern highlands (Mbulu district) of Tanzania (Ngowi *et al.*, 2004a) have found that the prevalence of porcine cysticercosis based on lingual examination was considered higher in pigs raised in households that were not using latrines than those using latrines. Furthermore, the study pointed out that the prevalence was generally higher in free-range (17.8%) than in indoor-raised (6.5%) pigs. Another study carried out in southern highlands of Tanzania (Boa *et al.*, 2006) based on tongue examination has revealed similar results as the absence of latrine in the households and free-range management of pigs showed very strong association with porcine cysticercosis.

Therefore based on the husbandry management systems in urban/peri-urban farming, porcine cysticercosis is present despite of confinements. It is a significant risk that there is high chances of acquiring the infection once the pig let out in this area.

Free-range management system is prohibited in urban/peri-urban pig farming and this is mainly due to the local government by-laws regulating livestock farming in urban areas which are more enforced than in rural/village farming. In fact the enforcement of these by-laws might also explain low prevalence in this study area.

Sarti *et al.*, (1992) pointed out that the prevalence of porcine cysticercosis is known to increase with free-ranging type of pig management as it accelerates free access to human faeces.

Intensive pig farming is highly encouraged in many areas of African countries even though the system has become stagnant and continues to meet challenges particularly in traditional setting. However, confinement of pig has been known to prevent porcine cysticercosis. Lekule and Kyvsgaard, (2003) emphasized on pig confinement as a successful method of minimise the risk of cysticercosis. Furthermore, the report observed that free-ranging of pigs is considered as one of the risk factors for porcine cysticercosis and it is, therefore, absolutely necessary to find ways to keep the pigs enclosed.

In most cases pork inspection in this region was done after slaughtering (61.1%) and ward livestock field officers were also responsible pork inspectors. Based on the fact that all of the slaughtering slabs were individually owned, the investigator observed that there was no specific time for slaughtering of pigs among the visited places and therefore the pork inspector was usually called even in the afternoon for inspection once the slaughtering process was over. In view of this it was not possible for the inspector to know the exactly number of pigs slaughtered in a given slaughtering facility as he always called at the end of the operation. This demonstrated inefficiency of the whole system of pork inspection in the study area. The situation was different in other places like Dar-es-Salaam city (Mkupasi *et al.*, 2011) where

the operation is synchronised within specific time and routine meat inspection was usually underway by specific meat inspectors.

The fact that, five out of seven of all cases of pork condemnation in the area were due to cysts revealed that porcine cysticercosis is one of the main conditions encountered during routine pork inspection. There are very little epidemiological reports about the prevalence of porcine cysticercosis in this region however, the pigs slaughtered in Dar-es-Salaam city originated from Dodoma has found to have high infection rates (8.2%) based on lingual and post-mortem examinations (Mkupasi *et al.*, 2011).

*T. solium* cysticercosis antigen detection by ELISA is known to detect only the live cysts in pigs (Nguekam *et al.*, 2003) and therefore the number of seropositive pigs found poses health risks to the consumers. Antigen detecting ELISA is a useful method because of its high sensitivity and specificity (86.7% and 94.7%) respectively (Dorny *et al.*, 2004).

## **5.2 African Swine Fever**

Antibody detection provide evidence of current and previous exposure to infectious agents and their assay is commonly employed in veterinary medicine as a relatively efficient and cheap means of detecting this exposure in both individual animals and populations (Thrusfield, 2000). In Morogoro Urban district, particularly in Mazimbu ward the outbreak of ASF was lastly reported in one of the commercial pig farm in February 2008 (Misinzo *et al.*, 2011) even though the disease was widely spread

among several pig keeping households (Table 7). The recent outbreak of ASF in Tanzania was only reported in December, 2010 in non-neighbouring Mbeya region (OIE, 2011).

