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The effect of delayed evisceration on the microbiological safety of black wildebeest (*Connochaetes gnou*) meat

By

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Submitted in partial fulfilment of the requirements for the degree

Magister Scientiae (Human/Animal/Ecosystem Health)

in the Faculty of Veterinary Science, University of Pretoria

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Declaration

I declare that this dissertation, which I hereby submit for the Master of Science degree in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, to be my own work and has not been previously submitted by me for degree purposes at another tertiary institution.

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Casper Vorster van Heerden

16 June 2016

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List of Abbreviations

cfu	Colony forming unit
DVTD	Department of Veterinary Tropical Diseases
MODS	Multiple organ dysfunction syndrome
VPN	Veterinary Procedural Notice

Summary

The effect of delayed evisceration on the microbiological safety of black wildebeest (*Connochaetes gnou*) meat

by

Casper Vorster van Heerden

Supervisor: Prof M van Vuuren

Degree: MSc (Human/Animal/Ecosystem Health)

Slaughter protocols for game harvesters are defined by the Department of Agriculture, Forestry and Fisheries in South Africa in Veterinary Procedural Notice (VPN 8). VPN 8 stipulates that game animals should normally be eviscerated in a hanging position within 2 hrs after being shot, and if bloating occurs evisceration must be done as an emergency, within 0.5 hr, or as soon as possible after bleeding by the game harvester. It would be beneficial to know if the concept of delayed evisceration has any deleterious effects on the microbiological safety of harvested black wildebeest (*Connochaetes gnou*) meat. The objective of this research project was to determine if delayed evisceration has an effect on enteric bacterial translocation across the intestinal mucosal barrier into the abdominal cavity. Sixteen harvested animals were grouped together in groups A, B, C, D and E, based on a time delay in evisceration. After each animal was eviscerated, an 8mm punch biopsy from the abdominal peritoneum was collected and placed into a 2 ml Biofreeze vial. In the laboratory, the biopsy samples were inoculated on blood and MacConkey agar plates and incubated for three days at 37°C. The bacterial species targeted for isolation were indicator bacteria for movement across the intestinal barrier. The quantifiable absence of bacteria from the samples that were subjected to bacterial isolation in this study indicates that bacterial translocation across the intestinal barrier is negligible in black wildebeest (*Connochaetes gnou*) exposed to delays in evisceration for up to 5 hrs. Evidence indicating that black wildebeest (*Connochaetes gnou*) meat exposed to bloating and a delay in evisceration is not safe for human consumption due to bacterial translocation is therefore lacking.

Chapter 1: Introduction

A notice was published in the Government Gazette No. 35706, 28 September 2012, in which the Minister of Agriculture invited public comments on the proposed Game Meat Scheme in terms of section 12 of the Meat Safety Act 40, 2000 (Act no. 40 of 2000). The invitation for comments was related to the implementation of the provisions of the Meat Safety Act in the game industry, in particular limited throughput game slaughter facilities and harvesting processes.

Food security and food safety and the potential impact it can have on the continent of Africa, cannot be over-emphasized (Godfray *et al*., 2010; Smith *et al*., 2013). The production of safe and quality-assured game meat can support the animal protein requirements in South Africa and provide game meat that is safe for consumption by all citizens in the country (Magwedere *et al*., 2013). To provide the consumer with safe and wholesome game meat, Wildlife Ranching South Africa (WRSA) applied for international certification for its game meat standard which is based on Food Safety Management Systems (ISO 22 000), VPN 8 and the proposed game meat scheme promulgated by the Department of Agriculture, Forestry and Fisheries (DAFF). Accreditation of the WRSA Game Meat Standard was obtained in 2015 from the International Standards Certification (ISC), a globally accredited certification body based in Australia, measured against the specifications of the International Organization for Standardization (ISO) ISO 9 001, ISO 14 000 and the ISO 22 000 Standards.

