Risk factors for bovine cysticercosis in a large commercial South African cattle feedlot

By

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Supervisors
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DECLARATION

I, Dirk J Verwoerd, student number 85071201, hereby declare that this dissertation, *Risk factors for bovine cysticercosis in a large commercial South African cattle feedlot*, is submitted in accordance with the requirements for the Magister of Science degree at the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, is my own original work and has not previously been submitted to any other institution of higher learning. All sources cited or quoted in this research paper are indicated and acknowledged with a comprehensive list of references.

Dirk J Verwoerd

12 June 2017

DEDICATION

To my loving wife; Liza and wonderful children; Lise-Marie, Danielle and Wilhelm:

‘There is a joy in madness sure,
Which none but madmen know...’

I am truly Blessed.

ACKNOWLEDGEMENTS

Prof Peter Thompson; for his support, advice and statistical expertise given during a time of high work pressure even though he was not part of the original team of study leaders when we embarked on this project.

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DISSERTATION SUMMARY

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Summary

Taeniid tapeworms have very simple lifecycles with the adult stage developing in the intestine of the obligate human host, after the ingestion of viable cysticerci in raw/undercooked meat. Bovine Cysticercosis follows epidemic outbreak patterns in a cattle feedlot. These peaks/point source epidemics have been related to contaminated feed or water, and/or to specific infected workers. Taeniosis in humans caused by *T. saginata* is a relatively mild condition; easy to control through regular deworming and improved personal sanitation practices, while positive cysticercosis carcasses caused by *T. saginata* result in very costly decontamination processes through freezing, that have a huge negative financial impact on the beef industry.

This study is a Retrospective Analysis of all abattoir data from 645 634 Karan Beef feedlot cattle slaughtered during 1 January 2009 to 31 December 2010. The main objective was to establish a scientific basis for the development of an effective cysticercosis risk avoidance and impact mitigation strategy at this feedlot. Positive cysticercosis carcasses based on standard meat inspection procedures by independent meat inspectors at the

Karan Beef Balfour Abattoir were related to their individual carcass classification and feedlot production histories as well as group/lot identities. A total of 17 561 cysticercosis infected carcasses were found, giving an overall prevalence of 2.72%.

Data were examined using Descriptive Univariate and Multivariable Analyses, followed by detailed univariate analyses of putative risk factors.

Individual risk factors were: Sex [Male (Bulls & Steers) vs Heifers], Arrival live weight groups (4), Breed, Days on Feed.

Lot based risk factors were: Buyer, Geographical origin/loading point, purchase channel, farm type, number of measles/lot, rainfall season when purchased, drinking water turbulence vs stagnation during their feeding period, risk period per geographical area (high vs low) using high/low population density areas during high/low rainfall periods as categories, Bovine Respiratory Disease Risk as proxy indicator for cysticercosis.

Cattle sourced via speculators and from small farms or communal herds as well as from certain dry areas where humans and cattle congregate/concentrate around limited water sources and similarly collection/concentration strategies such as holding stations, carried a significantly higher cysticercosis risk compared to controls. In addition cattle that arrived at a younger age (weight class) as well as cattle fed through the rainy season carried a higher risk. All of these factors indicate practical control measures in terms of purchase strategies, periods of more important staff deworming programmes and even future targeted vaccination strategies.

LIST OF ABBREVIATIONS

BRD	Bovine Respiratory Disease
DVTD	Department of Veterinary Tropical diseases
EC	Eastern Cape
FS	Free State
GAP	Good Agricultural Practice
GP	Gauteng
HS	Holding Station
ITM	Institute of Tropical Medicine
KZN	KwaZulu-Natal
MP	Mpumalanga
NC	Northern Cape
NW	North West
OR	Odds Ratio
P	P-value for statistical significance
Prev	Prevalence
UP	University of Pretoria
WC	Western Cape

1. INTRODUCTION

Cysticercosis and Taeniosis are cosmopolitan foodborne zoonotic infections associated with tapeworms where the former indicates a tissue infection caused by the larval cysticercus or metacestode stage, most commonly in pork (*Taenia solium*), pig organs (*Taenia asiatica*) and cattle (*Taenia saginata*). The mature parasite stage of these three Taeniid tapeworms occur in the human intestine while an aberrant self- infection cycle with *T. solium* only, can result in human neural cysticercosis, causing epileptiform fits, this is not the case with the cattle tapeworm *Taenia saginata* (Murrell et al., 2005).

These and other differences have often been overlooked in efforts to control the public health impact of these zoonoses. Neurocysticercosis is now accepted as one of the most important causes of epilepsy, particularly in developing nations characterised by poor sanitation, dependence on contaminated surface water, rapid increase in smallholder pig production and close contact between humans and pigs, and is now part of a World Bank sponsored global public health campaign ('Out of the Shadows; making mental health a global priority') (Anon, 2015; Slorach, 2011). Human cysticercosis control in eastern and southern Africa is coordinated by a dedicated working group of medical, veterinary, and social scientists established in 2002 (Anon, 2007). In terms of global public health significance *T. solium* was ranked 1st and *T. saginata* 19th out of 24 foodborne parasites evaluated by a joint FAO/WHO expert panel in 2012, using a multicriteria based ranking for risk management approach (Anon, 2012), clearly demonstrating where emphasis must be focussed.

Taenia saginata occurs worldwide but is of particular importance in Africa, Latin America, Asia and some Mediterranean countries. The prevalence of taeniosis caused by *T. saginata* can be classified into three broad categories: (i) highly endemic regions with prevalences > 10%, (ii) regions with moderate prevalences, and (iii) areas with a prevalence < 0.1% or where the condition is absent altogether. This situation is naturally a mirror image of the distribution and prevalence levels recorded for bovine cysticercosis (Pawlowski & Schultz, 1972; Lloyd, 1998; Froyd, 1965; Viljoen, 1937). The prevalence of bovine cysticercosis in Africa varies widely between countries and also between different studies conducted in the same country. Ethiopean studies have reported prevalences of 2.2% to 26.3% (Kumar & Tadesse, 2011) and 19.7% (Terefe, Redwan &

Zewdu, 2014) respectively, and even as high as 80%, with the situation increasing in Botswana and Nigeria (Pozio 1991). A recent retrospective study of abattoir carcass inspection data from over 1.4 million cattle slaughtered at 26 abattoirs between January 2010 and December 2013 in Gauteng Province, South Africa, reported an overall prevalence rate of 0.7%, with a significantly higher identification rate from feedlot cattle compared to those from non-feedlot origin (Qekwana 2016). Figure 1 below illustrate the interactions between the various introductory mechanisms and risk factors associated with *T. saginata* and (extensive) cow/calf cattle herds and (intensive) cattle feedlots against the backdrop of the very simple, direct lifecycle of the parasite.

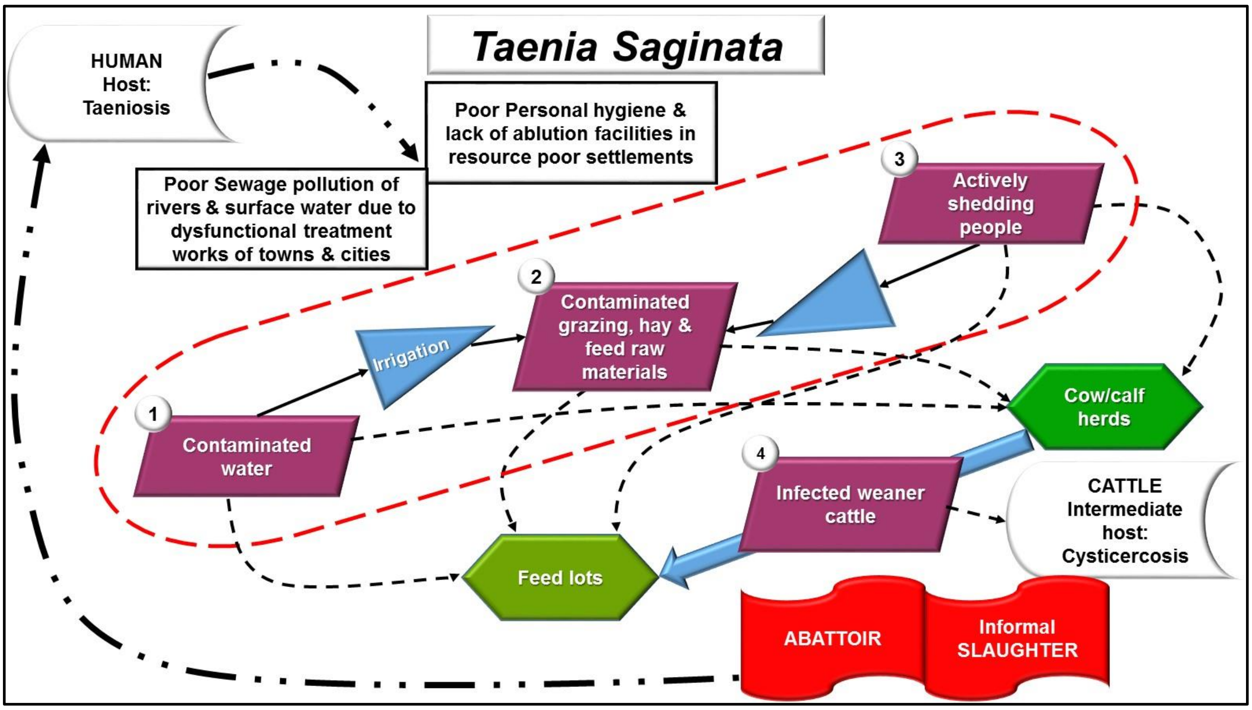

Figure 1 Risk factors and introductory mechanisms of *Taenia saginata* and beef feedlots. Numbers 1-4 indicate the main sources of *T. saginata* eggs for feedlot cattle.

(Drawn from information in literature as well as personal observations in beef cattle feedlots and abattoirs since 2001)

This study focus on the specific bovine cysticercosis risk factors related to the cattle profile of Karan Beef, the largest cattle feedlot in South Africa, situated near Heidelberg, Gauteng Province, retrospectively analysing data from 2009-2010. This period was chosen as representative of the situation before a structured, integrated cysticercosis management programme was put in place (from February 2011) and the data was analysed to provide a scientifically sound basis for the lot based risk profiling that forms part of this programme. By the end of 2012 this approach was clearly a success (Figure 2 below) and it was decided to investigate the 2009-2010 database more thoroughly and with the benefit of deeper insight and experience gained as fulltime staff feedlot veterinarian expand the range of putative risk factors, so that the eventual sharing of information/experience through this dissertation and resultant publication(s), can benefit the entire South African Feedlot Industry and consumers.

The Karan Beef Cysticercosis Management Programme rests on three pillars, based on a HACCP (Hazard Anaylsis of Critical Control Points) paradigm (Havelaar, 1994; Mortimore et al., 2013), (see Figure 1 above) whereby the major risk factors and introductory mechanisms as well as incorrect diagnoses are addressed. The effects of this programme are clear from the reduction in measles monthly prevalence (%) illustrated in Figure 2. To date (April 2017) the prevalence have remained in a narrow band around 0.5%. The three pillars are:

1. Regular, three-monthly deworming of all staff in the feedlot as well as holding stations, verified through a register and audited annually as part of the ISO 14001 Environmental Impact Assessment scheme.
2. Quality assurance of the independent meat inspection cysticercosis diagnoses at the Balfour Abattoir through second opinion (Experienced Veterinary Public Health Specialist Veterinarian or designated Senior Meat Inspectors) of all suspected cysticercosis carcasses. All lesions are sampled in formalin and this programme is supported by independent histological evaluation of lesions by an experienced Veterinary Pathology Specialist and regular communication between all parties (Verwoerd & Prozesky 2012).
3. Retrospective epidemiological analyses of slaughter data identifying risk factors and clusters of origin characterised by high levels of cysticercosis (this study). This information is combined with similar analyses that focus on respiratory health and growth performance achieved, leading to adjustments in calf purchase strategies.

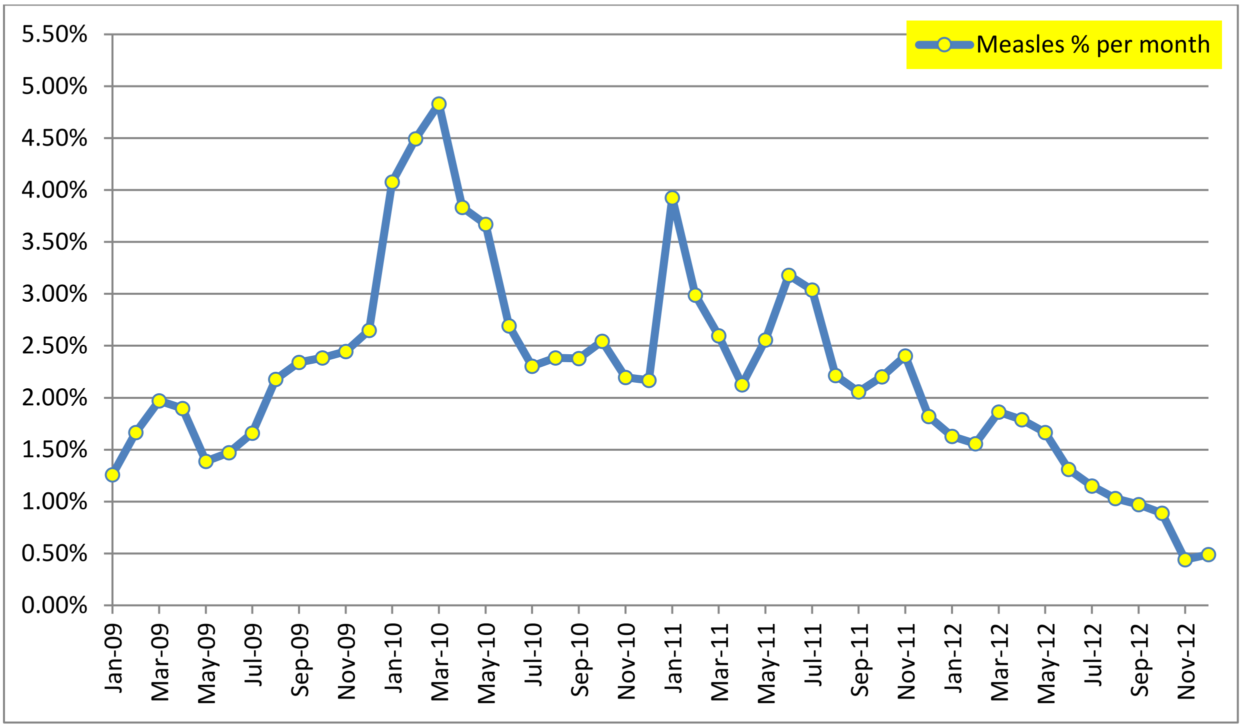

Figure 2 Prevalence (%) of measles in cattle slaughtered per month at Karan Beef Balfour Abattoir; January 2009 to December 2012, based on carcass inspection.

2. LITERATURE REVIEW

2.1 Taxonomy and morphology of Taeniid tapeworms

Tapeworms of the genus Taenia (*T. saginata, T. solium* & *T. saginata asiatica*) are flat, white or yellowish in colour, exceptionally long segmented intestinal parasites, measuring 1-12 m in their adult stage. A conspicuous feature of tapeworms is the lack of a mouth and digestive tract. The outer cellular cover, the tegument, functions in absorption, digestion, protection, and traction. The tegument envelops the entire tapeworm, with the most external layer a brush-border formed by microtriches that are microvilli like structures covered by a glycocalyx (thin layer of glycoproteins and mucopolysaccharides) (Hyman, 1951; Harrison & Bogitsch, 1991; Smith & McManus, 1989).

The head is called the scolex, and is the attachment organ. It has four suckers and a rostellum that are armed with hooks (*T. solium*), unarmed and sunken (*T. saginata*), or with rudimentary hooklets (*T. saginata asiatica)*. When hooks are present they are organised in two rows of 22-23 and range in size from 110-180 µm. This characteristic can be used in identification to species level. The scolex is the size of a pin head and is followed by a short undivided region; the neck, from which a long chain of proglottids/ segments (also termed the strobila) proliferate. The strobila has the appearance of a ribbon and may consist of more than a thousand proglottids. These gradually increase in size so that the posterior end of the tapeworm has the broadest, longest and oldest proglottids, gravid with eggs (Andreassen, 1989; Schmidt, 1986; Hyman, 1951; Harrison & Bogitsch, 1991; Flisser, 1989; Verster 1969).

Mature segments are hermaphroditic and contain several hundred testes, connected by fine sperm ductules that anastomose to form the vas deferens, which ends in the genital pore, forming a highly muscular cirrus. The female sexual system consist of one bilobulated ovary, connected to an oviduct. The vagina is a sinuous tube that connects the genital atrium to the oviduct while the vitelline glands are also connected to the oviduct. The oviduct, where fertilisation takes place, transforms into the central sac or uterus, once the gonads have attained maturity. Gravid proglottids resemble sacs full of eggs (50 000 to 80 000 each) and are approximately 0.5 cm wide by 1-2 cm long. The egg containing uterus develop 7-32 lateral branches, depending on the species. This is another characteristic that can be used in species identification; *T. solium* (7-11 branches), *T saginata* (12-32 branches) (Hyman, 1951; Schmidt, 1986; Harrison & Bogitsch, 1991).

The eggs of all taeniids are spherical, ranging in size from 20-50 μm, and are indistinguishable from each other by light and electron microscopy. When eggs are released from the definitive host, many are fully embryonated and infective to a suitable host, but others may be at different stages of maturation. The embryophore is a rigid structure made up of contiguous blocks of a keratin-like protein that surrounds and protects the oncosphere while the egg is in the external environment, rendering the eggs extremely resistant to a wide range of environmental temperatures. When eggs are ingested by a suitable intermediate host, the cementing substance that joins the embryophoric blocks is susceptible to enzymatic digestion which allows the oncosphere to be released (Andreassen, 1998; Flisser, 1998; Hyman, 1951; Schmidt, 1986; Harrison & Bogitsch, 1991).