The present cross-sectional serological study on ASF in sampled domestic pigs in Morogoro Urban district revealed no specific ASF antibody response based on indirect ELISA. The true negative result obtained in this study indicates absence of ASFV exposure among the sampled pigs. These results contradict with the similar cross-sectional study on ASF in domestic pigs in northern Tanzania (Swai *et al.*, 2005) which found the overall seroprevalence and farm prevalence to ASF antibodies in pigs to be 7% and 14.9% respectively. The possible explanation for the differences in these results could be based on the fact that the outbreak of ASF in northern Tanzania was occurred in September 2003 and the pigs were sampled in October same year. Therefore the possibility of getting more positive cases is increased since the antibody production peaks 7 – 10 days post-infection (CISA – INIA, 2008). Furthermore, the study found an association between the prevalence and proximity of pig farms to the border entries/exits that the pigs in close proximity to the border entries/exits had significantly higher prevalence and higher likelihood to seroconvert than those on the wildlife interface. However, these factors were not present in this study.

Free-range management system is not practised in urban/peri-urban pig farming and this is mainly due to the local government by-laws regulating livestock farming in urban areas which are more enforced than in rural/village farming. This could also

explain why all of the pig keeping households (Table 2) in the Morogoro Urban district had confined their pigs. The enforcement of these by-laws governing livestock farming in urban/peri-urban areas may have also contributed to the negative results obtained in this sero-prevalence study. This explanation can be confirmed by the serological study in two areas of southern Malawi (Allaway *et al.*, 1995) to determine the antibody against ASFV in domestic pigs sampled after the ASF outbreak. The study has revealed that the ASF outbreak spread were significantly associated with free ranging of pigs whereby the individuals who allowed their pigs to range free reported high frequency of ASF than those who permanently penned their pigs.

The interesting result was obtained in a study (Gallardo *et al.*, 2011) where the serum samples were positive for virus isolation based on PCR but the same samples were negative for ASFV antibody detection based on OIE-prescribed ELISA and Immunoblotting (IB) assay. In this study the outbreak of ASF in Uganda was reported in October 2007 and the blood sampling from the affected regions commenced after the outbreak. The study was conducted primarily to genotype ASFV isolates associated with disease outbreaks in Uganda in 2007. The fact that serum samples in pigs from east African countries gave negative results on OIE-prescribed ASF serological methods was also confirmed by the similar study (Perez-Filgueira *et al.*, 2006). The OIE-prescribed methods are usually based on the use of total ASF virus as antigen that includes many viral proteins. Gallardo *et al.*, (2011), pointed out that the possible explanation for the unexpectedly low seropositive antibody response with east African sera may exist in the immunogenetics of the

indigenous pig populations in this region and it is unlikely to be due to polymorphisms in immunodominant viral antigens.

The results of questionnaire survey suggested that most of the respondents in the study area were not aware of the ASF and few of them lost their pigs during the previous 2008 outbreak (Table 8). However, Mazimbu ward was the leading local administrative area representing higher proportion of pig keeping households by wards who lost their pigs during this attack. Mazimbu and Kihonda are neighbouring wards sharing their borders (Fig. 4). These two wards also had at least 8 slaughtering facilities (Table 5) located in different places and all receive pigs from several areas within and outside of Morogoro Urban district. The fact that these two wards had large number of slaughtering facilities and also receive pigs in large quantities and outside the district might accelerate the risk of ASF occurrence and thus the role of pork traders in disease transmission could be realised. This argument can be confirmed by the reports from the Ministry of Livestock and Fisheries Development which found that the 2008 ASF outbreak in Dar-es-salaam region was due to the transportation of live ASFV-infected domestic pigs by trader from Turiani-Mvomero district (one of Morogoro districts) for slaughter in Dar-es-salaam city. Therefore, the report confirmed that Turiani was the source of 2008 ASF outbreak in Morogoro (Mazimbu) and Dar-es-salaam regions.

## CHAPTER SIX

### 6.0 CONCLUSION AND RECOMMENDATIONS

This study has found that porcine cysticercosis might be present in Morogoro Urban district. The prevalence cannot be associated 100% with *T. solium* cysticercosis because of possible cross-reactivity of Ag-ELISA with *T. hydatigena*, whose

prevalence in the study area is currently unknown. The low prevalence observed in domestic pigs in the study area may largely attributed by the livestock husbandry practices governed and enforced by the local government by-laws in urban and peri-urban areas. The prevalence of porcine cysticercosis found in this study should not be underestimated but rather be considered as an important public health hazard alarming for effective control measures. This is due to the fact that pigs might have acquired the infection from human tapeworm carriers.

Public health education with emphasis on latrine use, discouraging street defecation and buildings of proper latrine with good sewage drainage system is highly recommended as one of the strategy to control and prevent the risk of *T. solium* cysticercosis transmission from human being to domestic pigs.