These ISO standards are industry benchmarks for environmental safety (ISO 14 000), food safety management (ISO 22 000) and quality management (ISO 9 001). ISC will therefore verify the auditing of game farms on which game is harvested, as well as the secondary production facilities, with the ultimate goal of providing a safe, quality-assured product that is traceable.

Slaughter protocols defined by the DAFF for game meat in South Africa in VPN 8 stipulates that harvested game should be eviscerated in a hanging position within 2 hrs after being shot, and if bloating occurs evisceration must be done within 0.5 hr, or as soon as possible after bleeding by the game harvester in the field.

The proposed draft game meat regulations under the Meat Safety Act (Act 40 of 2000) to be promulgated in 2016, needs to consider the outcome of this study.

The aims of this research project were:

- to determine if delayed evisceration of wildebeest following hunting has an effect on enteric bacterial translocation across the intestinal mucosal barrier into the abdominal cavity
- to determine if delayed evisceration compromises the safety of the meat for human consumption

Chapter 2: Literature Review

The factors that influence the quality and safety of a game carcass varies and can range from species differences to ambient temperature during harvesting, harvesting methods, day or night harvesting, shot placement, slaughtering techniques and secondary processing procedures (Hoffman *et al*., 2009a).

Specific carcass data parameters are also available relating to the different ungulate species including mountain reedbuck (*Redunca fulvorufula*) (Hoffman *et al*., 2008b), black wildebeest (*Connochaetus gnou*) (Hoffman *et al*., 2009d), blue wildebeest (*Connochaetes taurinus*) (Hoffman *et al*., 2011), blesbok (*Damaliscus dorcas phillipsi*) (Hoffman *et al*., 2008a), kudu (*Tragelaphus strepsiceros*) (Hoffman *et al*., 2009b; Hoffman *et al*., 2009c), impala (*Aepyceros elampus*) (Hoffman & Laubscher, 2009) and springbok (*Antidorcas marsupialis*) (Hoffman *et al*., 2007a; Hoffman *et al*., 2007b; Hoffman *et al*., 2007c; Hoffman *et al*., 2007d). These parameters include mean body and carcass weight, dressing percentage, initial and final pH of the meat, carcass temperature at 24 hrs post mortem, tenderness, colour, protein and lipid content, amino acid content and mineral (potassium, phosphorus, sodium, iron, copper) levels. In addition to the above-mentioned parameters, physical and chemical properties of game meat that have also been studied and documented in the southern African region include: drip loss, cooking loss, shear force and colour.

It is hypothesized that delays in evisceration may lead to a contaminated carcass or contaminated meat as a result of bacterial translocation across the intestinal barrier into the mesenteric lymph nodes and subsequently into the systemic circulation leading to widespread diffuse seeding of bacteria in distant carcass tissues, or into the peritoneal cavity leading to contamination (Gill *et al*., 1976). This observation is supported by the isolation of bacteria from the mesenteric lymph nodes in animals post mortem. However, documented evidence supporting this theory is not available. On the contrary, the bacteria isolated were identified as non-enteric bacteria belonging to genera that cannot grow in the intestinal tract of animals (Gill *et al*., 1976). Most human studies considered and explored the effects of pathogenic bacterial translocation in the diseased state (Gabe, 2001).

Berg (1995) proposes that even in healthy immunocompetent hosts, the indigenous enteric bacterial microflora translocates across the intestinal barrier in low numbers, but they are killed *en route* to or *in situ* in lymphoid organs and mesenteric lymph nodes. In addition, the efficiency of translocation differs between the bacterial species of the family *Enterobacteriaceae*.

Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli and *Proteus mirabilis* translocates with the greatest efficiency across the intestinal barrier to the mesenteric lymph nodes (Berg, 1995). When evaluating the animal models of translocation in Berg's review, the translocating bacteria are detected in the mesenteric lymph nodes before it is discovered in other locations or organs such as the liver, spleen, blood or peritoneal cavity (Berg & Hentges, 1983). Therefore, resident macrophages are tactically positioned to minimize bacterial translocation from the gastrointestinal tract. In addition, all components of the intestinal tract including mucosal immunity (secretory immunoglobulins), cell-mediated immunity (macrophages and T cells) and humoral immunity (serum immunoglobulins) aim towards reducing bacterial translocation. Therefore, in the healthy adult animal these translocating bacteria can hardly be cultured from the mesenteric lymph nodes or other extra-intestinal sites (Berg, 1995).

Gabe (2001) concluded that bacterial translocation is likely to occur to a minor extent in normal animals, and in the normal physiological state, but the intestinal immune system prevents the small numbers of bacteria that do manage to translocate across the intestinal mucosal barrier from entering the circulation. However, if, for some unrelated or external reason, the bacterial load becomes too great and/or the intact immune system's defenses are overwhelmed, bacteria may end up in the circulation.

In terms of the effect delayed evisceration has on the microbial safety of meat, Gill *et al.* (1976) stated that evidence from their work showed that samples of muscle and lymph nodes from uneviscerated lamb carcasses hung for 24 hrs at 20˚C, and which were incubated overnight remained free of bacteria. The assumption that delayed evisceration will lead to pollution of the meat by bacteria from the normal intestinal flora was refuted by these researchers, provided that the harvesting conditions were controlled and animals were killed outright, indicating that no animals were wounded with damage to their internal organs (Gill, 2007).

Proteolytic activity seems to be the major determining factor that could have an impact on the effect that delayed evisceration has on meat quality (Gill *et al*., 1978). According to Gill *et al*. (1978) microorganisms are not usually present in the muscle or lymph nodes of carcasses from healthy animals, nor does microbial migration occur from the intestine if evisceration is delayed for up to 24 hrs after death. Gill *et al.* (1978) concluded that unless tissue breakdown as a result of proteolytic activity results in rupture of the stomach or intestinal wall, there is no indication of bacterial translocation across the intestinal wall. Therefore, they concluded following their initial work on sheep carcasses that there is no reason to reject sheep carcasses for human consumption that has remained unevisceratedfor up to 24 hrs, based on the possibility that intestinal flora have invaded tissues post mortem.

In practice, as well as under experimental conditions, delayed evisceration does not lead to invasion of muscle or tissues by intestinal microflora, and as a quality check, visual inspection of such a carcass will be an adequate guide for judgment relating to the acceptability for human consumption (Gill & Penney, 1982). Unless rupture of the intestine is suspected, or holding conditions were such that general growth of surface microflora might have occurred, visual inspection that already is part of the meat inspection process should aid in the process of approving carcasses subjected to delays before evisceration.

It is important to recognize a few variables in the work done by Gill and Penney (1982) relating to this hypothesis. Firstly, there were species differences between ovine carcasses used by Gill and Penney (1982) and wild ungulates in Africa. Secondly, ambient temperature that is generally higher in an African context will be vastly different to the ambient temperature in the northern hemisphere reported by Gill and Penney (1982). Ambient temperature directly correlates with post mortem bloating due to the rapid multiplication of residual live intestinal bacteria at warmer temperatures (Gill & Penny, 1979) and subsequent intestinal rupture leading to contamination of carcass tissues. Thirdly, the abdominal cavities of the exsanguinated carcasses were opened by them to minimize the effects of bloating.

Therefore, the above-mentioned variables are important to consider in the interpretation of the results of similar studies.

Chapter 3: Experimental Design and Methods

3.1. Study location

The nature and aim of this research project determined that it is linked to a harvesting team willing to provide an opportunity to obtain biopsy samples under winter harvesting conditions. An opportunity presented itself to accompany the harvesting team of Mr Hans Beukes when a harvest was planned to take place. Sampling of animals were done during a commercial game harvesting process, which occurred on the SA Lombaard Nature Reserve near Bloemhof in the Northwest province of South Africa (Figure 1). The population from which the animals were harvested for game meat existed under natural conditions.