The process of infection by the oncosphere of *T solium* in pigs was described histologically (Yoshino, 1933a, b, c; De Aluja & Vargas, 1988; Blazek et al., 1981). The microscopic oncospheres as well as transitional forms were observed in the intestinal mucosa from 15 to 48 hours after the eggs were ingested. After intestinal penetration postoncospheral development leads to the formation of the cysticercus. Macroscopic cysticerci were identified in the liver, skeletal muscles and brain of pigs six days after infection, measuring around 0.3 mm, and 60-70 days later they had a fully developed scolex and measured 6-9 mm. Fully developed cysticerci measuring 8-15 mm were found 177 to 325 days post infection. At the initial stage of the infection, young cysticerci stimulate a minimal surrounding inflammatory reaction, while older parasites, or those treated with a cesticidal drug, stimulate an intense reaction that includes eosinophils, lymphocytes and macrophages (Yoshino, 1933a, b, c; Wilms & Merchant, 1980). These histologically differentiable characteristics allow accurate identification between cysticerci and non specific macroscopical lesions (false positives) identified during standard meat inspection as well as assist in the determination of when the infection most likely occurred; before entering the feedlot or during the feeding period (Verwoerd & Prozesky, 2012).

2.2 Life cycle of Taeniid tapeworms

Taeniid tapeworms (*T. saginata, T. solium* & *T. asiatica*) have very simple life cycles with the adult stage developing in the intestine of the obligate human host, after the ingestion of viable cysticerci in raw/undercooked beef (*T. saginata*), pork muscle (*T. solium*) or pork organs and intestines (*T. asiatica*) respectively. This condition is called 'taeniosis' in contrast to 'cysticercosis', the condition that describes cysts lodged in muscles, subcutaneously, central nervous system or organs of the intermediate host. Migration of larvae, which eventually lodge in nervous tissue and cause epileptiform fits, due to 'self-infection' by humans with *T. solium*, is called 'neurocysticercosis' (Murrell et al., 2005; Figure 3).

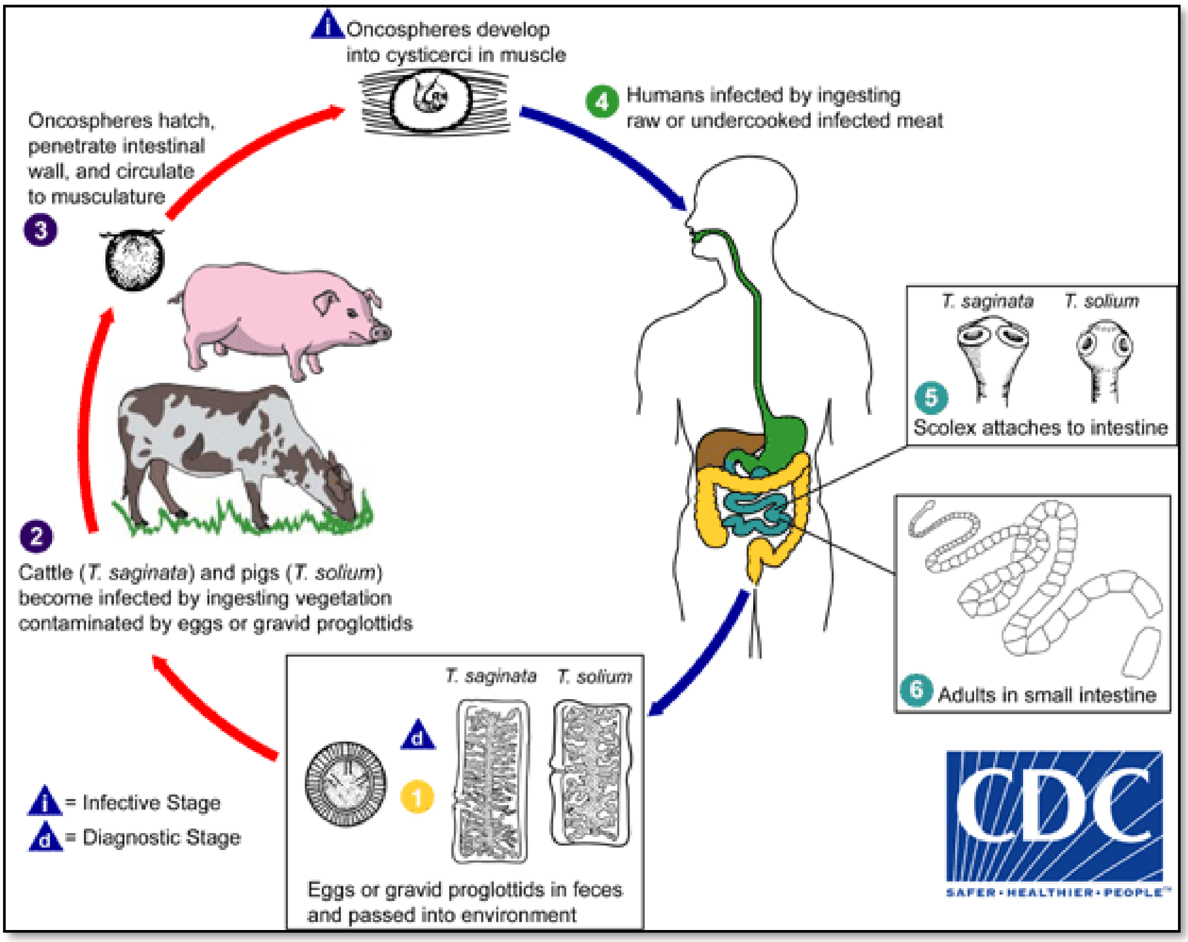

Figure 3 Life cycles of the *Taenia* spp. tapeworms

Taeniasis is the infection of humans with the adult tapeworm of *Taenia saginata* or *Taenia solium*. Humans are the only definitive hosts for *T. saginata* and *T. solium*. Eggs or gravid proglottids are passed with faeces 1; the eggs can survive for days to months in the environment. Cattle (*T. saginata*) and pigs (*T. solium*) become infected by ingesting vegetation contaminated with eggs or gravid proglottids 2; In the animal's intestine, the oncospheres hatch 3; invade the intestinal wall, and migrate to the striated muscles, where they develop into cysticerci. A cysticercus can survive for several years in the animal. Humans become infected by ingesting raw or undercooked infected meat 4. In the human intestine, the cysticercus develops over 2 months into an adult tapeworm, which can survive for years. The adult tapeworms attach to the small intestine by their scolex 5 and reside in the small intestine 6. Length of adult worms is usually 5 m or less for *T. saginata* (however it may reach up to 25 m) and 2 to 7 m for *T. solium*. The adults produce proglottids which mature, become gravid, detach from the tapeworm, and migrate to the anus or are passed in the stool (approximately 6 per day). *T. saginata* adults usually have 1,000 to 2,000 proglottids, while *T. solium* adults have an average of 1,000 proglottids. The eggs contained in the gravid proglottids are released after the proglottids are passed with the feces. *T. saginata* may produce up to 100,000 and *T. solium* may produce 50,000 eggs per proglottid respectively.

Life cycle image and information courtesy of DPDx.

The tapeworm reaches sexual maturity in approximately three months, whereafter it continuously produces gravid proglottids that are shed in faeces (*T. solium*) or actively ‘crawl’ out of the anus (*T. saginata*). Infected humans experience mild abdominal discomfort, weight loss and anal pruritis, clinical signs indistinguishable from a host of other conditions including multispecies intestinal helminthiasis (Dorny & Pratt, 2007).

Cattle become infected by accidental ingestion of parasite eggs during normal grazing behaviour or from contaminated raw material/hay in complete rations or water. The larvae migrate through the intestinal wall and distribute via the blood stream, forming visible cysts in muscles, subcutaneously, central nervous system or organs from as early as 21-30 d to 8-10 w after ingestion (Murrell et al., 2005; Table 1).

A strong immune response by the bovine host results in encapsulated, dead, calcified lesions that are non-infectious while the associated circulating antibodies can be used to detect a history of exposure using various serological assays. This high immunogenicity of the cysticerci forms the basis of the development of effective vaccines against Taeniid tapeworm infestation in the various intermediate hosts (Murrell et al., 2005; Lightowlers, 2010).

2.3 Prevention of Taeniosis and Cysticercosis

The prevention of infection of humans and subsequent environmental contamination with taeniid eggs is of paramount importance in both prevention and control schemes. The development of improved sanitation and hygiene practices have had a major impact on the occurance of cysticercosis in developed countries, and also amongst urban populations in developing countries, because of their effect on the transmission of taeniid eggs (Gilman et al., 1999). The transmission of taenia in rural areas or periurban informal settlements is greatly facilitated where the exposure of pigs and cattle to human faeces is high, however the installation of adequate sanitation and the adoption of safe animal husbandry practices are often problematical in such resource poor communities.

The relatively simple lifecycle of the parasite suggests that any interruption of the link between intermediate (cattle) and definitive/obligate (humans) hosts will lower the risk of contracting taeniosis/cysticercosis, major efforts have traditionally been on the identification and treatment of infected carcasses at slaughter, to prevent their assimilation in the human food chain. However, these zoonoses persist, especially in areas where poor sanitation, ineffective sewage treatment plants, irrigation with raw sewage/heavily contaminated surface water, free range or smallholder pig production and informal slaughter practices prevail. This is due to the enormous reproductive potential of Taeniid tapeworms where adults measuring 1-12 m release one or more gravid proglottids (segments 0.5 cm x 1-2 cm sized) daily, each containing 50 000 to 80 000 eggs (Schmidt, 1986; Hyman, 1951).

Thus effective prevention strategies must rely on multiple approaches following an integrated programme that consist of the following: (Mann, 1958; Gilman et al., 1999; WHO Guidelines on slaughterhouses1982; Gracy, 1999; Kyvsgard, Ilsoe, Henriksen & Nansen, 1990).

2.3.1 Meat carcass inspection to prevent human infection (taeniosis),

2.3.2 Improved farm livestock management to ensure that pigs and cattle are protected from ingesting feed or water contaminated with human faeces to prevent cysticercosis in these animals,

2.3.3 Screening and treatment of farm workers or regular (3 monthly), controlled deworming of all staff on large farming enterprises,

2.3.4 Proper treatment of sewage effluent and sludge to kill taeniid eggs, and the regulation of the use of effluent and sludge for agricultural purposes, (see discussion below).

2.3.5 Control of pig and cattle marketing systems to prevent clandestine marketing and slaughter practices that bypass certified official, inspected channels. These should include the provision of incentives to ensure owner compliance.

2.3.6 Health education of both farmers/livestock owners and the general public/consumers, especially on the risks associated with eating raw undercooked meat. Effective programmes target meat inspectors, primary health care workers, school teachers, pharmacists, butchers and food handlers, people involved in ritual/cultural/informal slaughter, campers, tourists and hunters.

Latrines should be provided to all farm workers, placed and managed in a manner that will ensure their correct use. Basic education and supervision is required in many instances to ensure compliance. Latrine waste and septic tank contents must be disposed of correctly, ensuring that it is not spread on pasture fieds used for the cultivation of crops. Several outbreaks cysticercosis storms have been associated with the mixing of septic tank contents with animal manure, spread on pastures (Nansen & Henriksen, 1986; Ilsoe, Kyvsgard, Nansen & Henriksen, 1990). The only efficient method to physically remove taeniid eggs from the effluent of secondary sedimentation tanks or dams seems to be sand filtration. To be effective the sand filter should have a depth of at least 0.6 m with sand of 0.5 mm effective size and 2.2 uniformity coefficient (Newton, Bennett & Figgat, 1949). Observations in practice and laboratory experiments indicate that the activated sludge treatment process has no apparent deleterious effect upon taeniid eggs and they have survived for five months in this process. In many sewage treatment plants the sludge is subjected to anaerobic, mesophylic, alkaline digestion at temperatures ranging from 28-34 ºC. Taeniid eggs survive even these conditions although an impractical detention time of 56 days seemed to be effective. Natural drying of sludge will also be ineffective as the elimination of taeniid eggs require low humidity and temperatures above 40 ºC.

A common agricultural practice is the deliberate use of raw sewage to fertilise pastures used for grazing or growing of livestock fodder, which naturally facilitate the infection of cattle. Livestock should not be allowed to graze pasture treated with undigested sludge for at least six months as the viability of taeniid eggs are greatly reduced after that time, although if the resting period is during winter, the lower temperatures will increase egg survival (Ilsoe et al., 1990; Nansen & Henriksen, 1986; Gemmel, 1977; Greenberg & Dean, 1958).

Using 'tracer calves' it has been found *T. saginata* eggs dispersed on pastures by deliberate irrigation with effluent, remained available and infective for at least 4-5 months or more at 32 ºC, although eggs failed to infect when silage was pressed and dried into pellets at temperatures exceeding 50-60 ºC during the pelleting process (Burger & Wilkens, 1982).

2.4 Diagnosis and treatment of Taeniosis and Cysticercosis

The pathobiology, clinical manifestations and treatment options of human cysticercosis, especially the neurocysticercosis manifestation caused by *T solium*, have been reviewed extensively in many standard texts e.g. Webbe (1994), and falls beyond the scope of this dissertation.

The detection and treatment of human taeniosis is however very important in the context of this epidemiological study, even though the condition is of moderate importance in human primary health care. The presence of a large worm in the intestinal tract, frequently accompanied by various other intestinal helminth parasites, causes abdominal and physical discomfort and interfere with the digestive/absorbtive processes. These are very non specific clinical signs so treatment is often stimulated by a general concern regarding intestinal helminthiasis. Classical parasitological techniques or simply questionnaires have limited value due to the intermittent shedding patterns of taeniid tapeworms (Kern & Pawlowski, 2002; Pawlowski & Schultz, 1972). Modern antigen capture ELISA immunological techniques have in recent times become available and shows significant promise for regular screening of farm workers upon employment or to induce a targeted treatment programme rather than a general (costly) preventative treatment cycle of all workers in large farming enterprises. The detection of coproantigens is based on the identification of parasite–specific antigens in the faeces of infected hosts using antibodies raised against parasite material, thus diagnosing current infections, even during prepatent/early infections when parasite eggs are absent from faeces. Genus specificity (*Taenia* spp. vs *Hymenolepsis* spp.) has been achieved and coproantigen levels drop to background levels within approximately one week of successful treatment. Taeniid coproantigens are stable for days in unfixed samples at room temperature and for months/years either in frozen samples or kept in formalin (Allan, Avila, Garcia-Naval, Mencos, Liu, Wen, Zhou, Stringer, Rogan & Zeyle, 1992; Allan, Wilkens, Tsang & Craig, 2003).

Currently used drugs effective for the treatment of taeniosis (WHO guidelines 1983):

2.4.1 **Niclosamide**: a halogenated salicylanilide, poorly absorbed from the intestine thus well tolerated and safe. The efficacy for taeniosis is about 85%, in a single dose of 2 g for adults, 1 g for patients < 35 kg, and 0.5 g for children < 10 kg. Side effects are neglible and associated with the disintegrating tapeworm.

2.4.2 **Praziquantel**: an acylated isoquinoline-pyrazine, well absorbed from the intestine thus active against tissue parasites such as cysticerci, liver flukes and schistosomes. The efficacy for taeniosis is about 95%, in a single dose of 5-10 mg/kg bodyweight for intestinal tapeworms, to 40-60 mg/kg for tissue parasites, continued for a few days. Adverse reactions are rare, mainly abdominal discomfort, pyrexia and urticaria.

2.4.3 **Albendazole**: a benzimidazole, is widely used against common nematode invasions such as ascariosis, trichiurosis and hookworm infections at 400 mg per adult once, showing some efficacy against tapeworms when given in much larger doses over a few days. Neurocysticercosis (*Taenia solium T*apeworm):

> 60 kg: 400 mg PO BID x 8-30 days

< 60 kg: 15 mg/kg/day divided BID PO x 8-30 days; not to exceed 800 mg/day

Hydatid (*Echinococcus* Tapeworm)

> 60 kg: 400 mg PO BID x 28 days, THEN 14 drug-free days x 3 cycles

< 60 kg: 15 mg/kg/day divided BID PO, no more than 800 mg/day x 28 days, THEN 14 drug-free days x 3 cycles

2.5 Detection and control of bovine cysticercosis (*Taenia saginata*)

Current detection of infected cattle is done according to a specific warm carcass inspection protocol based on organoleptic evaluation of so-called predilection sites (heart, diaphragm, masseter muscles, oesophagus and tongue) in abattoirs. These are the anatomical sites with highest density of cysts, while in some cases the triceps brachii has also been shown to have a higher density than the rest of the carcass. The inspection methodology is defined by the OIE and followed closely by all member states and described in detail in standard textbooks on meat hygiene (Murrell et al., 2005; Gracy, 1999; Kyvsgard et al., 1990; Maeda, Kyvsgard, Nansen & Bogh, 1996; McCool, 1979). The chronology of the development of cysticercosis cysts has been determined (Table 1) and show a very long potential infectious period although the strong host immune response has an apparent rapid effect in heart muscle and a delayed effect in other muscle tissue.

Table 1 Chronology of the development of cysticercosis cysts from ingestion of tapeworm eggs to calcification

D0	D11-D12	D21-D30	D30	D42	D70	D 112	Several Months	9 months
Ingestion of Tapeworm eggs	Migration to muscles; 1 mm cyst, visible only in thin slices	2-5 mm cysts in muscle, start of visibility to the naked eye	Start of degeneration/ calcification of cysts in heart	Cysts easily visible	Cysts start to be infectious to humans	Fully developed cysts	Start of degeneration in muscles other than heart	Full calcification of main cysts

(From: Dupuy et al., 2015; OIE 2008)

Meat inspection is notoriously subjective and depends on the experience and motivation of the meat inspector (Dorny, 2007). It is more efficient at detecting heavy infections than light/moderate infections (Kyvsgaard, Ilsoe, Henriksen & Nansen, 1990). This is particularly problematic in areas with a low background prevalence, for example in Denmark, with an estimated true animal level prevalence of bovine cysticercosis of only 0.06%, it was estimated that less than 15% of lightly infected cattle might be detected using standard meat inspection techniques with prescribed cuts in the predilection sites (Murrell et al., 2005; Kyvsgaard et al., 1990). The poor sensitivity (and specificity) of standard meat inspection practices was shown by several studies in different parts of the world (Walther & Koske, 1980; Murrell, 2005) in many cases using serology [Ag & Ab-ELISA] for comparison (Onyango-Abuje et al., 1996; Dorny, 2000; Van Kerckhoven et al., 1998; Bogh et al., 1996). A recent comparative study from Northern Spain found a prevalence of 1.1% using Ag-ELISA on a sample of 2073 cattle, compared to a prevalence of only 0.02% based on standard visual carcass inspection of 90 891 cattle in the same abattoirs over the same period (Allepuz, Gabriël, Dorny, Napp, Jansen, Vilar, Vives, Picart, Ortuño, Gutiérrez & Casal, 2012).