In addition, this cross-sectional study recommends for further extensive surveillance studies aiming at monitoring the porcine cysticercosis dynamics in urban and peri-urban pig farming so that more baseline information can be available not only for research purposes but even for design and implementation of long term control strategies.

This study has also found that there was no specific ASFV antibody response among the sampled domestic pigs in the study area. However this should not be interpreted as the absence of ASF in this region but rather these pigs were probably not exposed to ASFV. Basically, this study was implemented as a sero-surveillance to detect the antibody response against the ASFV in domestic pigs between the ASF outbreaks

and therefore the study should mainly be considered as one of the routine procedure in ASF diagnosis for effective disease control.

Based on the fact that, the ASF is endemic in the country due to the presence of reservoir hosts (soft ticks and warthogs) this study recommends for routine surveillance and monitoring of the disease in areas close to the presence of these reservoir hosts and those with history of absence of reservoir hosts.

The study has also observed the positive involvement of pork traders and the spread of ASF outbreak. Therefore, in Tanzania the outbreak of ASF is mainly propagated by the transportation of infected live pigs for pork consumption from areas where the disease originated to other newly non infected regions. This study recommends for the active monitoring, early detection and effective quarantine measures at the point of ASF occurrence so as to prevent the spread of the disease. With this effect, the ASF will be restricted and therefore the potential loss to the farmers associated with its occurrence would much be reduced.

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## APPENDICES

No.
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Appendix 1: Questionnaire administered to pig keeping households to assess pig production, knowledge and risk factors associated with the occurrence of porcine cysticercosis and ASF in Morogoro region, Tanzania 2010/2011.

### A. General information

- i. District \_\_\_\_\_ Division \_\_\_\_\_ Ward \_\_\_\_\_  
Street/village \_\_\_\_\_ Farming system \_\_\_\_\_
- ii. Date of interview \_\_\_\_\_ Name of enumerator \_\_\_\_\_

### B. Household characteristic

- i. Name of respondent \_\_\_\_\_ Gender 0 = male, 1 = female \_\_\_\_\_
- ii. Respondent's position in the household  
0= household head, 1 = wife of household head, 2 = child of household 3= animal worker, 4 = others (specify) \_\_\_\_\_
- iii. Age of the household head \_\_\_\_\_ (yrs)

iv. Gender of the household head: 0= male 1 = female \_\_\_\_\_

v. Education level of the household head

0 = No formal education, 1 = Adult education, 2 = primary: standard 1 – 4,  
3 = primary: standard 5 – 7, 4 = secondary: O - level, 5 = secondary, A-level, 6 =  
College/ university, 7 = others (specify) \_\_\_\_\_

### C. Pigs acquisition

- i. Usually from which locations do you acquire/purchase your pigs?  
0 = within the street, 1 = neighbouring streets, 2 = far streets, 3 = other districts within region, 4 = other region (specify) \_\_\_\_\_
- ii. When did you lastly purchase/receive a pig? \_\_\_\_\_
- iii. How many did you receive? \_\_\_\_\_
- iv. Where was each pig received from? 0 = within the street, 1 = neighbouring streets, 2 = far streets, 3 = other districts within region, 4 = other region (specify)
- v. What was the age of each pig? \_\_\_\_ 0=2-4months; 1=5-7months; 2=8-10; 3=1year; 4=more than 1year
- vi. What was the sex of each pig? \_\_\_\_ 0=male; 1=female; 2=both male and female
- vii. What was each pig intended for? a) \_\_\_\_\_ b) \_\_\_\_\_  
\_\_\_\_\_

viii. What are the important examinations you normally do to a pig before buying it

A. Examination	B. Tick	C. Rank	D. Explain your preference criteria
1. Presence of cyst(s)			
2. Body condition characteristic			
a. Length of body			
b. Size of the body			
c. Colour			
3. Other health status (specify at D)			
4. Background history/records ( i.e. reproductive & productivity (specify at D)			
5. Others (specify)			

### D. Commencement and trend of pig production

- i. When did you start keeping pigs? (year) \_\_\_\_\_
- ii. How is the trend of your pig numbers for the past months/years?

Month/year										
Pig No										

iii. What is your purpose of keeping pigs?

Purpose	Income generation	Domestic meat production	Manure production	Cultural (i.e. dowry)	Others (specify)
Indicate (tick)					
Order of importance					

iv. What is your current pig flock size (number) \_\_\_\_\_

v. What is your current herd structure

Type	Total Number	Type of pig (ecotype/breed)*
Breeding females (Sows)		
Breeding males (boar)		
Adult non castrated males (not for breeding )		
Adult castrated males		
Adult females (not for breeding)		
Pre – weaned male piglets		
Pre – weaned female piglets		
Weaned female piglets (2 – 4 months)		
Weaned non castrated male piglets (2 – 4 months)		
Weaned castrated male piglets (2 – 4 months)		
Grower females (5 – 8 months)		
Grower males non castrated (5 – 8 months)		
Grower males castrated (5 – 8 months)		
Total		

\* Type of pig (ecotype/breed): 0 = local, 1 = exotic, 2 = mixed (local & exotic), 3 = mixed (exotic & exotic), 4 = not known.

### E. Type of feeds and their sources

What are the main feed resources do you use during different period of the year

Type of feeds used	Indicate (tick)	Source of feed
Maize bran		
Rice bran		
Sunflower/cotton seed cake		
Lime		
Blood meal		
Mineral mix (pig mix)		
Green pasture		
Restaurant/kitchen left over		
Others (specify)		

**F. Pig shelter (enumerator to combine physical observation of shelter and interview)**

- i. Do you have specific shelter for your pigs  
0 = Yes, 1 = No
- ii. If yes, what type of pig shelter are you using  
0 = earthed floor, 1 = slated raised floor, 2 = slatted earthed level floor, 3 = concreted floor, 4 = others (specify) \_\_\_\_\_
- iii. Why did you opt for this production system(s)?  
1. \_\_\_\_\_  
2. \_\_\_\_\_  
3. \_\_\_\_\_  
**\*Production systems:** 1= Intensive system; 2= Semi-intensive system; 3= Extensive system
- iv. Condition of pig shelter (enumerator to make assessment of floor, wall and roof of shelter with following scores:  
0 = strong (highly protected can't offer free inlet or outlet of pigs), 1 = moderate (protected, however minimum effort can allow pigs out or in), 2 = weak (pig can get out and in when desires)
  - a. What is general condition of the shelter? \_\_\_\_\_
  - b. What is the specific condition of the floor? \_\_\_\_\_
  - c. Which material used for the floor? 0 = timber off cuts, 1 = tree/bamboo poles, 2 = cemented bricks, 3 = burned bricks, 4 = others (specify) \_\_\_\_\_
  - d. What is the specific condition of the wall? \_\_\_\_\_
  - e. Which materials used for the wall? 0 = timber off cuts, 1 = tree/bamboo poles, 2 = cemented bricks, 3 = burned bricks, 4 = others (specify) \_\_\_\_\_
  - f. Does a shelter have the roof? 0 = Yes, 1 = No
  - g. If yes, which materials used for the roof? 0 = thatched grass, 1 = iron sheet, 2 = bamboo trees, 3 = others (specify) \_\_\_\_\_
- v. How do you rate the importance of pig shelter  
0 = very important, 1 = important, 2 = less important, 3 = not important
- vi. According to condition of shelter, do the pigs or piglets ever escape from their shelters?  
0 = Yes, 1 = No
- vii. If yes, how frequently? 0 = always, 1 = only occasionally, 2 = during off (dry) seasons,  
3 = others (specify) \_\_\_\_\_

**G. Awareness and knowledge of African swine fever and porcine cysticercosis**

- i. Have you ever heard or experienced African swine fever? 0 = Yes; 1 = No
- ii. Have you ever lost your pigs due to ASF? \_\_\_\_\_ 0 = Yes, 1 = No
- iii. If yes, how many pigs? \_\_\_\_ 0 = All flock, 1 = Half of flock, 2 = Others, specify \_\_\_\_\_