A recent South African study examined the disparities in cysticercosis identification rates in 26 abattoirs in Gauteng Province between January 2010 and December 2013. Reporting rates differed by region, season (lower rates in summer, when the highest number of cattle were slaughtered) and production type (higher in cattle from feedlots and high-throughput abattoirs, where meat inspection was provided by independent service providers). The overall identification rate was 0.7% from more than 1.4 million carcasses examined, of which 97% came from non-feedlot sources (Qekwana et al., 2016). With only 41 776 (= +/- 3% of the 1.4 million carcasses) head slaughtered over 48 months

(+/- 44 per weekday) recorded as from feedlot origin, the results are certainly not representative of the situation in large commercial cattle feedlots in the province, and this origin allocation could very well be flawed data. In Gauteng there are more than 250 000 cattle on feed at any time, in nine large (10 000 head + capacity) and several smaller operations. Under typical South African feedlot conditions and management practices a 10 000 head capacity feedlot, with a feeding period of 120 days, will slaughter +/- 2 500 per month with an average of 125 per weekday. Although Karan Beef feedlot is situated in Gauteng Province, all Karan Beef cattle are slaughtered in its high throughput export approved abattoir (with independent meat inspection) in the neighbouring town of Balfour, Mpumalanga Province, thus do not form part of the data examined by Qekwana and co-workers in 2016. An earlier study from the North West Province in South Africa reported a cysticercosis prevalence of only 0.2% (Dzoma et al., 2011).

When heavy infections (e.g. more than 20 cysts) are detected the carcass is condemned, buried, rendered or incinerated. Lightly infected carcasses can be decontaminated by boiling for 3 h at a steam pressure of 0.5 athmospheres, with an internal temperature reaching 80 ºC, pickling in maximum 2.5 kg pieces pickled in salt for 20 days, or freezing. Freezing of beef carcasses at -20 ºC for 10 days is effective (Hajduk, Muller, Sallbieter, Eymer, Hiepe, Bruckner & Wilhelm, 1969; Mann, 1958; Van den Heever, 1969). In a carefully controlled study, it was shown that 12 and 16 week old *T saginata* cysticerci are much more susceptible to the lethal effects of freezing than 24 week old cysticerci. The time and temperature combinations required to ensure the death of all cysticerci irrespective of age were 360 h at 5 ºC, 216 h at 10 ºC and 144 h at -15 ºC or lower (Hilwig, Cramer & Forsyth, 1978).

2.6 Epidemiology and risk factors of *Taenia saginata*

In Denmark the major risk factors identified were access to open water streams carrying effluent from sewage treatment plants and organic farming, while there was also strong associations with gender (females contributed 54% of cysticercosis cases) as well as increasing age at slaughter (Kyvsgaard et al., 1991; Calvo-Artavia et al., 2013).

In Switzerland identified cysticercosis risk factors included the vicinity of grazing areas to railways or parking lots, leisure activities around these areas, use of purchased roughage, and organised public activities on farms attracting visitors (Flutch et al., 2008).

The association between cysticercosis and water was also demonstrated in Brazil. Data from 190 903 cattle slaughtered in 2012 from 556 farms in four states was analysed. Risk factors included access to open, uncontrolled drinking water sources and recreational fishing in or near particular farms. International accredited Good Agricultural Practice (GAP) include strict control and sanitation of drinking water used for livestock and, in export accredited farms where these practices were followed, the cysticercosis prevalence was significantly lower than in farms that did not follow this code (Rossi et al., 2015).

Several Ethiopian studies have examined the epidemiology and food safety implications of bovine cysticercosis in that country and due to particular local customs the high prevalence of bovine cysticercosis and the associated human taeniosis is a source of concern. In eastern Ethiopia there is an ever-increasing demand for 'Harar senga', meaning beef that is consumed raw or inadequately cooked in the *kurt, kitffo* and *dullet* traditions, driven by deep rooted cultural practices. Detailed questionnaires to 300 people in this area supported the suggestion that most of the infestation took place during the ingestion of these traditional dishes. Meat inspection revealed a prevalence of +/- 20% from this area, compared to 6.7% from Northeastern Ethiopia, 2.9% and 4.4% from south western Ethiopia, 11.3%, 3.0% and 26.3% from southern Ethiopia, 18.5% in northwest Ethiopia and 7.5% and 89.4% from around Addis Abeba in central Ethiopia (Terefe et al., 2014; Kumar & Tadesse, 2011).

A sero-epidemiological study of *Taenia saginata* cysticercosis was carried out in adult cattle in Zambia to determine the prevalence and study the influence of the farming system on the infection rate. Serum samples were examined for circulating parasite antigen by a monoclonal-based sandwich ELISA. Thirty-eight of 628 serum samples were found positive (prevalence 6.1%). Cysticercosis was significantly more prevalent in feedlots and in traditional farming systems than in dairy farms. The authors suggested that the continuous man to animal contact and the use of casual workers in feedlots may be factors that are conductive to *T. saginata* transmission (Dorny, Phiri, Gabriel, Speybroeck & Vercruysse, 2002).

Celine Dupuy and co-workers (2015) followed a novel approach in analysing French data from over 4.5 million cattle slaughtered during 2010. They tried to identify geographical areas where animals are infected by bovine cysticercosis and use this as a basis to develop focussed control measures and model a risk based meat inspection process. They followed an animal-herd level weighted analysis to take into account the uncertainty of the location where the animals became infected. The detection of clusters of infection indicated the need for further, detailed analysis to identify specific risk factors that in turn would suggest particular control measures. A similar philosophy was followed in this study using the Karan Beef database from 2009 to 2010, where cattle were sourced from all over South Africa to the Karan Beef feedlot.

Cysticercosis follows epidemic outbreak patterns in a cattle feedlot or intensive grazing on irrigated pasture. This can happen against a background of longterm high or low endemic prevalence. These point source epidemics have been related to contaminated feed raw materials e.g. potato by-products (Yoder, 1994), imported copra meal fed to Australian feedlot cattle (Jenkins et al., 2013), feed and/or water contamination by infected workers (Slonka, 1978; Murrell, 2005) as well as sewage-irrigated pastures (Fertig, 1985; Rickard, 1977) and feedlot cattle drinking water contaminated by human sewage waste (Lees, Nightingale, Brown, Scandrett & Gajadhar, 2002). The high prevalence of cysticercosis in cattle grazing the (treated) sewage irrigated pastures of the Melbourne municipal sewage works (Rickard, 1977) triggered research and development of an anti-cysticercosis vaccine (Lightowlers et al., 2003).

Similar large scale common source exposure to human sewage also occurs in South Africa when non-feedlot cattle are grazed in vleis and floodplains surrounding informal settlements, municipal areas or any area inundated with raw sewage from overwhelmed or non-functional sewage treatment plants. According to the most recent Green Drop Report (July 2011) from the office of the Minister of Water and Environmental Affairs, less than half of the 821 municipal sewage works in South Africa are functional. Thus sewage-contaminated water constitutes a growing risk factor for cysticercosis in cattle in South Africa. A parallel situation was reported from Matabeleland, Zimbabwe where cattle from communal sources had a significantly higher prevalence of cysticercosis than cattle from feedlots (Sungirai et al., 2014).

3. PROBLEM STATEMENT

Although *T saginata* is a zoonosis, it is of relatively low public health concern as taeniosis in humans can relatively easily be treated along with regular wide spectrum deworming practices against intestinal helminthiasis, even in resource poor communities, on a primary health care level. The major concern is the massive economical impact of the obligatory decontamination procedures (carcass freezing for 10 days at -20 ºC). The cost of decontamination is carried by producers, abattoirs and/or feedlots (Roberts, Murrell & Marks, 1994). Karan Beef financials indicate that a retained measles carcass looses approximately 20% of its monetary value, which means for most of the time this penalty far exceeds the potential profit margin. With no visual ante mortem indication of a bovine's cysticercosis status identifiable, consistent epidemiological risk factors can be very useful in terms of targeted cattle purchase policies. There are little published data from South Africa on bovine cysticercosis risk factors that can be used as part of potential risk avoidance-/mitigation strategies.

3.1 Hypotheses

Ho = There is no association between the identified putative risk factors and the prevalence of bovine cysticercosis in Karan Beef feedlot cattle based on independent post slaughter carcass inspection at the export-approved large volume throughput Karan Beef Balfour Abattoir.

H1 = There is an association between identified putative risk factors and the prevalence of bovine cysticercosis in Karan Beef feedlot cattle based on independent post slaughter carcass inspection at the export approved large volume throughput Karan Beef Balfour Abattoir.

4. MATERIALS AND METHODS

4.1 Study population

Karan Beef is a large commercial cattle feedlot, purchasing an average of 30 000 cattle every month, with an annual throughput of approximately 335 000 during the period 2009 and 2010. The exact figures are: 331 963 and 341 028 respectively. This represent approximately 35% of the beef produced in South Africa. Cattle are obtained from across South Africa and Namibia. The rations that are used include raw materials similar to all large commercial SA cattle feedlots, with the exception of silage. The Karan Beef Feedmill manufactures approximately 1 500 tonnes of feed every day which are distributed during three feedings, using five rations (Starter-, Intermediate-, Finisher-, Marketing- and Hospital/Holding Station Ration formulations) in a step up challenge feeding model whereby the energy levels as well as kg fed are gradually increased in concert with adaptation, increasing consumption and daily growth (> 1.5 kg per day). This volume of feed means a narrow spectrum of raw materials can be used to obtain a consistent, high quality product. No raw material from animal/fish origin are used, only by-products from the grain and sugar industries. Raw materials are: hominy chop, high moisture rolled maize, gluten, wheat bran, sunflower oilcake meal, cotton seed, bagasse, molasses, eragrostis hay and wheat straw. The supply of raw materials are secured through longterm contracts and due to fluctuating availabilities are often stored for long periods; from grass bale stacks (> 12 months) to most of the others for several weeks in enclosed stores/bunkers. Hominy chop can only be stored for about 30 days maximum and forms the bulk of the ration at more than 50% inclusion, so this product is characterised by a very high turnover. In addition, water is obtained from surface water sources (rivers), while the labour force is also comparable to workers at other SA feedlots.

Holding stations are satellite ‘mini-feedlots’ under Karan Beef management and function as collection points for small groups of cattle. In addition they follow a rumen- and feed bunk adaptation programme for short periods (minimum 2 weeks) on the Karan Beef starter ration while responding immunologically to an initial profile of vaccines, all under a low stress pen environment. Cattle from distant source areas are trucked to holding stations by preference, but if these are filled to capacity, the cattle go directly to Karan

Beef feedlot near Heidelberg, Gauteng, and their origins are reflected as the loading point (see Appendix 1).

Table 2 Geographical location of Holding Stations and their source areas

Holding Station: nearest town and capacity	Cattle origins
Cookhouse HS: 2 500 head	Inland Eastern Cape-, Southern- & some Western Cape districts
Grahamstown HS: 1 500 head	Coastal Eastern Cape & Transkei districts
Indwe HS: 2 000 head	Eastern Cape Karoo, Stormberg, inland Transkei & Ciskei
Christiana HS: 6 000 head	Southern & Western Free State-, Northern Cape-, far North West Province districts

4.1.1 Data sources and management

4.1.2 All feedlot health, feeding and production data are captured on the WALCO Feedyard management software [iTa], based on a unique six figure individual animal identity ear tag number linked to the lot number (date code) applied to all new cattle at processing.

4.1.3 All abattoir data are captured on SCANPLANT Abattoir management software.

4.1.4 Various reports, set up by the Karan Beef IT team, using data from both the abovementioned software systems, are collated by SAP Crystal Reports Version 1.8 software [Logicity] based on selected parameters e.g. dates, periods, lot numbers, etc.

4.1.5 Raw data are exported to Excel spreadsheets [Microsoft] either directly or indirectly via the Crystal reports, allowing analyses.

4.1.6 Respiratory disease treatment data had many inaccuracies, so for the purpose of this study the raw data was cleaned up through the following steps to reflect a more accurate Bovine Respiratory Disease profile:

- Only treatments using a respiratory treatment code and regimen used on day one for a first pull was allowed.

- Individual animal numbers can appear only once in the database.
- Incorrect matching of pull codes with treatment regimens were discarded, e.g. if an animal was pulled for lameness but received a respiratory treatment regimen based on the computer history captured.
- Cut off values were changed to 15% and 30% to more realistically reflect the long-term seasonal patterns of BRD at Karan Beef.

4.2 Experimental design

This was a cross-sectional study involving the retrospective analysis of all abattoir data from approximately 650 000 cattle slaughtered during 1 January 2009 to 31 December 2010. This period reflects 24 months that were characterised by dramatic highs and lows in the prevalence of bovine cysticercosis and when no specific control strategies were instituted (see Figure 2).

Only data from feedlot cattle transported from the company's own feedlot in Heidelberg with a standing capacity of 130 000 head at that time, were used in this analysis. Currently (from 2015) the standing capacity is 140 000.

4.3 Experimental procedures and data analysis

Positive cysticercosis (= 'measles') carcasses based on standard meat inspection procedures by independent meat inspectors at the Karan Beef Balfour Abattoir, during the period 1 January 2009 to 31 December 2010, were related to their individual carcass classification and feedlot production histories as well as group/lot identities (See Table 3).

4.3.1 Univariate Analysis

Individual variables i.e. sex ('male' & female), arrival live weight grouped in weight categories) and 'breed' were based on these allocations done during the processing procedures 24 hours after arrival at the feedlot. Other group-level variables were identified in the following way: every truckload of cattle is assigned a coded lot number (date plus chronological number) on arrival at Karan Beef. This is linked to the purchase history (e.g. date, geographical origin, buyer, etc.) as well as particulars related to the processing regimen received by every individual in this lot. Different processing regimens are used that correspond to the health risk appraisal done at offloading and after 24 hours rest. This is a composite of bovine respiratory disease risk (high, medium or low) and specific geographical considerations such as tick-borne diseases, liver fluke, etc.

These datasets in each category were combined with production and carcass classification data from the Karan Beef Balfour Abattoir to build master spreadsheets for the analysis, allowing comparative risk analyses for the following (Table 3) identified potential risk factors in 2x2 tables:

Table 3 Characteristics of the study population: Individual and lot based risk factors used in Descriptive Univariate Analysis and Multivariate Analysis

Individual Animal Identification Number: 6 figures	Eartag: all data is electronically linked to this number in combination with the Lot number
Lot Number = single truck load	Date code plus sequential number; e.g. the 5^{th} lot that arrived on 21 June 2010 = 10 062 105
Processing Date (B)	Usually +/- 24h after arrival
Slaughter date (C)	At Balfour abattoir
Days on Feed (D)	C minus B = actual total feeding period. More or less than the average of 120 days used in this analysis.
Live/Dead Weight (E)	Weight of carcass immediately after stunning and exsanguination
ADG = Average Daily Gain	(E-A)/D
Hospital history	The entire treatment history is captured in detail, but for this analysis only respiratory treatment history (Yes or No) were used to determine BRD risk categories per lot.
Sex	Male (Bulls & Steers) vs Heifers. Unknown sex category due to operator error (night shift) and/or computer error.
Arrival live weight (A)	All cattle are sorted into production pens during processing, based on a sex and weight classification: BG: < / = 160 kg; GR: 161-210 kg; STR: 211-250 kg; GSTR: > 251 kg
Breed	Only breeds that were consistently allocated over the study period with a high level of confidence were used. Breed allocations e.g. Bonsmara, Beefmaster, Red Angus that could easily be confused in lightweight weaner calves were grouped as 'other'. Unknown breed is a designation allocated to commercial crossbreds of indeterminate breed.
Buyer	17 different buyers used during the study period
Geographical origin/loading point	Various provinces and/or subregions within them compared: Gauteng, Free State, North West, KwaZulu-Natal, Limpopo, Northern Cape and Namibia, Western Cape, Eastern Cape
Geographical origin/loading point	Per magisterial district and related human densities as per SENSUS 2011 coupled with broad land use categories
Geographical origin/loading point	Ecoregions i.e. a combination of vegetation type and rainfall
Purchase channel	Auction vs breeding farm vs holding station
Farm type	Combination of geography, farming system and vegetation type

Odds ratios (OR), 95% upper- and- lower levels and p-values were computed using chi square calculations while additional univariate analyses used standard OR formulae from Medicalc freeware (www.medicalc.org).

4.3.2 Multivariate Analysis

Due to the large dataset available, all independent variables, irrespective of their association with the outcome variable (presence of cysticercosis) on univariate were considered for inclusion into a multilevel logistic regression model were first eliminated by selecting the biologically most meaningful one for inclusion. Clustering within lot was accounted for by the modelling of lot as a random effect. Statistical significance was assessed at $P < 0.05$. Statistical analysis was done using Stata 14 (StataCorp, College Station, TX, U.S.A.).

4.3.3 Detailed Univariate Analysis

Further detailed univariate analyses were done to try and understand the epidemiological drivers of cysticercosis specifically related to the Karan Beef Feedlot better, hopefully identifying practical critical control points suitable for an effective control programme. Factors considered in these analyses are shown in Table 4 below.

Table 4 Characteristics of the study population: Individual and lot based risk factors used in detailed univariate analysis

a) Number of measles/lot	< / = 3% /lot (average/background as control) vs > / = 9% /lot (if one lot from a specific buyer and origin combination had a prevalence of > / = 9%, all lots with that combination were grouped and evaluated as a suspected 'measles high risk profile'
b) Rainfall season	(winter – vs summer-) Using a high population density area from each; Gauteng vs Western Cape; George and Cape Town respectively
c) Drinking water	Blesbokspruit and Suikerbosrand river confluence: River turbulence = High Risk; River stagnation = Low Risk. This is determined from rainfall data generated by the Karan Beef weather station. Compare with cysticercosis status of cattle purchased locally, also with all cattle purchased.
d) Risk period per geographical area	(High vs Low) using high/low population density areas during high/low rainfall periods as indicators. Any permutation with 'high' = High Risk; low + low = Low Risk.
e) BRD Risk	Retrospective analysis of crude morbidity per lot were used to assign lots to a High- Medium- or Low Risk for BRD category. This is standard practice at Karan Beef to determine repeatable epidemiological patterns and thus adjust the level of intervention needed per lot. Long-term patterns indicate a strong association between Small Lot/Auction calves/Smallholder origin/communal cattle purchased through agents = High Risk designation for BRD metaphylaxis vs Large Lot, non-holding station calves, in most cases originating from single large commercial cow-calf herds as Low Risk for BRD. Thus an attempt to use a well established BRD risk association to investigate whether it can also be used as a proxy to predict Cysticercosis risk to ultimately determine the economic justification of purchases from such marginal sources.