- iv. When did you lost your pigs? \_\_\_\_ 0=Past 12 months, 1=Past 2 years, 2=Others, \_\_\_\_\_
- v. If it was ASF, can you briefly describe it (clinical signs/post mortem picture)?  
\_\_\_\_\_  
\_\_\_\_\_
- vi. Do you know how pig gets infected? \_\_\_\_\_ 0= Yes, 1= No
- vii. If yes, please explain \_\_\_\_\_  
\_\_\_\_\_
- viii. If yes, where did you get the information? 0 = from my fellow pig keepers, 1 = extension officers, 2 = from researchers, 3 = from pig traders, 4 = media (radio and television), 5 = formal education (knowledge through reading), 6 = others (specify) \_\_\_\_\_
- ix. Have you ever bought/receive meat from wild pig? \_\_\_\_\_ 0= Yes; 1= No
- x. If yes, how frequently? \_\_\_\_\_ 0 = always/often, 1 = only occasional
- xi. Do you know if there is a relationship between the disease in pigs and wild pig meat? \_\_\_\_ 0= Yes; 1= No
- xii. Have you ever feed your pigs with leftover of wild pig meat? \_\_\_\_\_ 0= Yes; 1= No; 2= Don't remember
- xiii. What efforts did you put to control the disease? \_\_\_\_\_ 0= Slaughtering, 1= Selling, 2= Treatment, 3= Others (specify) \_\_\_\_\_
- xiv. Was there involvement of village/local government to control the disease? \_\_\_\_\_ 0= Yes, 1= No
- xv. If yes, what did they recommend? \_\_\_\_\_ 0= Slaughtering, 1= Selling, 2= Others (specify) \_\_\_\_\_
- xvi. Have you ever heard or experienced about cysts in pigs? \_\_\_\_\_ 0 = Yes, 1 = No
- xvii. If yes, when did you get aware of the disease for the first time? (year)  
\_\_\_\_\_
- xviii. Briefly explain your understanding on the disease  
\_\_\_\_\_  
\_\_\_\_\_
- xix. Do you know how pigs get infected with cyst? 0 = Yes, 1 = No
- xx. If yes, please indicate the causes of the infestation  
1. \_\_\_\_\_  
2. \_\_\_\_\_
- xxi. If yes, where did you get the information on the disease  
0 = from my fellow pig keepers, 1 = extension officers, 2 = from researchers, 3 = from pig traders, 4 = media (radio and television), 5 = formal education (knowledge through reading), 6 = others (specify)  
\_\_\_\_\_
- xxii. How serious is porcine cysticercosis in this village. 0 = non-existence, 1 = it is present but not serious, 2 = moderate serious, 3 = it is serious problem, 4 = I am not aware
- xxiii. Have you ever encountered cases of cysticercosis infection in your pig herd?  
0 = Yes, 1 = No, 2 = not sure
- xxiv. If yes, which methods do you use to understand/diagnose the infected pig  
1. \_\_\_\_\_

2. \_\_\_\_\_

#### H. Hygiene: extent of latrine use, water access and use

- i. Presence and use of latrine (enumerator should request permission to assess the latrine)  
0 = present and being used, 1 = present but not used, 2 = the construction started, 3 = absent
- ii. Type of latrine 0 = pit latrine, 1 = flushing toilet, 2=others (specify)  
\_\_\_\_\_
- iii. For household using latrine, the interviewer should assess the following
  - a) The status of walls 0= completed/strong with enough protection, 1 = incomplete/weak
  - b) The status of roof 0 = reasonable strong, 1 = present but week, 2 = latrine has no roof
  - c) Is the latrine having a closing door? 0 = Yes, 1 = No
  - d) Latrine base floor 0 = earthed, 1 = cemented, 2 = timber floor,
  - e) Presence of human faeces on the floor surface or elsewhere around the latrine: 0=Yes, 1= No.
- iv. Which are the sources of water for your household? 0 = tap water, 1 = shallow borehole, 2= deep borehole, 3 = springs, 4 = river, 5 = others (specify) \_\_\_\_
- v. Do you ever do anything with the water before drinking it? \_\_\_\_\_ 0= Yes; 1= No
- vi. If yes, what do you do? \_\_\_\_\_ and why? \_\_\_\_\_ 

No.
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Appendix 2: Questionnaire administered to pork traders to assess the status of pork inspection and knowledge of porcine cysticercosis in urban/peri-urban areas of Morogoro region, Tanzania, 2010/2011.