5. RESULTS:

Data, captured from all lots processed during the study period, included 17 561 measles-positive cattle from a database of 645 634 head analysed, an overall prevalence of 2.72% based on carcass inspection. A total of 217 condemned (all causes) carcasses were excluded from the analysis even though < 5% of these were condemned because of severe cysticercosis (> 20 cysts per carcass), but unfortunately the Walco database did not reflect reasons for condemnation. These condemned carcasses were removed from the slaughter line and sent to the byproduct plant for rendering into carcass meal. Less than 5% of cattle had incomplete histories, no weight or sex allocated, etc., and these were excluded from the relevant calculation so the totals in the different categories are not always the same (see below in Results). This was mainly a problem in the 2009 data, and most of these data capture errors were eliminated from 2010 after a software upgrade and several personnel changes.

Over the last few years (2012-2016) with a larger slaughter volume of 350 000 to 400 000 per annum and a stable cysticercosis prevalence of around 0.5%, about 1-2 condemnations per month (average total condemnations from all causes = 10-15 per month) occured as a result of severe levels (> 20 cysts per carcass) of cysticercosis.

In addition to the 5% mentioned above, experience has shown that there is an approximately 1% identity tag loss, plus around 2% human error in typing the identity tag number correctly into the Scanplant software system at the abattoir.

Total number of cattle slaughtered from January 2009 to December 2010 (24 months) from Karan Beef feedlot at the Karan Beef Balfour Abattoir was 672 991 head. Due to the reasons mentioned above data from 27 357 head were excluded from the analyses. Data from 645 634 head slaughtered were incorporated in the master database analysed; a data capture rate of 95.94%.

5.1 Univariate Analysis

Statistically significant differences in the different risk factor groups were recorded between cattle breeds, buyers, farm types, purchase channels, centre (with most towns and periurban areas), wet East, dry West and holding stations, as well as between different ecoregions. This formed the basis of the detailed univariate analyses below. The statistically significant difference in risk between 'unknown' sex and both male and female cattle cannot be interpreted and illustrate the difficulty in working/analysing a database with such deficiencies in the data capture process. The number is too big to leave out of the entire analytical process. 'Unknown' sex and breed allocations come from long periods in both 2009 and 2010 when the processing facility was operated using two teams in a day shift and night shift schedule to accommodate large numbers of incoming cattle. Both allocations were very difficult at night with high speed processing, so uncertainties were recorded as 'unknown'. Correct allocations per group were done the next day by daylight through sorting. Planning started in 2009 and finally in 2013 a brand new state of the art processing facility was built with the two teams using two parallel lanes plus squeeze shuts, thus eliminating night shift related errors and imperfections.

Table 5 Measles prevalence in categories of cattle defined by independent variables and univariate significance using Pearson's Chi square calculation.

Risk Factor		Number of cattle	Prevalence (%)	P-value
Sex	Female	98 185	2.22	0.673
	Male (Oxen & Steers)	337 205	2.25	
	Unknown	210 244	3.71	< 0.001
In weight	20 x Categories with 50 kg increments; 50-350 kg	645 622	2.72	0.515
Breed	Brahman	24 468	1.66	< 0.001
	Brahman X	16 439	1.97	
	Brown Swiss	5 792	1.92	
	Brown Swiss X	1 789	2.29	
	Hereford	4 331	2.05	
	Hereford X	391	2.05	
	Nguni	8 073	2.02	
	Nguni X	1 772	2.54	
	Other (Breeds inaccurately allocated)	209 460	2.31	
	Unknown (Commercial X's)	373 119	3.09	
Buyer	CDB	23 207	2.66	< 0.001
	CDP	45 758	2.92	
	DF	11 969	2.30	
	DO	76 655	2.77	
	FA	21 007	2.59	
	FVH	41 165	2.50	
	FM	79 769	2.78	
	FL	23 038	2.67	
	GJ	44 488	2.30	
	JB	104 032	2.93	
	JDB	167	2.40	
	KB	8 244	2.66	
	KVDL	1 329	1.28	
	L	98 581	2.74	
	LB	1 292	2.01	
	PH	8 782	2.37	
	PK	56 151	2.78	
Region: province or part thereof	Eastern Cape	90 306	2.81	< 0.001
	Free State Central	44 929	2.82	
	Free State North	23 773	2.39	
	Free State South	52 659	2.69	
	Gauteng Province	2 887	2.50	
	KwaZulu-Natal	33 837	2.40	
	Limpopo	14 128	2.60	
	Mpumalanga	41 150	2.51	
	Namibia	21 461	2.82	
	Northern Cape	85 395	2.81	
	North West Province Central	3 778	2.59	
	North West Province South	104 782	2.89	
	North West Province West	85 415	2.72	
	Springbokflats	4 619	2.73	
	Western Cape	15 067	2.54	

Farm type	Coastal livestock & small grains	778	**2.44**	**< 0.001**
	Coastal livestock, dairy, wine & fruit	14 366	2.55	
	Commercial & communal mix	21 461	2.82	
	Extensive bushveld cattle	36 686	2.61	
	Extensive large- & small livestock	5 738	2.74	
	Holding station	177 914	2.83	
	Kalahari extensive large & small livestock	82 616	2.81	
	Karoo extensive large & small livestock	7 622	3.12	
	Mixed crops & livestock	252 643	2.68	
	Sourveld large & small livestock cold	869	3.45	
	Sourveld extensive large & small livestock	5 050	2.59	
	Sourveld extensive large & small livestock cold	12 212	2.10	
	Sourveld extensive large & small livestock warm	26 970	2.41	
	Speculators & small farms	286	5.24	
Purchase channel	Agent/Auction/Speculator	322 318	2.74	0.001
	Direct from breeding farm	114 213	2.56	
	Holding station	208 681	2.78	
Centre, East, West & Holding Station	Holding station	208 681	2.78	0.001
	N1 Centre & Periurban	41 650	2.80	
	N1 East WET	165 617	2.58	
	N1 West DRY	229 264	2.76	
Ecoregion	Bushveld cattle	30 068	2.52	< 0.001
	Bushveld commercial & communal mix	5 154	2.99	
	Cash Crops & Thornveld	180	5.00	
	Coastal WC & winter rainfall	15 430	2.60	
	Eastern Cape Central	27 671	2.84	
	Eastern Cape North, Transkei & Stormberg	26 134	2.82	
	Eastern Cape South & Coastal	23 496	2.64	
	Grass Karoo	6 228	3.36	
	High rainfall grassland & crops	165 097	2.51	
	Highveld cattle & crops	297	2.36	
	Highveld/Bushveld transition	32 980	2.52	
	Kalahari	101 293	2.81	
	Karoo	7 510	2.77	
	Low rainfall, grass, savanna & crops	193 372	2.88	
	Low-Med rainfall Bush- & Grass mix	10 302	2.70	
Human Density	High density informal	31 888	2.71	0.729
	High density periurban	32 876	2.74	
	Low density small towns	539 779	2.72	
	Medium density big towns	19 208	2.60	
	Namibia commercial & communal mix	21 461	2.82	

5.2 Results multivariable analysis

Table 6 Factors associated with measles in feedlot cattle using a multivariable mixed-effects logistic regression model, adjusted for clustering by lot (group variable)

Number of Observations	645 200
Number of Groups	8 014
Observations per group	Min = 1
	Ave = 80.5
	Max = 544

Table 7 Results of the multivariable mixed-effects logistic regression model analysis of associations between putative risk factors

Putative Risk Factor	Level	Odds Ratio	95% Confidence Interval		P-value
In weight: production group categories (kg)	< / = 160	1			
	161-210	0.951	0.899	1.007	0.086
	211-250	0.947	0.894	1.003	0.067
	> 250	0.953	0.895	1.015	0.135
Breed	Brahman	1			
	Brahman X	1.17	1.008	1.360	0.039*
	Brown Swiss	1.17	0.942	1.447	0.155
	Brown Swiss X	1.39	1.001	1.927	0.049*
	Hereford	1.28	1.011	1.623	0.040*
	Hereford X	1.29	0.632	2.617	0.486
	Nguni	1.22	1.010	1.470	0.038*
	Nguni X	1.53	1.113	2.091	0.009*
	Other (Breeds inaccurately allocated)	1.41	1.271	1.569	< 0.001*
	Unknown (Commercial X's)	1.88	1.698	2.092	< 0.001*
Purchase channel	Agent/Auction/Speculator	1.08	1.026	1.139	0.004*
	Direct from breeding farm	1			
	Holding station	1.017	0.953	1.086	0.606
Human density	High Density Informal	1.037	0.948	1.136	0.424
	High Density Periurban	1.059	0.965	1.161	0.225
	Low Density Small Town	1			
	Medium Density Big Town	0.996	0.895	1.108	0.943
	Namibia commercial cattle farms	1.009	0.905	1.126	0.860
Ecoregion	Bushveld Commercial Cattle Farms	1.047	0.956	1.148	0.322
	Bushveld Commercial & Crops	1.139	0.933	1.389	0.200
	Cash Crops & Thornveld	1.805	0.777	4.192	0.169
	Coastal Western Cape & Winter Rainfall	0.942	0.822	1.079	0.386
	Eastern Cape Central	1.090	0.987	1.204	0.087
	Eastern Cape North & Transkei	1.142	1.033	1.264	0.009*
	Eastern Cape South & Coastal	1.026	0.923	1.142	0.631
	Grass Karoo	1.269	1.074	1.500	0.005*
	High Rainfall Grassveld	1			
	Highveld Commercial cattle & Crops	0.982	0.425	2.266	0.965
	Highveld/Bushveld transition	1.020	0.934	1.114	0.660
	Kalahari	1.090	1.024	1.161	0.007*
	Karoo	1.062	0.900	1.252	0.474
	Low Rainfall, Grass, Savanna	1.131	1.074	1.190	< 0.001*
	Low-Medium Rainfall Bushveld	1.023	0.886	1.183	0.754

*statistically significant difference

Although there was no significant association between starting mass and presence of measles, there was a tendency for lighter animals (≤ 160 kg) to be more at risk of having measles. The Brahman breed had the lowest odds of measles infection, with all other breeds significantly more likely to be infected, with the exception of Brown Swiss and Hereford crosses. These two breeds represent very small numbers from specific, dedicated breeders. Brahmans are generally used as pure or foundation for cross breeding in the warmer parts of the country due to their inherently higher heat tolerance and resistance to ticks. Purchase through an agent, auction or speculator was associated with an increased odds of measles compared to cattle purchased directly from breeding farms. There were no significantly different odds of measles infection between cattle procured from areas with different human densities. Using High Rainfall Grassland as the baseline, only cattle originating from the Eastern Cape North and Transkei-, Grass Karoo-, Kalahari- and Low Rainfall Grass, Savanna ecoregions had significantly higher odds of measles infection.

Results detailed univariate analyses: Lots were grouped according to various parameters or combinations of parameters, based on their associated geographical loading points, in a stepwise fashion, working from large, crude collections to smaller, more specific, detailed combinations of recognisable characteristics in an effort to identify practical sets of characteristics that will allow Karan Beef to deploy a sensible discriminatory purchase strategy in essence to 'blacklist' repeat offenders and stop purchasing those calves in future.

5.2.1 Geographical areas associated risks compared

See Appendix 1 for map showing the geographical locations/loading points used by Karan Beef during the period 2009 and 2010.

Table 8 Groups/lots from various geographical origins compared: provinces and subregions

	Gauteng (GP)	Free State (FS)	Eastern Cape (EC)	KwaZulu-Natal (KZN)	Northern Cape (NC) & Namibia	Limpopo, North West (NW) & Spring-bok flats	Mpumalanga (MP)	Western Cape (WC)
Measles POS	609	2 919	2 028	1 488	2 986	6 285	953	403
No. cattle	24 654	109 376	72 656	59 615	106 134	225 432	39 392	15 527
Prev	2.5%	2.7%	2.8%	2.5%	2.8%	2.8%	2.4%	2.6%
OR	1.02	1.11	1.16	1.03	1.17	1.16	**CONTROL**	1.07
95% CI min	0.92	1.03	1.07	0.95	1.08	1.08		0.96
95% CI max	1.13	1.19	1.25	1.12	1.26	1.24		1.21
P	0.68	0.008	0.0002	0.45	< 0.0001	< 0.0001		0.23

Table 9 Results of univariate analysis: Geographical areas associated risks compared - Gauteng

	Gauteng	Auction: small farms & plots, GP East	Highveld/ Bushveld transition farms	Karan Beef close small farms & plots	Randfontein Auction: small farms & plots, GP west	Vaal Triangle periurban
Measles POS	609	139	81	156	55	178
Measles NEG	24 045	4 627	3 817	6 422	1 975	7 204
No. cattle	24 654	4 766	3 898	6 578	2 030	7 382
Prev	2.5%	2.9%	2.1%	2.4%	2.7%	2.4%
P	0.14	0.01		0.33	0.12	0.26
OR	1.19	1.42	**CONTROL**	1.14	1.31	1.16
95% CI min	0.94	1.07		0.87	0.93	0.89
95% CI max	1.51	1.87		1.5	1.86	1.52

5.2.2 Geographical areas associated risks compared: Free State Province subregions

Table 10 Results of Univariate Analysis: Groups/Lots from various geographical origins within Free State Province compared

	Free State	FS Central	FS East & Lesotho Border	FS North	FS Northeast: High, cold & wet	FS South	Vaal Triangle & Vaaldam South
Measles POS	2 919	1 155	996	211	108	424	25
Measles NEG	106 457	40 566	37 972	9 068	4 352	13 781	718
No. cattle	109 376	41 721	38 968	9 279	4 460	14 205	743
Prev	2.7%	2.8%	2.6%	2.3%	2.4%	3.0%	3.4%
OR	1.11	1.22	1.12	**CONTROL**	1.066	1.32	1.49
95% CI min	1.03	1.05	0.97		0.84	1.12	0.98
95% CI max	1.19	1.42	1.31		1.35	1.56	2.28
P	0.008	0.008	0.12		0.59	0.001	0.061

Table 11 Traditionally the N1 highway that runs diagonally from the northeast to the southwest through this province, is regarded as the line between the 'dry west' and the 'wet east' while Bloemfontein, surrounded by peri-urban small farms and plots and its satellite towns, representing the highest population density in the province, straddle this informal division as the 'centre'.

Free State	N1 Centre & peri-urban	N1 East WET	N1 West DRY
Measles POS	555	2 014	350
Measles NEG	16 953	74 758	14 746
No. cattle	17 508	76 772	15 096
Prev	3.2%	2.6%	2.3%
OR	1.38	1.14	**CONTROL**
95% CI min	1.2	1.01	
95% CI max	1.58	1.27	
P	0.0001	0.031	

5.2.3 Geographical areas associated risks compared: North West Province + Limpopo + Sprinbokflats (= 'Bushveld' region) and extensive grassland/ crops in southwestern part of NW Province

Table 12 Results of Univariate Analysis: Groups/Lots from various purchase channels/eco-geographical origins within North West Province and Bushveld region compared

	Lim, NW & SF	Back grounder & Pivots	Bushveld cattle farms	Cash crops & Thornveld	Christiana HS	Grass & Maize	Speculators & small farms
Measles POS	6 285	243	645	141	2 891	1 500	865
Measles NEG	219 147	8 707	24 469	4 711	97 620	51 392	32 248
No. cattle	225 432	8 950	25 114	4 852	100 511	52 892	33 113
Prev	2.8%	2.7%	2.57%	2.9%	2.9%	2.8%	2.61%
OR	1.16	1.06	**CONTROL**	1.14	1.12	1.22	1.01
95% CI min	1.08	0.91		0.94	1.03	1.12	0.92
95% CI max	1.24	1.23		1.37	1.22	1.34	1.13
P	< 0.0001	0.45		0.18	0.008	< 0.0001	0.74

5.2.4 Geographical areas associated risks compared: Mpumalanga Highveld

Table 13 Results of Univariate Analysis: Groups/lots from various farming type/trade channel zones in Mpumalanga Province

	Mpumalanga	Commercial & communal mix	Highveld cattle & crop farms	Speculators & small farms
Measles POS	953	372	382	199
Measles NEG	38 439	12 355	18 787	7 297
No. cattle	39 392	12 727	19 169	7 496
Prev	2.4%	2.9%	2.0%	2.7%
OR		1.48	**CONTROL**	1.34
95% CI min		1.28		1.28
95% CI max		1.71		1.6
P		< 0.0001		0.0009

5.2.5 Geographical areas associated risks compared Namibia, the Northern Cape and Central Karoo

Table 14 Results of Univariate Analysis: Groups/Lots from various geographical/ecological zones in Namibia and Northern Cape Province compared

	NC & Namibia	Namibia	NC: Karoo & central	Kalahari
Measles POS	2 986	606	140	2 240
Measles NEG	103 148	20 860	4 685	77 603
No. cattle	106 134	21 466	4 825	79 843
Prev	2.8%	2.82%	2.9%	2.81%
OR	1.17	1.01	1.04	**CONTROL**
95% CI min	1.08	0.92	0.87	
95% CI max	1.26	1.1	1.23	
P	< 0.0001	0.89	0.7	

Table 15 Northern Cape farming type/trade channels

Northern Cape	NC: big herds (direct)	NC: small herds & speculators
Measles POS	1 942	438
Measles NEG	68 242	14 046
No. cattle	70 184	14 484
Prev	2.8%	3.0%
OR	**CONTROL**	1.09
95% CI min		0.98
95% CI max		1.22
P		0.09

5.2.6 Geographical areas associated risks compared Western Cape Province

Table 16 Results of Univariate Analysis: Groups/Lots from various geographical/ecological zones in Western Cape Province compared

	Western Cape	WC: Coastal	WC: Swartland small grains	WC: Karoo	WC: wine & fruit
Measles POS	403	335	20	2	46
Measles NEG	15 124	12 353	819	95	1 857
No. cattle	15 527	12 688	839	97	1 903
Prev	2.6%	2.6%	2.38%	2.1%	2.42%
OR	1.07	1.11	**CONTROL**	0.86	1.01
95% CI min	0.96	0.7		0.2	0.6
95% CI max	1.21	1.75		3.76	1.73
P	0.23	0.65		0.84	0.96

5.2.7 Geographical areas associated risks compared Eastern Cape Province

Table 17 Results of Univariate Analysis: Groups/Lots from various geographical/ecological zones in Eastern Cape Province compared.

	Eastern Cape	EC: Karoo	EC: Cookhouse HS	EC: Grahamstown HS	EC: Indwe HS	EC: Stormberg
Measles POS	2 028	423	785	621	48	151
Measles NEG	70 628	13 413	26 881	22 841	1 896	5 597
No. cattle	72 656	13 836	27 666	23 462	1 944	5 748
Prev	2.8%	3.1%	2.8%	2.6%	2.5%	2.6%
OR	1.16	1.25	1.15	1.07	**CONTROL**	1.07
95% CI min	1.07	0.92	0.86	0.8		0.77
95% CI max	1.25	1.69	1.55	1.45		1.48
P	0.0002	0.15	0.34	0.64		0.7

5.2.8 Geographical areas associated risks compared KwaZulu-Natal Province

Table 18 Results of Univariate Analysis: Groups/Lots from various geographical/ecological zones in KwaZulu-Natal compared

	KwaZulu-Natal	Griqualand East	KZN Midlands	North KZN; All	North KZN; Danhauser Speculator*	North KZN; Newcastle-Normandien Speculator*	North KZN; Farms
Measles POS	1 488	794	194	500	350	103	47
Measles NEG	58 127	30 348	8 148	19 631	12 284	4 420	2 927
No. cattle	59 615	31 142	8 342	20 131	12 634	4 523	2 974
Prev	2.5%	2.5%	2.3%	2.5%	2.8%	2.3%	1.6%
OR	1.03	1.1	**CONTROL**	1.07	1.77	1.45	**CONTROL**
95% CI min	0.95	0.94		0.9	1.3	1.02	
95% CI max	1.12	1.29		1.27	2.41	2.05	
P	0.45	0.25		0.79	0.0003	0.04	

*****These agents, loading from Dannhauser and Newcastle, purchase mainly communal cattle

5.2.9 Geographical areas associated risks compared; different eco-geographical combinations

Table 19 Results of Univariate Analysis: Groups/Lots from various combinations of geographical-, ecological zones and seasonal rainfall patterns in SA and Namibia, including holding stations as separate entities

Eco-geographical-, rainfall-, land use, human density- & HS zones/regions		Karoo	Bush veld cattle farms	Christiana HS	Cook house HS	Grahams-town HS	Indwe HS	All HS Combined	Coastal Summer Rainfall
Measles POS	18 186	183	756	2 891	785	621	48	4 345	390
Measles NEG	653 701	6 301	28 629	97 620	26 881	22 841	1 896	149 238	14 606
No. cattle	671 887	6 484	29 385	100 511	27 666	23 462	1 944	153 583	14 996
Prev	2.7%	2.8%	2.6%	2.9%	2.8%	2.6%	2.47%	2.8%	2.6%
OR		1.15	1.05	1.17	1.15	1.08	1	1.15	1.05
95% CI min		0.99	0.97	1.11	1.07	0.99	0.75	1.1	0.95
95% CI max		1.34	1.13	1.23	1.25	1.17	1.34	1.21	1.18
P		0.07	0.27	< 0.0001	0.0003	0.1	0.99	< 0.0001	0.3

Table 19 continued	Coastal WC & winter rainfall	Grass Karoo	High rainfall grassland & crops	Highveld/ Bushveld transition farms	Kalahari	Low-med rainfall grassland	Periurban cities & big towns high human density	Stormberg
Measles POS	390	378	3 326	74	2 846	2 466	3 260	151
Measles NEG	14 606	11 953	131 698	3 314	98 463	88 575	114 913	5 597
No. cattle	14 996	12 331	135 024	3 388	101 309	91 041	118 173	5 748
Prev	2.6%	3.1%	2.46%	2.2%	2.8%	2.7%	2.8%	2.6%
OR	1.05	1.25	**CONTROL**	0.88	1.14	1.1	1.12	1.07
95% CI min	0.95	1.12		0.7	1.08	1.04	1.07	0.9
95% CI max	1.18	1.4		1.12	1.2	1.16	1.18	1.26
P	0.3	< 0.0001		0.3	< 0.0001	0.0003	< 0.0001	0.43

*See Appendix 1 for towns/loading points allocated to these zones

5.2.10 Cysticercosis prevalence groups compared

Table 20 Results of Univariate Analysis: Groups/lots in high or low measles prevalence risk groups comparison

Variable	Group: Buyer & Origin	Measles Positive	Measles Negative	Preva-lence	OR	95% L	95% U	P
Number of measles/lot: **Background as control: all lots with average < / = 3% lot vs lots grouped where one had > / = 9% / lot.** **Only direct, non holding station lots**	All buyers & all origins with low prevalence	7 954	355 400	2%	**CONTROL**			
	CDB* Free State Central: Kroonstad	170	5 003	3%	1.52	1.30	1.77	< 0.01
	CDP* Northwest West: Coligny	115	2 927	4%	1.76	1.46	2.12	< 0.01
	CDP* Northwest West: Lichtenburg	496	13 145	4%	1.69	1.54	1.85	< 0.01
	FM* North Cape: Kuruman	906	26 404	3%	1.53	1.43	1.64	< 0.01
	FM* North Cape: Olifantshoek	487	13 649	3%	1.59	1.45	1.75	< 0.01
	FM* North Cape: Van Zylsrus	158	4 236	4%	1.67	1.42	1.96	< 0.01
	JB Free State Central: Bloemfontein	141	3 425	4%	1.84	1.55	2.18	< 0.01
	L* Free State Central: Marquard	62	1 433	4%	1.93	1.50	2.50	< 0.01
	PK* Free State Central: Rouxville	82	2 615	3%	1.40	1.12	1.75	< 0.01

*Initials of buyer/agent

5.2.11 Seasonal rainfall and human density risks compared

Table 21 Results of Univariate Analysis: Groups/Lots in Seasonal Rainfall in specified high human population density geographical areas comparison

Variable	Group	Measles positive	Measles negative	Preva-lence	OR	95% L	95% U	P
Seasonal rainfall: Summer vs Winter **High human population density areas compared.**	Gauteng: DRY winter (May-Aug)	187	7 792	2.3%	**CONTROL**			
	Gauteng: WET summer (Jan-Apr & Sep-Dec)	459	17 091	2.6%	1.12	0.94	1.33	0.20
	Western Cape: George & Cape Town: DRY summer (Jan-Apr & Sep-Dec)	227	8 561	2.6%	1.10	0.91	1.34	0.32
	Western Cape: George & Cape Town: WET winter (May-Aug)	81	2 902	2.7%	1.16	0.89	1.51	0.26

5.2.12 Local rainfall at Karan Beef Feedlot and river turbulence risks compared

Table 22 Results of Univariate Analysis: Groups/Lots; risk comparison using Local/Gauteng seasonal rainfall and associated river turbulence as risk determinants

Variable	Group	Measles Positive	Measles Negative	Preva-lence	OR	95% L	95% U	P
Rainfall & River Turbulence risk	Low Risk	3 743	117 090	3.1%	**CONTROL**			
	High Risk	14 151	419 214	3.3%	1.06	1.02	1.1	0.01

5.2.13 BRD risk profile categories as Cysticercosis risk proxy compared

Disease Risk is determined through retrospective analysis of data from the Karan Beef First Pull Crystal Report which sorts through the Walco database based on set date parameters and give all individual cattle data based on first entry to hospitals for any reason. This is a standard approach to determine levels of intervention at arrival or processing based on established respiratory disease (BRD) risk profiles. Approximately

70% of first pulls are for respiratory treatments, 20% for digestive system conditions and 10% for various other diagnoses such as lameness/cripples, tick-borne disease, etc. The question is therefore whether this established BRD risk profiling can also be used to characterise a parallel cysticercosis risk.

Table 23 Results of Univariate Analysis: Groups/lots; Disease Risk Categories as risk determinants

All Cattle	BRD High Risk: > / = 30% Crude Morb	BRD Medium Risk: > 15% < 30% Crude Morb	BRD Low Risk: < / = 15% Crude Morb	BRD Medium + Low Risk
Measles POS	7 620	5 627	4 251	9 878
Measles NEG	278 358	206 348	140 208	346 556
No. cattle	285 978	211 975	144 459	356 434
OR	**CONTROL**	0.996	1.11	1.04
95% CI min		0.96	1.07	1.01
95% CI max		1.03	1.15	1.07
P		0.83	< 0.0001	0.009
Prev	2.7%	2.7%	2.9%	2.8%

Non HS Cattle	BRD High Risk: > / = 30% Crude Morb	BRD Medium Risk: > 15% < 30% Crude Morb	BRD Low Risk: < / = 15% Crude Morb	BRD Medium + Low Risk	All HS combined BRD Risk
Measles POS	5 661	3 293	2 183	5 476	6 389
Measles NEG	207 868	121 880	73 175	195 055	223 288
No. cattle	213 529	125 173	75 358	200 531	229 677
OR	1	**CONTROL**	1.1	1.04	1.06
95% CI min	0.97		1.05	0.99	1.01
95% CI max	1.05		1.17	1.09	1.11
P	0.72		0.0004	0.09	0.009
Prev	2.7%	2.6%	2.9%	2.7%	2.8%

5.3 Summary of statistically significant results

Table 24 Identifiable statistically significant risk factors that can be used practically to identify lots with a high risk profile for cysticercosis, using the origin map in Appendix 1.

Risk Factor determined for	P-Value	Conclusion
Geographical origin	< 0.01	Compared to cattle loaded in Mpumalanga Highveld as control (lowest prevalence), cattle from the following regions: Free State Eastern Cape, Northern Cape plus Namibia and Limpopo, North West & Springbokflats combined had a significantly higher risk for cysticercosis.
Gauteng Province	0.01	Compared to cattle delivered to Karan Beef in small loads (< 40 animals/lot) in a variety of multipurpose trucks, trailers, etc. (not Karan Beef or similar cattle trucks), originating from small farms, plots, communal cattle around townships, etc. that showed the highest cysticercosis prevalence, cattle that arrived at Karan Beef in dedicated cattle trucks (90-115 per lot), from large farms/cow calf herds, in the Highveld/Bushveld transition districts have a significantly lower cysticercosis risk (p=0.01). There is a strong suggestion that cattle from the western part of GP, mainly channelled through the Randfontein auction, also carry a higher cysticercosis risk (P=0.12)
Free State Province	< 0.01	Compared to cattle originating from the northern Free State (lowest prevalence) characterised by extensive high rainfall grassveld and crop farming, cattle from the central region (Bloemfontein and surrounding towns), characterised by periurban sources, grazing and crops, as well as those loaded from the south and southeast, characterised by lower rainfall grazing, some scattered crops as well as communal cattle from former homelands, have a significantly higher cysticercosis risk.
Free State Province	< 0.01	If we divide the province from an arbitrary traditional viewpoint into a 'dry west' and 'wet east' using the diagonal N1 highway that runs through Bloemfontein in the center, a similar picture emerges. The cattle sourced from the area close to Bloemfontein and surrounding plots, small farms and township communal cattle as well as from nearby satellite towns carry a significantly higher cysticercosis risk.
North West Province, Limpopo & Bushveld combined	< 0.01	The Bushveld area characterised by patches of dryland maize/sunflower crop farming and/or extensive grazing, often in combination with/interspersed with game farms had a significantly lower cysticercosis prevalence than all other source designations from these northern regions of South Africa.
Mpumalanga Highveld subregions	< 0.01	Cattle combined from a mixture of commercial and communal sources as well as those collected by opportunistic speculators and from small farms, carried a significantly higher cysticercosis risk than cattle sourced mainly from large commercial cattle and crop farms.
Northern Cape	0.09	Cattle from small herds and speculators had a strong indication of a significantly higher cysticercosis risk compared to cattle sourced directly from large commercial herds.
Eastern Cape Province	0.15	There is a strong suggestion of a statistically significant higher risk for cysticercosis in cattle from the EC Karoo region (P=0.15), compared to cattle that were channelled through the three holding stations in the region or cattle from the cold, pristine Stormberg area.
Northern KwaZulu-Natal Province	< 0.01	There is a statistically significant higher cysticercosis risk associated with cattle sourced by the Dannhauser speculator from mainly communal cattle sources in the northern KZN, with a strong suggestion of a similar association (P=0.04) from a comparable trade channel centred in nearby Newcastle-Normandien, compared to cattle from the same eco-geographical area sourced from large herds of commercial farmers.
Eco-geographical zones	< 0.01	Compared to cattle from high rainfall areas with extensive grazing cattle from the following origins carried a significantly higher cysticercosis risk: Cookhouse HS, Christiana HS, All HS combined, Grass Karoo, Kalahari, Low-Medium rainfall Grassland, Periurban big cities and towns.

Buyer plus geographical origin profile with 1/more lots with a very high cysticercosis prevalence	< 0.01	A lot with a particular buyer plus geographical origin profile that register a very high cysticercosis prevalence (> / = 9%) is a good indicator that other lots with the same buyer plus origin profile will have a significantly higher cysticercosis risk compared to all lots with a low prevalence (< / = 3%) of cysticercosis.
High Summer Rainfall in high human population density areas	0.20	Cattle born and kept until weaning/feedlot entry, in high summer rainfall areas, carry a higher cysticercosis risk compared to cattle born and kept during the dry winter period. All from the same high human population density geographical origin. The same trend is not true when high winter rainfall vs dry summer months are compared.
Rainfall & River Turbulence risk	0.01	Cattle with their feeding period during the local summer high rainfall and high river water turbulence phase carries a higher cysticercosis risk than those fed during the dry winter (May to August) period.
BRD morbidity	< 0.01	Low Risk BRD groups (morbidity < / = 15%), as well as the combination of Low plus Medium BRD risk carried a higher cysticercosis risk than those groups with high (> / = 30% morbidity.
BRD morbidity: Non HS cattle	< 0.01	Low Risk BRD groups (morbidity < / = 15%), carried a higher cysticercosis risk than those groups with higher morbidity.

6. DISCUSSION

Studies on human-pig tapeworm (*Taenia solium*) dominate the scientific literature and historically these perspectives have been extrapolated without critical appraisal to the human-cattle tapeworm (*Taenia saginata*) and the perspectives on effective and sensible critical control points to assure food safety reflected in the carcass inspection regulations of most countries are essentially similar. However there are several very important distinctions between these two human parasites that have enormous economic implications for food animal production and thus food security.

Table 25 Differences between human-pig tapeworm (*Taenia solium*) and human-cattle tapeworm (*Taenia saginata*) that are important for effective control measures [Adapted from Murrell (2005)]

Human-Pig Tapeworm (*Taenia solium*)	Human-Cattle Tapeworm (*Taenia saginata*)
1. Larval stage can infect not only pigs but also humans to cause cysticercosis.	1. Larval stage can only infect cattle to cause cysticercosis.
2. Gravid proglottids containing eggs are shed passively in the faeces only.	2. Gravid proglottids containing eggs are disseminated by actively crawling out of the anus as well as passively shed in the faeces.
3. Substantial evidence indicate that *Taenia solium* is the cause of Human cysticercosis/neurocysticercosis and related epilepsy. Conditions often difficult/dangerous to treat.	3. Very little if any direct evidence link *Taenia saginata* with human disease other than relatively innocuous taeniasis (adult tapeworm in the intestinal tract), a condition easily treated with standard antihelmintics.

Several studies on bovine cysticercosis have highlighted the poor sensitivity and specificity of standard carcass inspections based on so-called predilection sites due to the subjectivity inherent in this approach (Murrell et al., 2005; Kyvsgaard et al., 1990; Walther & Koske, 1980; Murrell, 2005; Allepuz et al., 2012).

The calculation of sensitivity and specificity is central to further statistics such as deriving the true animal prevalence from the apparent animal prevalence as well as risk attributes. To calculate the true animal prevalence of cysticercosis Calvaro-Artavia and co workers in Denmark assumed that the meat inspection specificity = 1.

This is fundamentally flawed as there is no possibility that meat inspection by anybody anywhere can be 100% correct in identifying a suspected *Taenia saginata* cyst as such. It is easy to mistake a small fat deposition or connective scar tissue for a *Taenia saginata* cyst, especially if it is old and calcified, and there is only one on the cut surface examined in the few seconds available. Occasional carcasses display a large number of cysts, these are easy to identify and result in obligatory carcass condemnation under the South African Meat Hygiene Act (Act No 40, 2000) (see Figure 4 below).