#### A. General information

- i. District \_\_\_\_\_ Division \_\_\_\_\_  
Ward \_\_\_\_\_ Street/village \_\_\_\_\_
- ii. Date of interview \_\_\_\_\_ Name of enumerator \_\_\_\_\_

#### B. Pork trader characteristics

- i. Name of respondent \_\_\_\_\_ Gender 0 = male, 1 = female \_\_\_\_\_
- ii. Category of pork trader \_\_\_\_ 0 = slaughtering and sell to retailers; 1 = pork retailer; 2 = both, as pork slaughter and retailer
- iii. If slaughtering and sell to retailers, what is the average number of pigs slaughtered \_\_\_ and estimated total weight (kg) of pork obtained per day \_\_\_\_\_
- iv. If pork retailer, what is the estimated weight (kg) of pork purchased per day \_\_\_\_\_

- v. Source of pigs slaughtered \_\_\_\_ 0 = within the village, 1 = neighbouring villages, 2 = far villages, 3 = other districts within region, 4 = other region (specify) \_\_\_\_\_

### C. Pigs acquisitions

What period (month) and particulars (age group, weight, and sex) for the pigs purchased in the past one month?

Pig particulars	Month		
No purchased			
Source			
Age group*			
Estimated weight (kg)			
Sex: 1 = male, 2 = female			

Age group\* = 1 = weaned piglets, 2 = grower (4 – 8 months), 3 = adult

### D. Pork inspection

- ii. How do you know that your pork is fit for human consumption?

\_\_\_\_\_

\_\_\_\_\_

- iii. If he/she indicates presence of meat inspection, then who inspect your pork?

\_\_\_\_\_

- iv. When does the inspection take place? \_\_\_\_ 0 = Before slaughter; 1 = After slaughter; 2 = Any other explanation \_\_\_\_\_

- v. Have your pork ever been condemned? \_\_\_\_ 0 = Yes; 1 = No.

- vi. If yes, what were the causes of condemnation you can remember?

1. \_\_\_\_\_

2. \_\_\_\_\_

3. \_\_\_\_\_

vii. What abnormal conditions have you ever encountered in your pork?

1. \_\_\_\_\_

2. \_\_\_\_\_

3. \_\_\_\_\_

THIS IS THE END OF THE INTERVIEW

THANK YOU VERY MUCH FOR YOUR COOPERATION

Appendix 3: Enzyme – linked immunosorbent assay for the detection of circulating

*Taenia solium* cysticerci antigens (Ag-ELISA) in serum

The monoclonal antibodies used were B158C11A10 used as first – capturing antibody and a biotin labelled B60H8A4 used as a second – detecting antibody.

The serum samples were pre-treated using freshly prepared 5% ( $\frac{w}{v}$ ) Trichloroacetic acid (TCA) (Sigma-Aldrich) dissolved in reverse osmotic de-ionised water (RO – DI water). Pre-treatment was done to breakdown immune-complexes to obtain free circulating antigen so as to increase the specificity and sensitivity of the assay. A 5% TCA solution was prepared by dissolving 1 g of TCA in 20 ml. of RO – DI water. The serum samples were thus pre-treated by mixing an equal volume of serum and 5% TCA and mixed thoroughly by vortexing. For the negative control sera, 75µl of

serum was used while 150µl of serum was used for the pre-treatment of positive control and the test sera. These mixtures of sera and 5% TCA solution were incubated for 20 minutes at room temperature and mixed again by vortexing before centrifuged.

The mixture was centrifuged at 12 000 r.p.m for 9 minutes and the supernatant of the same volume of the added sera were removed and aliquoted into eppendorfs. The pH of the collected supernatant was raised by adding an equal volume of 75µl sodium carbonate/bicarbonate buffer (0.610 M) at pH 10 (neutralization buffer) to the supernatant of the negative control sera and 150µl neutralization buffer to the supernatant of positive control and the test sera. 100µl of this mixture at final serum dilution of 1:4 was used in the Ag-ELISA protocol.

All wells in a plate was coated with 100µl of capturing antibody (B158C11A10) diluted at 5 µg/ml in carbonate buffer (0.06M, pH 9.6) except for the 2 wells for the substrate control in which 100µl of coating buffer were put. The plate was incubated at 37°C for 30 min. while shaking. After coating the wells were washed once with PBS – T20 (washing buffer) and the plate drained thoroughly on blotting paper.