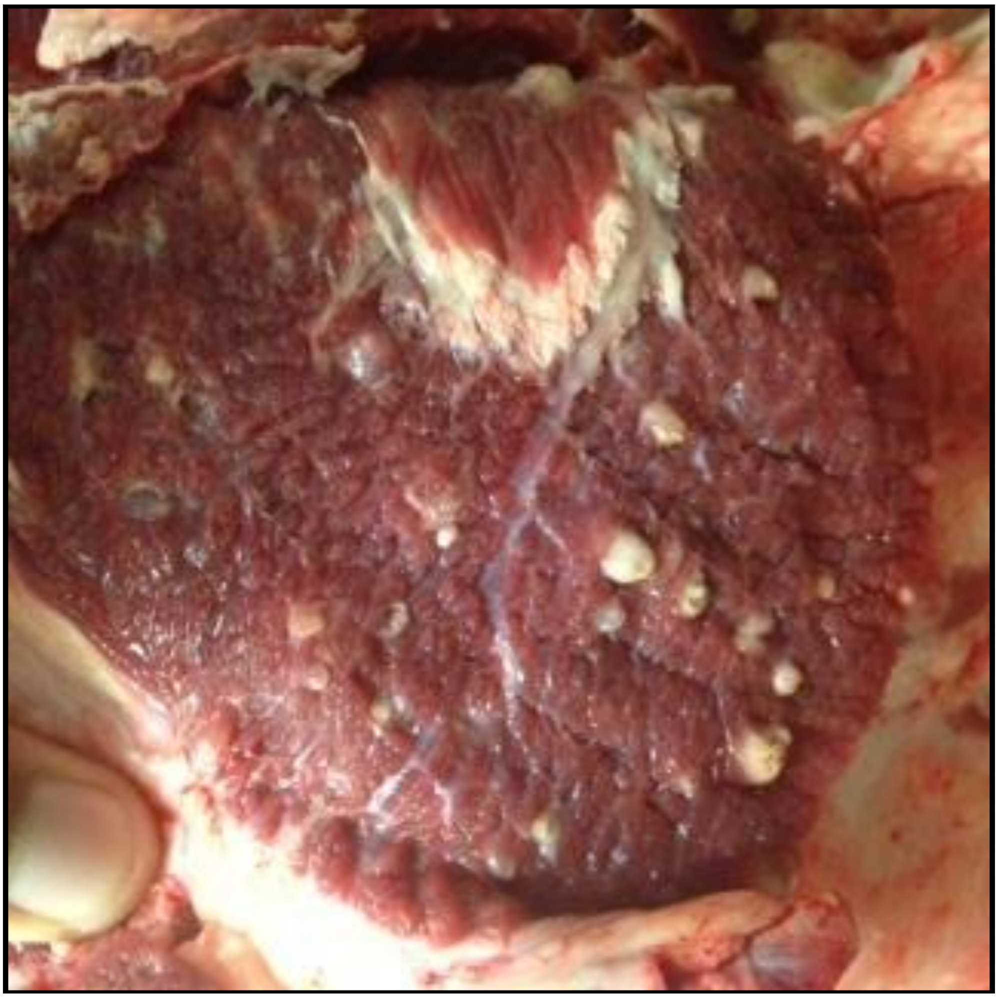

Figure 4 Severe cysticercosis infestation resulting in obligatory carcass condemnation in South Africa. Picture taken at the Karan Beef Balfour abattoir.

It is therefore important to not rely on only carcass inspections as the critical control point regarding the risk of introducing viable *T. saginata* cysts in beef into the human food chain, but rather rely on an integrated approach where the epidemiology of high cysticercosis prevalence cattle is also considered.

6.1 Sex and age/production weight categories associated risks compared

Cattle demonstrate a strong immune response to *Taenia saginata*, a characteristic used in the development of effective anti cysticercosis vaccines (Lightowlers et al., 2003; Lightowlers, 2010). Males and females mature at different rates and under the influence of different hormone profiles, suggesting a different capability to eliminate or prevent infection by parasites (Kyvsgaard et al., 1991; Calvaro-Artavia et al., 2013). Immune responses behave in temporal patterns in response to exposure and thus it is possible that different arrival weight categories (as a proxy for age) can represent different susceptibilities and/or abilities to encapsulate cysts or prevent infestation/migration of *Taenia saginata*. It is therefore scientifically plausible that the risk for cysticercosis as determined when feedlot cattle are slaughtered at a very similar endpoint/live weight can be different between the sexes or arrival weight categories.

In this study there is no statistically significant difference between the cysticercosis risk for heifers vs 'male' (intact bulls plus steers) cattle.

There is a statistically significant difference between the cysticercosis risk only for the very lightest (= youngest) weight category compared to the heaviest (= oldest) category as control (chosen because they had the lowest prevalence). This is suggested when the randomised sample of individual cattle data is used for the calculation (p=0.15) and confirmed when the entire dataset of trial period cattle is used (p=0.01). This category represent approximately 2-4% of incoming cattle and often represent mildly stunted animals or those weaned early and thus forced to eat vegetation/anything including foreign material while they were still supposed to drink from their mothers.

These results are in contrast to those reported from northern Europe, where there was a significantly higher cysticercosis risk association with female cattle as well as younger cattle of both sexes (Kyvsgaard et al., 1991; Calvaro-Artavia et al., 2013; Bogh et al., 1996; Dorny & Praet, 2007). The particular management practices of relatively small cattle herds in environments characterised by long cold winters (housing) and summers with relatively high rainfall and mild temperatures are dramatically different to those practised with relatively large herds under extensive, dry and hot African conditions. So the increased cysticercosis risk in these particular age/sex cohorts could be a confounding effect of the farming conditions of a particular geographical region rather than inherently (physiological) higher susceptibility. Alternatively the inaccuracy of correct sex allocation and data recording during night shifts in 2009, although corrected visually (not electronically) the next day is a more significant flaw than previously appreciated and acts as a major confounder for the sex risk analysis. The electronic reports unfortunately used this flawed database for sex allocation of individual cattle rather than the correct one at slaughter. This situation has been remedied since then, both in terms of the modern, state of the art processing facility as well as adjustments to the electronic reporting systems.

6.2 Multivariate analysis discussion

The higher risk for measles infection attributed to lighter animals (< / = 160 kg) could be the result of early weaning or poor mothering so that these individuals had to forage at an earlier, less immunocompetent age than normal, and thus were exposed to heavier parasite infestations at a younger age. Animals that are clearly stunted as determined by their horns/horn stumps and fall into this very light weight category, are not accepted by the feedlot as they have limited/no growth potential, thus this weight category reflects chronologically very young calves. The host immune response to parasites were thus diluted and more cysticerci allowed to establish in the bovine intermediate host for *T. saginata*. Another speculative mechanism that could explain the observed trend would be that lighter weight calves that were purchased with their heavier brothers and sisters from the same breeding herds, were born later in the season, coinciding with a heavier parasite challenge during their first few months of age, when most susceptible and in the middle of the rainy season. Other analyses in this study suggest a higher measles challenge during periods of high rainfall (= high river turbulence, as well as increased runoff). Further studies are needed to explore these potential mechanisms as they could have a direct bearing on the most optimal timing (calf age and season characteristics) for vaccination if such a strategy is ever rolled out.

The lower risk of measles infection displayed by pure Brahmans could be as a result of their natural geographical distribution in mostly warm and dry regions, and in extensive commercial herds. The high risk associated with some of these ecoregions (see below) is most likely associated with clustering around limited water sources or on contaminated communal grazing areas, while pure Brahmans are less dependent on water and usually come from big commercial herds on uncontaminated grazing with significant browsing in the Bushveld/Kalahari regions. Brown Swiss and Hereford crosses represent very small numbers from specific, dedicated breeders.

Cattle obtained via agents, auctions or speculators are characterised by a huge variation in breeding origins as these lots are assembled from various sources including small numbers of ‘out of season’ calves from large commercial herds, calves from small herds, mixed farming enterprises, emerging and communal farmers. They are often fed grass/hay from dubious origins such as cut from roadsides, before being loaded in big enough parcels to feedlots. A single cattle truck sized lot of 80-100 head can consist of cattle from 10 or more original owners. Considering the lifecycle and transmission routes of *T. saginata*, there are several scientifically plausible mechanisms explaining the higher risk for measles infection in this profile of cattle.

The data do not show a significant difference in odds for measles infection between cattle procured from areas with different human densities, probably because the human density data per magisterial district (see Appendix 3) is a poor indicator of contamination of grazing areas as the scale is too large/imprecise. In addition, the loading point within a particular district does not represent only cattle from within that magisterial district, but rather a poorly defined general source area, often stretching over several magisterial districts or even just the adjacent one. Loading points are chosen for ease of accessibility, existing loading facilities, and/or the proximity to the nearest accurate weighbridge.

Compared to High Rainfall Grassland for example the Stormberg area and the north-eastern Free State and Drakensberg foothills as the baseline, only cattle originating from the Eastern Cape North and Transkei, Grass Karoo-, Kalahari- and Low Rainfall Grass, Savanna ecoregions had significantly higher odds of measles infection. During the study period 2009 to 2010, the first of these were a major channel for cattle from emerging and communal farmers to Karan Beef and this could explain the higher measles risk. Since then the buyers and agents used to obtain calves from that general area were changed, mainly because these cattle also performed very poorly in terms of respiratory health and performance. In addition the holding stations servicing that area were either closed and/or replaced with different ones, under better management and in better facilities, so that the entire health risk profile for cattle from these areas has changed significantly. Cattle from the dry Grass Karoo-, Kalahari- and Low Rainfall Grass, Savanna ecoregions are often obtained from smaller cattle herds where the major farming enterprise is sheep and goats, and/or from situations where human and animals have to share limited water sources. Once again, these regions represent areas where buyers and agents where changed since 2009 to 2010 and holding stations were established in conjunction with different, focussed preventative health programmes employed. Their health profile have also changed significantly.

6.3 Geographical risks compared

The epidemiology of the *Taenia saginata* lifecycle is strongly influenced through shedding of infective segments by human hosts, thus it is scientifically plausible that cattle from different geographical origins, where they are exposed to different levels of infestation of their grazing and/or drinking water as a result of informal settlements, poor ablution facilities, sanitation, personal hygiene and/or dysfunctional sewage treatment plants (see Figure 1 in Introduction), can demonstrate different risk profiles to cysticercosis.

6.3.1 Provinces and sub regions

This is a first level comparison to determine broad levels of risk association between provinces or regions that ecologically or epidemiologically fit together.

Compared to cattle loaded in Mpumalanga Province as control (lowest cysticercosis prevalence), cattle from the following regions: Free State Province, Eastern Cape Province, Northern Cape + Namibia combined, Limpopo + North West + Springbokflats combined, had a significantly higher risk for cysticercosis ($p < 0.01$). A large percentage of Mpumalanga Highveld origin calves come from extensive commercial cattle/crop farms. This broad paradigm of risk comparison have little practical value except to serve as a platform for more detailed analysis.

6.3.2 Gauteng Province subregions

Based on the epidemiology of *Taenia saginata*, it is scientifically plausible that if we compare cattle from the same general geographical region, cattle from small herds or low numbers of calves available (communal cattle around settlements) have more exposure to contaminated grazing or -water than cattle from large herds that in this area typically graze in large natural veld camps or on harvested maize, sorghum, sunflower or soya lands.

Compared to cattle delivered to Karan Beef in small loads (< 40 animals/lot) in a variety of multipurpose trucks, trailers, etc. (not Karan Beef or similar cattle trucks), originating from small farms, plots, communal cattle around townships, etc. that showed the highest cysticercosis prevalence, cattle that arrived at Karan Beef in dedicated cattle trucks (90-115 per lot), from large farms/cow calf herds, auctions, or speculators/backgrounders with loading points close to the major towns in the Vaal triangle (Vereeniging, Sasolburg, Meyerton, Parys) have a significantly lower cysticercosis risk ($p < 0.01$). The latter typically originate from farms where cash crops (maize, sorghum, sunflower or soya) is the major agricultural activity and cattle are used to clean up waste material after harvest (winter) while grazing on natural veld in summer.

6.3.3 Free State Province subregions

Based on the epidemiology of *Taenia saginata*, it is scientifically plausible that if we compare cattle from the same general geographical region, cattle from small herds or low numbers of calves available (communal cattle) have more exposure to contaminated grazing or -water than cattle from large herds that in this area typically graze in large natural veld camps. This hypothesis was true for Gauteng, would it also hold true for the Free State or is there a Gauteng specific confounder? In farms roughly north of Bloemfontein, both east and west, crop farming is also common and cattle are used to clean up harvest waste.

Compared to cattle originating from the northern parts of the Free State (lowest prevalence) characterised by extensive high rainfall grassveld and crop farming, cattle from the central region (Bloemfontein and surrounding towns), characterised by informal and communal sources, grazing and crops, as well as those loaded from the south/southeast, characterised by lower rainfall grazing, some scattered crops as well as communal cattle from former homelands, have a significantly higher cysticercosis risk. If we divide the province from an arbitrary traditional viewpoint into a 'dry west' and 'wet east' using the diagonal N1 highway that runs through Bloemfontein in the center, a similar picture emerges. The cattle sourced from the area close to Bloemfontein and surrounding plots, small farms and township communal cattle as well as from nearby satellite towns and big towns along the N1 highway carry a significantly higher cysticercosis risk.

Cattle from extensive cattle farms in high rainfall areas have less opportunities for cysticercosis infection than cattle from higher population density areas, often characterised by shared grazing with informal ablutions. Lower rainfall areas as well as all other situations where cattle are concentrated facilitate the infection of cattle with Taenia eggs. All situations where cattle are concentrated facilitate the infection with Taenia eggs, especially if the grazing areas are contaminated by informal ablutions.

6.3.4 North West Province + Limpopo + Sprinbokflats (= 'Bushveld' region) and extensive grassland/crops in southwestern part of North West Province; in combination with the southern parts of the Limpopo Province as well as the districts known colloquially as the Springbokflats is generally described as the 'Bushveld'. Here it is combined with extensive grassland/crops in the southwestern part of the province.

Based on the epidemiology of *Taenia saginata*, it is scientifically plausible that if we compare cattle from the same general geographical region, cattle from small herds or low numbers of calves available (e.g. plots, small farms, communal township cattle) have more exposure to contaminated grazing or -water than cattle from large herds that in this area typically graze in large natural veld camps. This hypothesis was true for Gauteng and the Free State Province, would it also hold true for the traditional Bushveld area (North West Province, Limpopo & Springbokflats) as well as extensive grassland/crops in the southwestern part of the North West Province? There is also a strong suggestion that proximity to high density human populations is a significant cysticercosis risk factor. While the North West Province consist of a kaleidoscope of different land uses, from extensive mining around Brits-Rustenburg corridor, small farms and communal cattle in the former Bophuthatswana homeland areas to dryland marginal crop farming with extensive grazing in the low rainfall western districts, and dry savanna bushveld in the northern districts, the trading of cattle is a very dynamic affair with lots of buying and selling by layers of speculators, backgrounding, etc. Due to the dry environmental conditions and the sweetveld grazing there is very little seasonal breeding practiced so that variable numbers of calves are offered for sale throughout the year. This is in contrast to higher rainfall, sourveld grazing in the eastern parts of the country, where a more concentrated breeding season approach has to be followed. It therefore makes sense to compare similar purchase channels/similar ecological profiles in terms of cysticercosis risk.

The dry western area characterised by dryland maize/sunflower crop farming and/or extensive grazing, as well as cattle via the Christiana HS had a significantly higher cysticercosis prevalence, compared to cattle from the Bushveld ($p < 0.01$). This latter origin category represent very few if any cattle from small farms/communal township cattle or communal 'homeland' cattle, or any variation of intermediate phase, and were loaded and trucked in most cases directly from their birth farms to Karan Beef feedlot.

6.3.5 Mpumalanga Province subregions

This is a diverse group, but based on the epidemiology of *Taenia saginata*, it is scientifically plausible that if we compare cattle from similar eco-geographical areas where rainfall and farming methods are comparable but trade channels differ, combined into recognisable profiles for specific lots, there could be some with higher risk than others. Periurban and township communal cattle find their way to Karan Beef through intermediaries: 'backgrounders', speculators or with auctions as the collection and combination channel. Large commercial crop farmers typically also run large cow/calf herds and these were generally loaded and transported directly to Karan Beef.

There was a statistically significant difference between the groups tested in terms of cysticercosis risk. Similar to the patterns observed in other provinces, cattle sourced from highly comingled/mixed groups, including communal and commercial cattle via speculators and middlemen traders, carried a higher cysticercosis risk compared to cattle sourced from commercial farms in the higveld. It was not possible to distinguish between cattle from primarily crop farms and those with livestock as the primary agricultural activity. All situations where cattle are concentrated facilitate the infection with Taenia eggs, especially if the grazing areas are contaminated by informal ablutions

6.3.6 Namibia, Northern Cape Province and Central Karoo

These areas represent the cattle from the driest parts of South Africa; with annual rainfall below 400 mm (most areas below 200 mm). Major ecological zones include the dry Kalahari semi-desert/savanna, as well as the dry scrublands of the Karoo.

There was no statistically significant difference between the groups tested in terms of cysticercosis risk, but a suggestion of significance (P=0.09) in the difference between cattle sourced via speculators and small herds and those from mainly larger herds.

If the Northern Cape cattle are further divided into those originating mainly from big commercial herds (direct transactions) vs those from small herds (speculators/ intermediaries), there is a significantly higher cysticercosis risk associated with the latter category. Even in the driest part of South Africa the same trend as in higher rainfall areas is visible; small herds and intermediate phases where cattle are collected or congregate, increase the cysticercosis risk.

6.3.7 Western Cape Province subregions

This province represents the winter rainfall area of South Africa, with subregions distinctly dominated by the nominated major agricultural activities. Beef cattle herds are generally small, dispersed and kept on marginal lands, while the area is better known for its Dairy industry clustered in groups of large Dairy Farms, often next to or associated with pig farms. The Western Cape Karoo region is better known for its ostriches and only very small numbers of beef cattle were sourced here.

There were no statistically significant differences between the groups tested in terms of cysticercosis risk.

6.3.8 Eastern Cape Province subregions

The Eastern Cape Province has a very wide variation of ecological types, rainfall patterns as well as concentrations of urban and periurban towns and metropolitan areas as well as rural, communal cattle owners in the former Ciskei and Transkei homelands. Based on the epidemiology of *Taenia saginata*, it is scientifically plausible that if we compare cattle from different eco-geographical areas that represent these different profiles, we will find statistically significant risk profiles. The Grahamstown holding station sourced cattle from the area south to southeast of the town, towards the coast, as well as from the former Ciskei (King Williamstown, Bisho, etc.) and the Komga corridor between these two former homelands (East London to the Kei river). The habitat is valley bushveld with a distinct bimodal seasonal rainfall pattern with most rain falling in the summer months. Cookhouse HS received cattle from the central part of the province and Indwe HS accommodated the overflow of cattle from these areas when the other HS were full as well as cattle from the immediate vicinity in the Stormberg. The EC Karoo region is centred on the so-called Grass Karoo/Bo-Karoo towns of Burgersdorp, Steynsburg, Bethulie, etc., south of the Gariep dam and Orange River, an area with the lowest rainfall of these five groups.

There is a suggestion of a statistically significant higher risk for cysticercosis in cattle from the EC Karoo region (P=0.15), compared to cattle sourced from/via the other entities. This is counter intuitive because of the generally hot and dry environmental conditions which are regarded as detrimental to the survival of parasite eggs. However, under these conditions cattle often congregate for long periods around the few waterpoints and wetter/more vegetation areas such as vleis, (dry) watercourses and human habitation. This is a mainly sheep farming area, with cattle herds generally small, often given supplements in the vicinity of limited water points on commercial farms (thus concentrating them) while a significant number of cattle were also communal cattle grazing on municipal ground surrounding these towns and informal settlements.