Blocking to avoid non-specific reactive sites was done by adding 150µl per well of PBS-T20/1% NBCS and then the plates were incubated on a shaker for 15 minutes at 37°C. Without washing the plate, 100µl of pre-treated sera at a dilution of ¼ was added in the designated wells and the wells for the substrate and conjugate controls were filled with 100µl of blocking buffer. The plate was incubated at 37°C for 15 minutes while shaking. The plate was drained and washed five times.

100µl of detecting antibody (B60H8A4) diluted at 1.25 µg/ml in PBS – T20/1% NBCS (blocking buffer) was added in all wells, except the 2 wells for the substrate control in which 100µl of blocking buffer were put in these wells. The plate was then incubated while shaking at 37°C for 15 minutes. The plate was drained and washed five times with PBS – T20 as above.

100µl of streptavidin – horseradish peroxidase (Jackson Immunoresearch Lab, Inc.) diluted at 1/10 000 in PBS – T20/1% NBCS to act as conjugate was added in all wells, except the 2 wells for the substrate control in which 100µl of blocking buffer was put in these wells. The plate was incubated at 37°C for 15 minutes while shaking. After incubation, the plate was washed 5 times with washing buffer.

One tablet of the chromogen/substrate, orthophenylene diamine (OPD) was dissolved in 6 ml of RO-DI water. This preparation was done in a dark recipient. Just before the addition of OPD on the plate, 2.5µl of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added. Then 100µl of this solution was added to all wells and incubated at room temperature for 15 minutes in the dark without shaking.

The reaction was stopped by addition of 50µl H<sub>2</sub>SO<sub>4</sub> (4N) in each well. The plates were read using an ELISA reader (Labsystem Multiskan RC) at 492 nm. The 492 nm filter is the most important one, as it is used to measure the maximum absorption.

The cut-off was calculated using a modified Student *t*-test (Sokal *et al.*, 1981) programmed in MS Excel sheet, by comparing the optical density of each serum sample with a series of 8 negative reference serum samples at a probability level of  $P < .001$ . A serum sample was considered as positive when the ratio (optical density of test sample/optical density cut-off) was  $\geq 1.0$ .

Source: *Detection of viable metacestodes of Taenia spp. in human, porcine and bovine serum samples with the use of a monoclonal antibody – based sandwich ELISA*. Institute of Tropical Medicine, Department of Animal Health, Antwerpen (Antwerp), Belgium.

Appendix 4: Enzyme – linked immunosorbent assay for detection of African swine fever antibody (Ab-ELISA) in pig serum

1. *Preparation of Buffers:*

- i. Coating buffer for ASF antigen (0.05M carbonate/bicarbonate buffer), pH 9.6

One carbonate/bicarbonate tablet was dissolved in one litre of distilled water and pH adjusted to 9.6. The preparation was stored at +4°C.

- ii. Washing buffer (PBS, pH 7.4)

One PBS sachet was dissolved in one litre of distilled water to yield 0.01M phosphate buffered saline, 0.0138M NaCl, 0.0027M KCl, pH 7.4 at 25°C.

2. *Diluent for Sera and Conjugate (PBS/Tween 20 )*

One PBS sachet was dissolved in one litre of distilled water to yield 0.01M phosphate buffered saline, 0.0138M NaCl, 0.0027M KCl, pH 7.4 at 25°C. 0.5 ml. of Tween 20 was then added to yield PBS – Tween 20, 0.05%.

### 3. *Preparation of Chromogen – OPD*

This should be prepared when required. Ortho-phenylenediamine (OPD) one tablet was dissolved in 75ml. distilled water and agitated to dissolve completely.

### 4. *Preparation of substrate - H<sub>2</sub>O<sub>2</sub> (3%)*

One tablet of Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was dissolved in 10ml. distilled water and stored at +4°C in the dark. Just before the addition of OPD to the ELISA plate, hydrogen peroxide should be added at 4-1 per ml. of OPD (e.g. 40 µL of H<sub>2</sub>O<sub>2</sub> in 10 ml. OPD), mixed thoroughly and used immediately.

### 5. *Stopping Solution (1M sulphuric acid)*

16.1 ml. of concentrated Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was added in 200 ml. distilled water. Water was measured first and sulphuric acid added slowly.

### 6. *Disinfectant Solution*

25.0g of VIRKON powder was added to 5 litres of tap water.