6.3.9 KwaZulu-Natal (KZN) Province subregions

KwaZulu-Natal North is an area of high rainfall bushveld north of the Tugela river, some crop farming around Dundee, the rest extensive grazing. There are significant numbers of communal cattle in the area, mainly Nguni types and crosses and a particular speculator located in Dannhauser sourced mostly from the latter. Several of his lots showed a particularly high prevalence of cysticercosis, so this area was evaluated with him in/out. The KZN Midlands centres around the towns of Mooiriver and Ladismith and being halfway from the coast to the central Drakensberg escarpment, experience cold winters with frost. It is a high rainfall, sourveld plus irrigated pasture and some crop farming area, the hub of the KZN dairy industry, thus a very high concentration of cattle for different purposes as well as high numbers of labourers per farm associated with cattle farming in this area. East Griqualand is a scenic area centred around the towns of

Underberg and Kokstad, of high rainfall, extensive sourveld grazing plus various crops, in the foothills of the southern Drakensberg with regular annual snow in winter. During this time cattle are concentrated in small camps or on post harvest lands where they share water-, lick and grazing resources. Based on the epidemiology of *Taenia saginata*, it is scientifically plausible that if we compare cattle from different eco-geographical areas that represent these different profiles, we will find statistically significant risk profiles.

There is a statistically significant higher cysticercosis risk associated with cattle sourced by the Dannhauser speculator from mainly communal cattle sources in the northern KZN, compared to cattle from the same eco-geographical area sourced from large herds of commercial farmers ($P < 0.01$ & $P=0.04$). Communal cattle often graze in vleis and open grassland surrounding towns, townships and informal settlements, where ablutions are informal.

6.3.10 Eco-geographical combinations

Combinations of easily recognisable general characteristics associated with the loading points of each lot were grouped into similar categories. These include eco/geographical descriptions e.g. high rainfall – vs low rainfall grassland, Stormberg, Kalahari, Grass Karoo, Karoo etc., management profiles e.g. holding stations, and proximity to high human density localities. Such 'footprints' allow allocation of risk categories with corresponding preventative strategies, in this case cysticercosis risk.

Several combinations/permutations of eco/geographical- plus human density/ management profiles show a statistically significant higher cysticercosis risk ($P < 0.01$) compared to the control cattle that originate from extensive high rainfall grazing areas. This includes counterintuitively, cattle from the very dry Namibia and Northern Cape as well as the cold and wet Stormberg. There could be several reasons for this, but a common denominator is that in all cases, including these extremes of weather and rainfall, have periods where cattle congregate in high densities around scarce resources such as food and open/shared water. These are often shared with or in close proximity to human habitation or settlement. During this study period most Namibian cattle were sourced in the northern region, many from communal sources, and collected in a contracted facility that functioned as a holding station at Otjiwarongo. This type of cattle/human concentration is generally not the case in extensive high rainfall grazing situations.

Several of these categories also have many lots that originate from speculators and backgrounders that purchase from small herds/communal cattle or these are directly taken to the holding stations. Other risk comparisons above have already suggested that these factors combine to create a significantly higher cysticercosis risk than cattle from mainly large commercial herds. A few such high prevalence loads in an eco/geographical category can influence the prevalence and thus act as a confounder. All of these descriptions have a common denominator: there is a significant concentration of cattle around feed/water sources, including all holding stations, often where single or a small number of human carriers can infect large numbers of cattle.

6.4 Cysticercosis prevalence groups compared

If a lot with a very prevalence of cysticercosis (≥ 9%) is detected, will other lots bought by the same person and loaded from the same place, i.e. probably with a very similar history, carry a higher cysticercosis risk than the background? If so it can serve as a reliable 'alert signal' and stimulate a detailed examination of where and how such cattle are collected before purchase so that obvious high-risk sources can be eliminated. Karan Beef buyers each have a network of informants, secondary agents known as 'runners' as well as small scale speculators that supply them with either information regarding calves that become available or the calves themselves. In the table the official buyer/agent is indicated with his initials*.

All categories tested showed a statistically significant higher risk of cysticercosis compared with the background, in other words it seems as if the alert signal of a single lot with very high prevalence is a useful indicator to stimulate a detailed analysis of a particular buyer and loading point combination.

6.5 Seasonal rainfall patterns in South Africa and human density

Based on the epidemiology and ecology of *Taenia saginata* it is scientifically feasible that Taenia eggs will have a higher survival rate and thus more infectious opportunities during warm and wet seasons compared with other combinations. Two specific, high human density areas were selected i.e. Gauteng vs Gape Town plus George.

There is a strong suggestion (P=0.20) that cattle exposed during their 6-9 months before entering the feedlot to high summer rainfall, carry a higher cysticercosis risk than cattle that spent most of their pre-feedlot lives in the dry, cold winter months, all from the same high human density geographical area. High humidity plus warm temperatures create potentially a friendlier microclimate for cysticercosis eggs to survive in human faeces or in free segments, until ingested by the intermediate bovine host.

6.6 Local rainfall patterns (Heidelberg-Gauteng) and concomitant river (Blesbokspruit and Suikerbosrand rivers) turbulence

Putative risk from literature and expert opinion statement: 'Contaminated river water is an important driver of cysticercosis infestation of cattle in the Karan Beef Feedlot during their feeding period'. To investigate this it must be assumed that rainfall measured at Karan Beef Feedlot is an accurate indicator for rainfall in the Blesbokspruit and Suikerbosrand River catchments and thus correlated with periods of turbulence/strong flow, as well as that river flow/turbulence causes more Taenia eggs to be suspended in the water column, at the level of approximately 1 m below surface where the inlet pumps draw from, compared to periods of stagnation when these eggs have time to settle in the bottom sediment.

Table 26 Rainfall measured at Karan Beef feedlot, Heidelberg, and river turbulence risk category

Month	Rainfall (mm)	Total per cycle	Turbulence =Risk
Jan-09	140		
Feb-09	84		High Turbulence = High Risk
Mar-09	52		
Apr-09	0	**276**	
May-09	24		
Jun-09	9		Low Turbulence = Low Risk
Jul-09	0		
Aug-09	22	**55**	
Sep-09	2		
Oct-09	64		High Turbulence = High Risk
Nov-09	107		
Dec-09	112	**285**	
Jan-10	214		
Feb-10	136		High Turbulence = High Risk
Mar-10	14		
Apr-10	46	**410**	
May-10	24		
Jun-10	0		Low Turbulence = Low Risk
Jul-10	0		
Aug-10	0	**24**	
Sep-10	0		
Oct-10	18		High Turbulence = High Risk
Nov-10	96		
Dec-10	175	**289**	

The standard production programme at Karan Beef entail three cycles/population turnovers of roughly four months each, average days on feed = 120 days, although some heavier cattle are fed for only 90 days (GSTR) and lighter cattle for 150 days (BG). The confluence of the Blesbokspruit and Suikerbosrand Rivers is on the Karan Beef estate and although the pump station is in the latter river, about 300 m from the confluence, the seasonal flow patterns in the larger Blesbokspruit determine where the drinking water for the cattle actually comes from.

There is indeed a statistically higher risk (P=0.01) for cysticercosis during periods of high rainfall and the associated higher turbulence in the river water column at the pumpstation when all cattle arriving during the study period from all areas are considered. Several helminth specialists (Jan van Wyk, Joop Boomker personal communication) have suggested that the contaminated river water used at Karan beef is a significant driver for the cysticercosis prevalence observed, and this result supports that suggestion. High rainfall periods coincide with active flowing, turbulent water, keeping suspended cysticercosis eggs in the water column at a depth where the pump station pulls drinking water, alternatively the regular flushing of contaminated material into the river system through summer thunder storms accompanied by heavy downpours causes a high level of contamination in the entire river water column compared to dry winter periods when these eggs settle in stagnant water in the bottom sediment.

6.7 BRD risk profile categories as Cysticercosis risk proxy compared

Retrospective evaluation at slaughter (‘closeout’) of the health and production performance of lots received at Karan Beef is done as a standard operating procedure, in line with the practice at most large feedlots in the world. This allows the recognition of epidemiological patterns that remains similar over time and can thus be used as part of a predictive expectation of health and performance of cattle sourced from particular areas, trade channels, seasons, etc. This approach forms part of the fabric of preventative veterinary interventions based on Risk Profiling and appropriate prophylactic/ metaphylactic processing regimens and adaptation phase management in large cattle feedlot populations. This existing standard practice focuses on respiratory disease management, but the platform can potentially be extended to include all factors that affect the economic sustainability of purchasing and feeding cattle with a particular epidemiological footprint.

Problems with data: With arbitrary cut off values of 20% and 30%, there is a statistically significant ($P < 0.01$) lower cysticercosis risk associated with cattle from lots that recorded BRD morbidities higher than 20% during this period.

Using the raw data in this way unfortunately accommodates many operator mistakes (e.g. where follow up treatments on day 5 or 8 would still be entered as 'treatment 1') and inaccuracies allowing individual cattle to be present more than once as during this period (2009) first treatments were reflected per treatment category, in other words an animal could be pulled for respiratory treatment, develop bloat after recovery and be treated for that and maybe later pulled again for lameness and all three treatments/pulls would be present in the data. The 2010 to 2011 period was also when several intensive comparative evaluations of antibiotics used during the standard treatment protocols were conducted, often using a cross over experimental design to limit personal bias as a major confounder for or against a particular compound or regimen by a specific hospital team. The way of entering data into Walco at the hospitals was changed in 2010 to allow more accurate, less complex data analysis.

When all cattle in the study period database are categorised into High, Medium or Low risk, the very poor health performance registered by the Namibian (via Otjiwarongo HS) as well as Zastron HS (contracted out) and Grahamstown HS (contracted out) cattle act as a major confounder. These cattle were sourced from communal areas, small auctions and leftovers after speculators had sold the better-looking calves to others and poor management on the holding stations could not turn this around. All three holding station operations were closed in 2010 and 2011 respectively so that cattle from these geographical areas were since then sourced through other, direct purchase channels. Low BRD risk cattle alone or in combination with medium risk lots showed a significantly higher cysticercosis risk with P values of < 0.0001 and 0.009 respectively. Cattle channelled through the Christiana, Indwe and Cookhouse holding stations have consistently performed very well in terms of respiratory health; at least half of pull rates registered by lots from non-HS sources in any given period. They also received a large percentage of their calves from similar trade channels as mentioned above, but managed to establish a solid health base through exceptional management. This was shown to be the key success factor for this satellite facility concept in turning high BRD risk calves to low BRD risk.

In addition, cattle from holding stations experience a two week period of exposure to potential drivers of cysticercosis infestation e.g. infected/shedding worker(s), contaminated hay, common source drinking water. So once again the holding station channel could be a major confounder in testing the central question; 'How and When are cattle infected with cysticercosis?'

The adaptation period in holding stations establishes a significant benefit in terms of respiratory health so this low category of BRD risk is dominated by cattle from the various holding stations. Here a single human carrier can infest a relatively large number of cattle as they are concentrated and share water and feed troughs.

However, with all holding station cattle split out from the database the cattle with low and medium BRD risk had significantly higher cysticercosis risk compared to cattle with a high BRD risk (high crude morbidity). The latter category were dominated by cattle transported directly from relatively pristine, disease free environments, which then succumbed to the very high infectious respiratory disease challenges during the adaptation phase (first 30 days) in the feedlot. In addition, any low BRD risk lots come from dry areas with associated concentration points as explained above. During subsequent years (2012 to 2016) three new holding stations were established in Kuruman, Verkeerdevlei and Graaff-Reinet, to provide a smoother, less stressful adaptation to these cattle, while the purchase procedures and reception protocols at existing holding stations were critically reviewed and adapted. This new strategy has stood the test of time in terms of BRD management at Karan Beef, but from the data analysed in this study there is no sense in pursuing a general 'BRD risk avoidance' purchase strategy with a concomitant bonus the lowering of cysticercosis risk.

7. GENERAL ASPECTS AND LIMITATIONS OF THIS RETROSPECTIVE STUDY

7.1 Retrospective studies in general are not suitable for rare diseases or inaccurately diagnosed diseases/conditions

The prevalence of cysticercosis in this study was only 2.72%, based on standard abattoir carcass inspection data. The limitations of using such a subjective, experience and motivation driven 'diagnostic tool' was discussed in the Introduction Section. This is a recognised weakness of using such data, but the approach is still used extensively in studies worldwide. In this study, the very large dataset analysed gave more credibility to the results. In addition, the assumption is that the human factor is relatively constant as all cattle analysed were slaughtered in the same abattoir, with minimal changes in the meat inspector personnel over the study period. This is different from other studies that used country- or regional data from many abattoirs (Calvo-Artavia et al., 2012 & 2013; Dorny et al., 2000; Dupuy et al., 2015; Qwekana et al., 2016).

7.2 Standard records that were not designed for the study results in poor quality of available data

This study used standard production data captured by the Karan Beef electronic management systems. Due to aged infrastructure during the study period, personnel changes in the processing team and the limitations of night shift cattle handling due to high numbers of incoming cattle over short periods, some data such as sex and breed allocations were done inaccurately at times or for significant periods not at all. This detracts significantly from the value of analysing these variables or putative risk factors. While sex allocation could be corrected visually the next morning (but not captured electronically) and is important for production purposes, breed allocation remains a purely academic exercise at Karan Beef as we do not factor breed differences into our production system at all. Some breeds e.g. Nguni cattle do not justify the same price structure at purchase due to their poorer growth potential compared to most other breeds, but during the production period all breeds are mixed.

7.3 There is an absence of data on potential confounding factors because the data were recorded in the past

A major emphasis of this study is the investigation of buyer-source-geographical area (loading point) associations with cysticercosis prevalence. In many cases the loading point is a poor indicator of exact geographical origin of calves due to the fact that large cattle trucks simply cannot reach the very remote rural areas and thus cattle are often transported in smaller parcels to a convenient 'near' loading point, not always the same one for cattle from a particular district or farming type. In addition, some loading points reflect a mixture of self-bred and backgrounded (bought in) calves, also many loads consist of calves from more than one owner and the purchase channel allocation was then assigned based on the status of 'most' of the calves in the load. These different sources carry inherently a different risk profile for cysticercosis. The accurate capture of the exact history of every calf was not done during the study period, and although this aspect was addressed since then and is much improved currently, the level of accuracy we desire will only be possible once an official national electronic identification scheme is rolled out in South Africa.

Rainfall patterns in the source areas during the 6-10 months (calf age) before the loading date were not recorded and broad, SA Weather Service rainfall data is not accurate enough to factor into an analysis of the impact of such patterns on the associated cysticercosis risk. A general positive association in terms of high (summer) rainfall (as measured at Karan Beef) and associated turbulence of the river systems that provide the Karan Beef feedlot cattle with drinking water, suggest that a similar dynamic could be very relevant also in the localised, source geographical areas.

7.4 Differential losses to follow up can introduce selection bias in retrospective studies

There were strong indications that cattle from speculators that collect small numbers of calves, even singles, from small scale or emerging farmers and communal herds carry a higher cysticercosis risk than groups of cattle that were procured through other purchase channels and farming types. These cattle also have a higher morbidity and mortality rate, thus this category experience a higher 'attrition rate' from arrival to slaughter. The potential use of BRD risk as a proxy for cysticercosis risk was discussed in depth in 6.6

above. The diagnosis of cysticercosis is made only after slaughter, so the representation of the cysticercosis prevalence in such groups as a true reflection of its status on arrival will potentially be less accurate than the situation in comparative groups sourced through other channels, thus introducing bias to the analyses of such data.

8. PRACTICAL APPLICATIONS OF CYSTICERCOSIS RISK PROFILES

A focus on specific trade channels and geographical origins that carry consistently a disproportionate cysticercosis risk (e.g. the KZN: Dannhauser speculator) is a feasible and pragmatic approach that was instituted by Karan Beef in February 2011 as part of the successful integrated cysticercosis mitigation strategy mentioned above. The circumstantial evidence from this dataset regarding the potential risk for completion of the *T. saginata* lifecycle by concentrating large numbers of cattle in holding stations with exposure to single/few infected staff members, similar to what has been described in feedlot 'outbreaks' were brought to the attention of senior management and thus the Karan Beef obligatory staff deworming programme was extended to include all HS staff.

With effective cysticercosis vaccines developed against both *T. saginata* and *T. solium* (Lightowlers et al., 2003; Lightowlers, 2010) and the latter actively promoted as a preventative measure in free ranging pigs (Anonymous, 2007), it is feasible that such a commercial product against *T. saginata* could be available in future. Considering the relatively low prevalence of cysticercosis in the general cattle population that enter South African feedlots, it will be important to focus such a measure on identifiable higher risk (high prevalence) lots/origins to attain an optimum cost-benefit for the additional expense incurred. Effective cysticercosis control/prevention/impact mitigation programmes will always consist of an integrated approach e.g. what is attempted in the Karan Beef Cysticercosis programme explained above in the Introduction. Cysticercosis vaccination of cattle should be done in conjunction with regular effective deworming and personal hygiene education of people at a Primary Health Care level in communities/areas that has shown consistently high levels of infestation taeniosis in people and/or cysticercosis in cattle) e.g. those identified in this study.

9. CONCLUSIONS AND RECOMMENDATIONS

Karan Beef is similar to other large commercial cattle feedlots in South Africa in terms of the cattle procurement dynamics, basic production methodology, procurement, production and use of feed raw materials in on site feed factories, the use of surface water as source of drinking water for the cattle as well as the labour force profile so the cysticercosis data presented here is likely to be representative of SA feedlot cattle, and conclusions reached about the relationships between risk factors and efficacy or not of control measures at feedlot level could probably be extrapolated to the rest of the SA feedlot industry.

From this series of risk analyses on data from 2009 and 2010, several variables were consistently associated with a higher cysticercosis risk in cattle sourced from all over South Africa to Karan Beef Feedlot. Cattle sourced via speculators and from small scale producers or communal herds as well as from certain dry areas where humans and cattle congregate/concentrate around limited water sources and similarly collection/ concentration strategies such as holding stations, carried significantly higher cysticercosis risk compared to controls. In addition, cattle that arrived at a younger age as well as cattle fed through the rainy season carried a higher risk. All of these factors point to practical control measures in terms of purchase strategies, periods of more important staff deworming and even future targeted vaccination strategies. Key concepts are; concentration of cattle and common source exposure to *T. saginata* contaminated feed, grazing and/or water.