## ***Test method***

Two, 5 litres, buckets of "VIRKON" disinfectant was prepared at 1/200 (25g in 5litres). One bucket was kept near the sink and the other at the workbench.

ASF positive antigen (VPPA, batch number A - 389 supplied by INIA) was diluted according to INIA recommendation **1 in 1 600** in coating buffer (50µL of antigen plus 80 ml. of coating buffer). 100µL of the diluted antigen was placed (coating step) to each well of NUNC - PolySorp ELISA microplates and incubated overnight (18 hrs.) at +4°C in sealed plastic bags.

In order to fasten the dilution of sera and simplify the whole process of working with sera, round bottom well microplates (haemagglutination plates) similar to ASF ELISA plates were used. The plates were half – divided as shown below:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Column number 1 and 2 were reserved for ASF control sera while the rest for the test sera. The sera to be tested were then recorded on the ASF ELISA worksheets.

The next day the whole contents of coated plates were discarded into disinfectant and the plate(s) were washed 6 times in wash buffer. The plates were blot to dry onto paper towelling and discarded into the disinfectant.

The serum samples, ASF positive control serum (C+, batch number PPA 90, supplied by INIA), ASF negative serum (C-, batch number PPA 49, supplied by INIA) and ASF limit control serum (CL, batch number PPA 8) were diluted **1 in 30** (3.3 µL of sera plus 96.7 µL PBS/Tween 20) using round bottom well microplates.

The ASF ELISA worksheets were used as a reference to add test and control sera. Therefore 100µL per well of pre-diluted test and control sera were added to coated plates by using a multi-channel micropipette. All sera were tested in duplicate. ASF positive control serum was added to wells C1 and C2, D1 and D2. ASF limit control serum was added to wells E1 and E2, F1 and F2, ASF negative control serum was added to wells G1 and G2, H1 and H2. The plates were then incubated at 37°C for 1 hour on incubator shaker. Following the ASF ELISA worksheets, 40 test sera were tested in each plate.

The contents of plate(s) were discarded into disinfectant and the plates washed 6 times in wash buffer. The plates were blotted to dry on paper towelling.

A stock solution of Protein-A horseradish peroxidase conjugate was prepared **1 in 5 000** in PBS/Tween 20 (9.6µL conjugate in 48ml. PBS/Tween 20) and 100µL of conjugate was added per well and incubated at 37°C for 1 hour in a shaker.

The contents of the plate were discarded into the disinfectant and the plates washed 6 times in wash buffer and the plates were blotted to dry on paper towelling.

The ELISA reader was switched on to allow warm up 30 minutes before the plates were read. Since the reader was directly connected to the computer, filter 492nm was then selected.

Addition of substrate solution: OPD tablet was dissolved completely in 75 ml. of distilled and 40µL of H<sub>2</sub>O<sub>2</sub> solution was added in each 10 ml. of OPD solution. 100 µL of substrate solution was then added per well and incubated at room temperature in the dark for 10 minutes. A new ELISA plate, to be called a "blanking" plate was simultaneously prepared by addition of 100µL per well of the chromogen/substrate solution to column 1, wells A to H.

After 10 minutes of incubation at room temperature, the reaction mixture was stopped by addition of 1M sulphuric acid, 100µL per well and repeated also for column 1 in the "blanking" plate.

The "blanking" plate was first used to blank the ELISA reader before the plates can be read. The Multiskan SX reader was used and the blanking step was as follows:

The "blanking" plate was placed on the carriage of the ELISA reader and at the "In" message the "STEP" key was pressed. The plate was then automatically drawn into the machine under the detectors and stopped when column 1 is in position. The "blank" message was printed out. The "BLANK" key was pressed and the LED display showed the blanking proceeding message. The blanking was accepted by the message "Blank OK Col. 1 Ready" and it was printed out. The "In" message was automatically displayed indicating that the reader is ready to read the test plates.

The first plate was placed, containing the test sera, on the carriage and the "START" key was pressed. The plate was automatically entered the machine and the optical density (OD) of each well measured.

The colour intensity is proportional to the amount of specific antibody bound to the antigen. ELISA results should always be evaluated in relation to the controls.

A serum was considered ASF antibody **positive** if it has an OD value **greater** than **twice** the mean OD value of the control ASF negative serum.