Further studies should explore the sex and age susceptibility differences found in this study compared to European studies as this can have profound implications on developing targeted preventative measures in terms of vaccination, key infection periods and places etc. especially in European small cattle herds.

Another key question in understanding the immune response of cattle to *T. saginata* that carry enormous financial implications, is whether the status of a single cyst (soft and viable vs hard, calcified and non-viable) found during standard meat inspection is indeed an accurate representation of all other cysts in the carcass, not found visually. In other words a carcass might be ‘cysticercosis positive’ but carry no infective risk thus do not justify expensive decontamination procedures requiring freezing.

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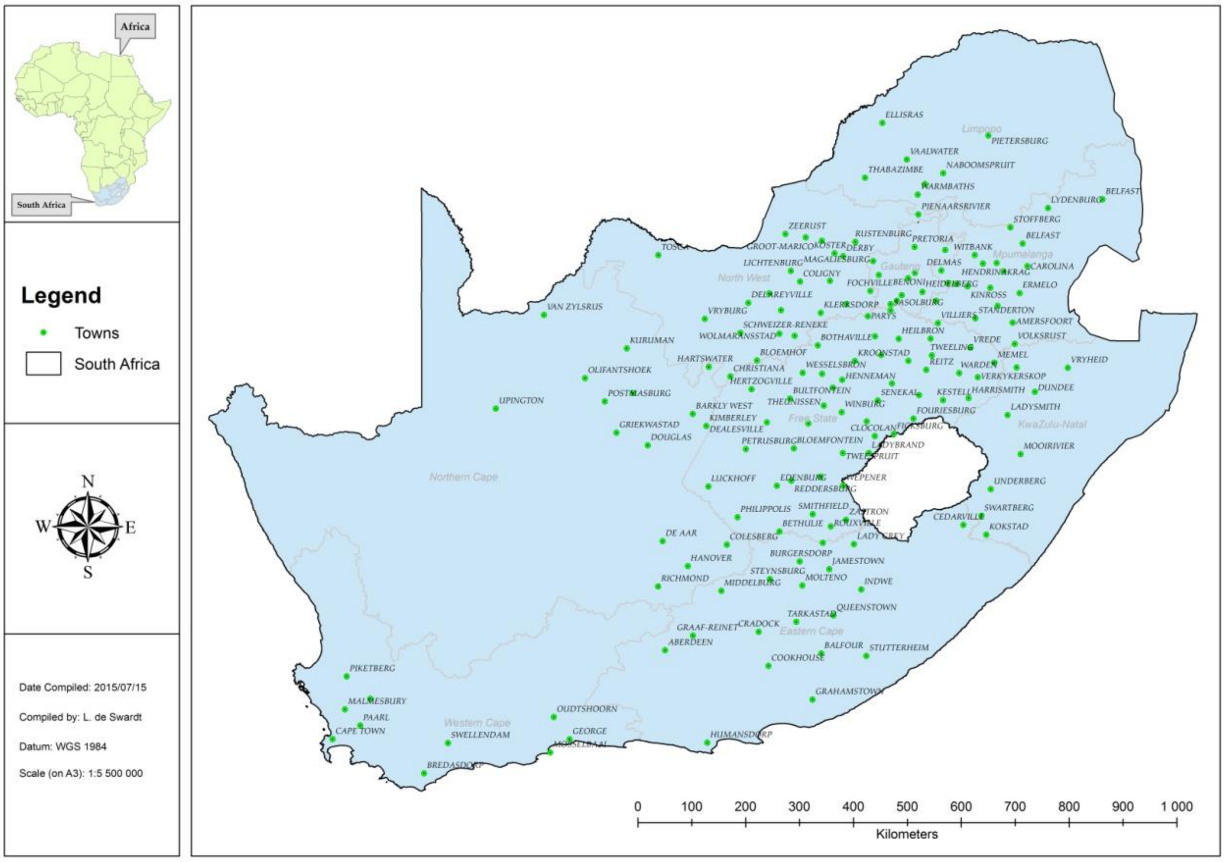
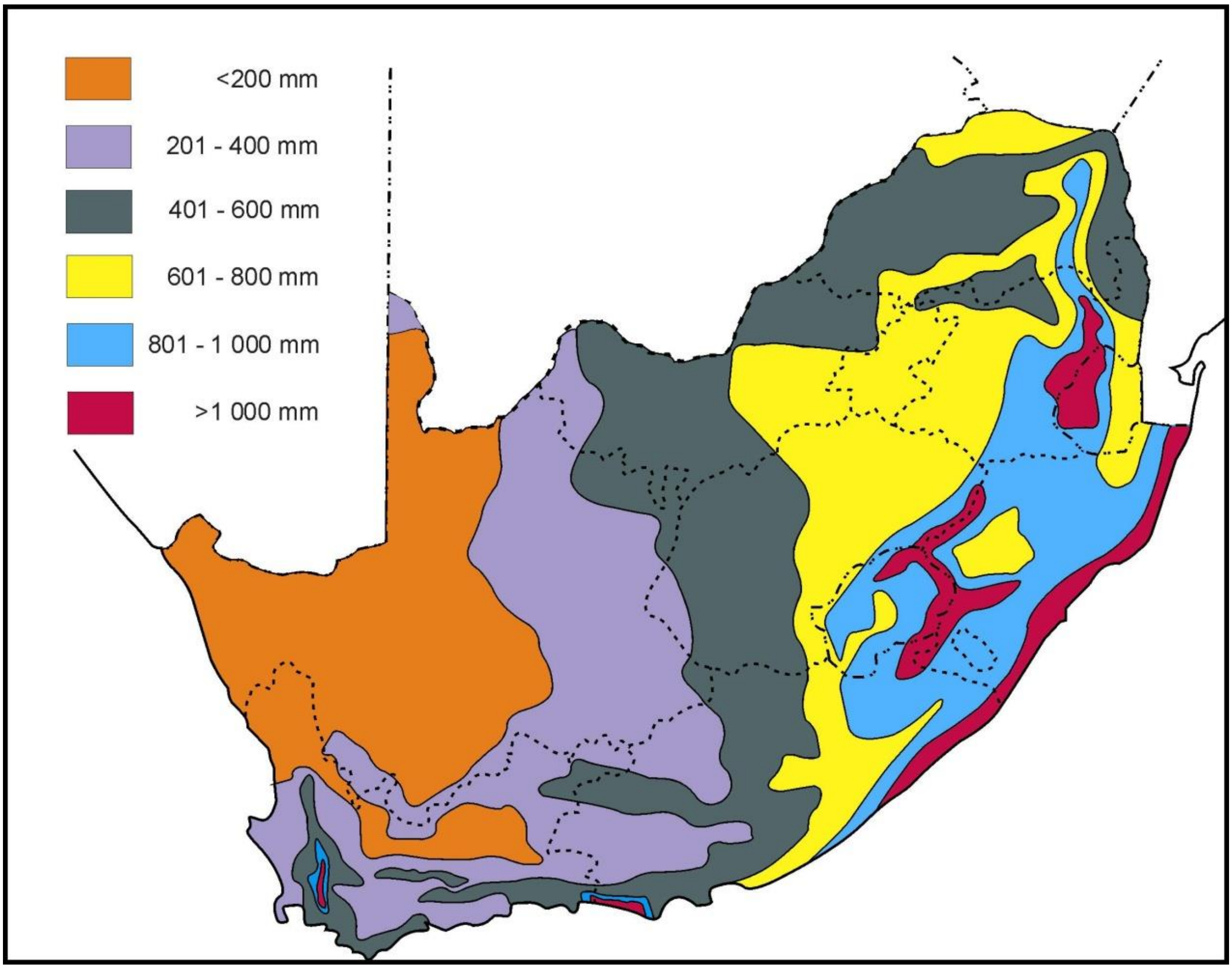
Appendix 1

Loading points/nearest town in the case of silo's or auction yards used for this purpose for cattle purchased by Karan Beef Feedlot: January 2009 to December 2010



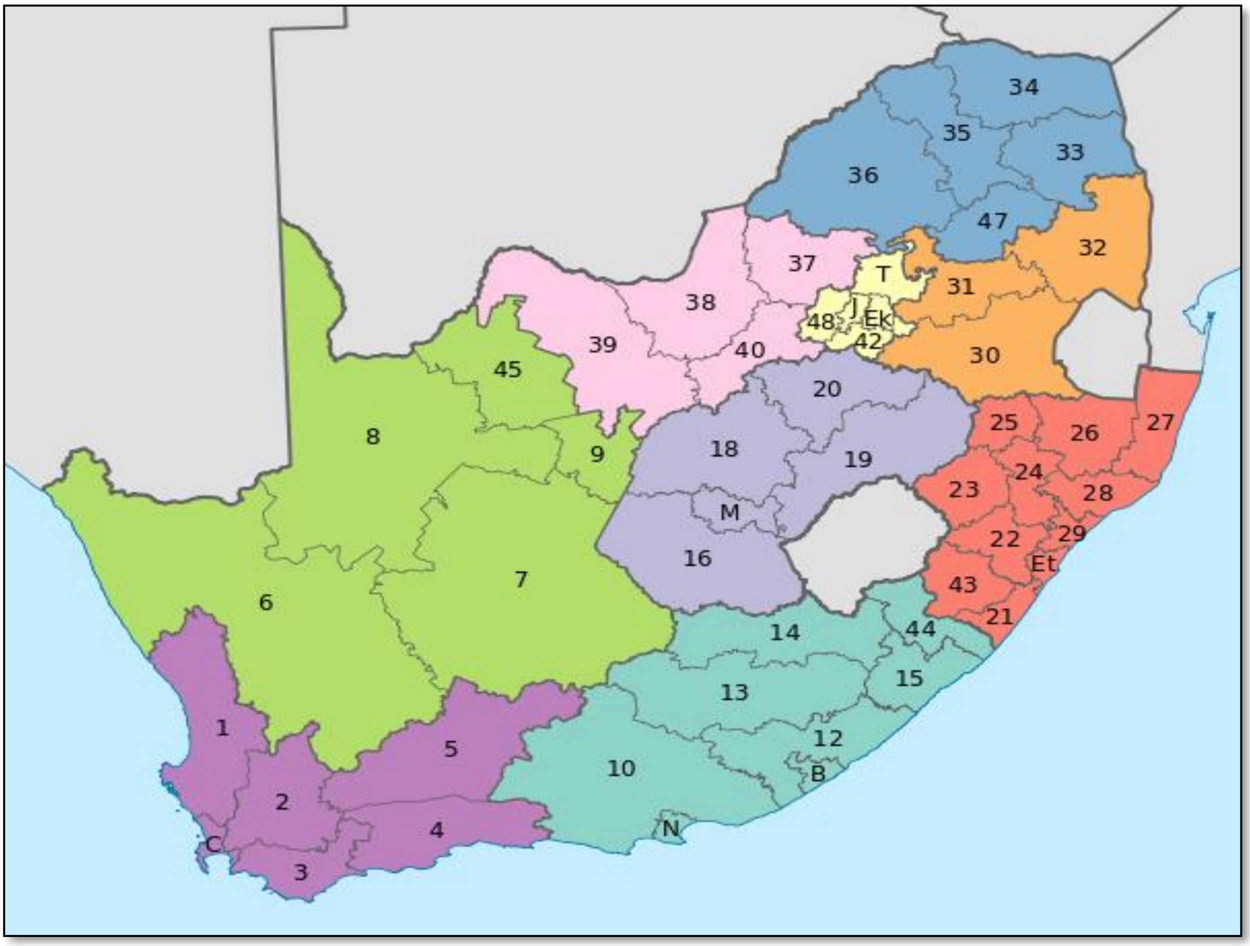
Appendix 2

Rainfall in South Africa: 1973 to 2013



Appendix 3

Map and table of district municipalities and metropolitan municipalities related to sensus 2011 figures, used to correlate loading points of cattle to human densities



Name	Code	Province	Seat	Area (km²)	Population	Pop. Density (per km²)
West Coast District Municipality	DC01	Western Cape	Moorreesburg	31.104	391,766	12.6
Cape Winelands District Municipality	DC02	Western Cape	Worcester	22.309	787,490	35.3
Overberg District Municipality	DC03	Western Cape	Bredasdorp	11.405	258,176	22.6
Eden District Municipality	DC04	Western Cape	George	23.331	574,265	24.6
Central Karoo District Municipality	DC05	Western Cape	Beaufort West	38.854	71,011	1.8
Namakwa District Municipality	DC06	Northern Cape	Springbok	126.836	115,842	0.9
Pixley ka Seme District Municipality	DC07	Northern Cape	De Aar	102.727	186,351	1.8
ZF Mgcawu District Municipality	DC08	Northern Cape	Upington	102.524	236,783	2.3
Frances Baard District Municipality	DC09	Northern Cape	Kimberley	13.518	382,086	28.3
Sarah Baartman District Municipality	DC10	Eastern Cape	Port Elizabeth	58.194	450,584	7.7
Amathole District Municipality	DC12	Eastern Cape	East London	21.043	892,637	42.4
Chris Hani District Municipality	DC13	Eastern Cape	Queenstown	36.695	795,461	21.7
Joe Gqabi District Municipality	DC14	Eastern Cape	Barkly East	25.663	349,768	13.6
OR Tambo District Municipality	DC15	Eastern Cape	Mthatha	15.968	1,364,943	85.5
Xhariep District Municipality	DC16	Free State	Trompsburg	37.674	146,259	3.9
Lejweleputswa District Municipality	DC18	Free State	Welkom	31.930	627,626	19.7
Thabo Mofutsanyana District Municipality	DC19	Free State	Phuthaditjhaba	32.637	736,238	22.6
Fezile Dabi District Municipality	DC20	Free State	Sasolburg	21.301	488,036	22.9
Ugu District Municipality	DC21	KwaZulu-Natal	Port Shepstone	5.047	722,484	143.2
uMgungundlovu District Municipality	DC22	KwaZulu-Natal	Pietermaritzburg	8.934	1,017,763	113.9
uThukela District Municipality	DC23	KwaZulu-Natal	Ladysmith	11.326	668,848	59.1
uMzinyathi District Municipality	DC24	KwaZulu-Natal	Dundee	8.589	510,838	59.5
Amajuba District Municipality	DC25	KwaZulu-Natal	Newcastle	6.911	499,839	72.3
Zululand District Municipality	DC26	KwaZulu-Natal	Ulundi	14.799	803,575	54.3
uMkhanyakude District Municipality	DC27	KwaZulu-Natal	Mkuze	12.821	625,846	48.8
uThungulu District Municipality	DC28	KwaZulu-Natal	Richards Bay	8.213	907,519	110.5
iLembe District Municipality	DC29	KwaZulu-Natal	KwaDukuza	3.269	606,809	185.6
Gert Sibande District Municipality	DC30	Mpumalanga	Ermelo	31.841	1,043,194	32.8
Nkangala District Municipality	DC31	Mpumalanga	Middelburg	16.758	1,308,129	78.1
Ehlanzeni District Municipality	DC32	Mpumalanga	Nelspruit	27.896	1,688,615	60.5
Mopani District Municipality	DC33	Limpopo	Giyani	24.489	1,092,507	44.6
Vhembe District Municipality	DC34	Limpopo	Thohoyandou	21.349	1,294,722	60.6
Capricorn District Municipality	DC35	Limpopo	Polokwane	16.988	1,261,463	74.3
Waterberg District Municipality	DC36	Limpopo	Modimolle	49.504	679,336	13.7
Bojanala Platinum District Municipality	DC37	North West	Rustenburg	18.333	1,507,505	82.2
Ngaka Modiri Molema District Municipality	DC38	North West	Mafikeng	27.889	842,699	30.2
Dr Ruth Segomotsi Mompati District Municipality	DC39	North West	Vryburg	44.017	463,815	10.5
Dr Kenneth Kaunda District Municipality	DC40	North West	Klerksdorp	14.642	695,933	47.5
Sedibeng District Municipality	DC42	Gauteng	Vereeniging	4.177	916,484	219.4
Harry Gwala District Municipality	DC43	KwaZulu-Natal	Ixopo	11.127	461,419	41.5
Alfred Nzo District Municipality	DC44	Eastern Cape	Mount Ayliff	6.859	801,344	116.8
John Taolo Gaetsewe District Municipality	DC45	Northern Cape	Kuruman	27.283	224,799	8.2
Sekhukhune District Municipality	DC47	Limpopo	Groblersdal	13.426	1,076,840	80.2
West Rand District Municipality	DC48	Gauteng	Randfontein	4.087	820,995	200.9
Buffalo City Metropolitan Municipality	BUF	Eastern Cape	East London	2.536	755,200	297.8
City of Cape Town Metropolitan Municipality	CPT	Western Cape	Cape Town	2.460	3,740,026	1,520.3
Ekurhuleni Metropolitan Municipality	EKU	Gauteng	Germiston	1.924	3,178,470	1,652.0
eThekwini Metropolitan Municipality	ETH	KwaZulu-Natal	Durban	2.292	3,442,361	1,501.9
City of Johannesburg Metropolitan Municipality	JHB	Gauteng	Johannesburg	1.645	4,434,827	2,695.9
Mangaung Metropolitan Municipality	MAN	Free State	Bloemfontein	6.284	747,431	118.9
Nelson Mandela Bay Metropolitan Municipality	NMA	Eastern Cape	Port Elizabeth	1.959	1,152,115	588.1
City of Tshwane Metropolitan Municipality	TSH	Gauteng	Pretoria	6.345	2,921,488	460.4

Appendix 4

Animal Ethics Approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Risk Indicators and management procedures associated with the Control of Bovine Cysticercosis (taenia saginata) in a large commercial South African Cattle Feedlot**
PROJECT NUMBER	**V036-15**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. DJ Verwoerd**

STUDENT NUMBER (where applicable)	**UP_85071201**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**n/a**	
NUMBER OF ANIMALS	**n/a**	
Approval period to use animals for research/testing purposes		**November 2015-November 2016**
SUPERVISOR	**Prof. D Abernethy**	

<u>*KINDLY NOTE:*</u>

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 30 November 2015
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15