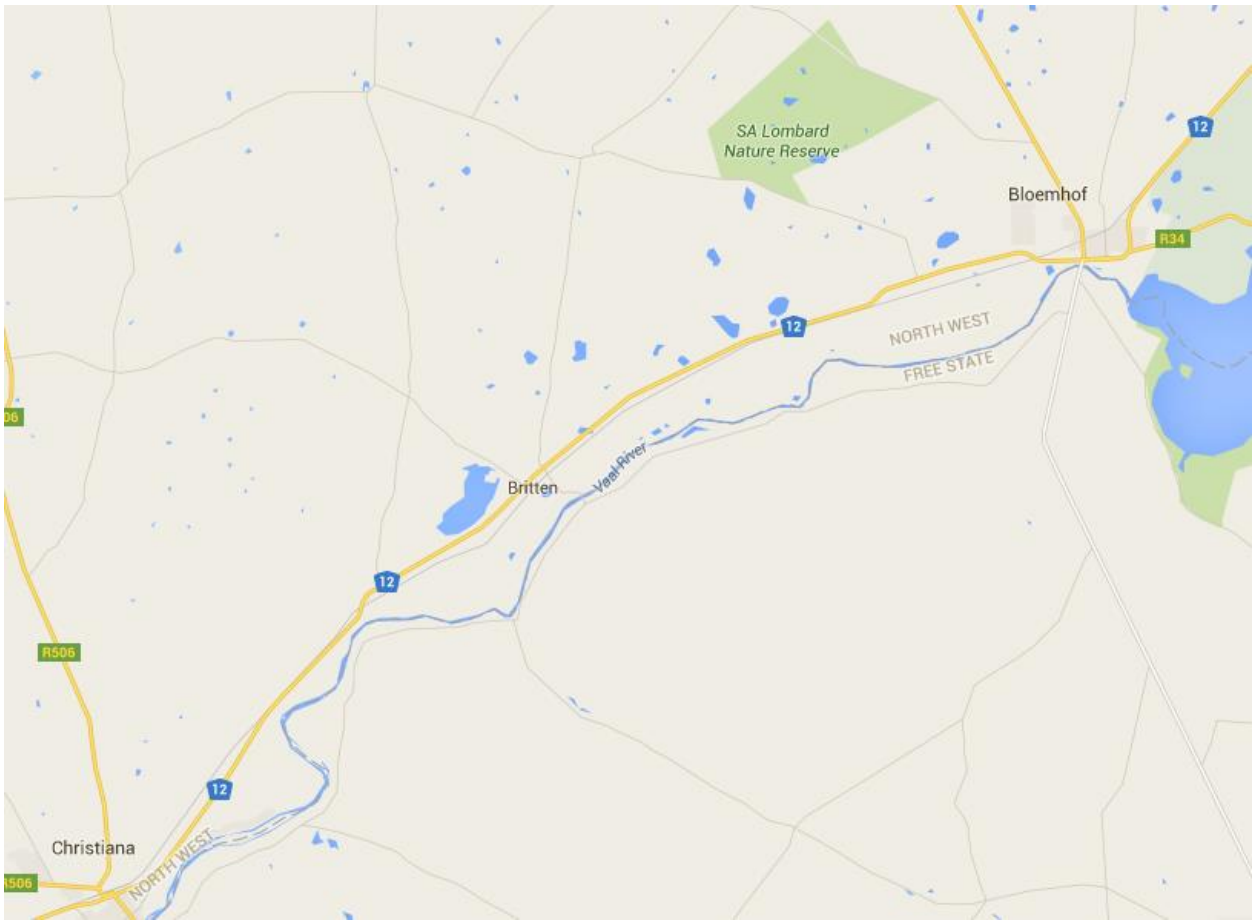

Figure 1 Map illustrating location of the SA Lombaard Nature Reserve between Bloemhof and Christiana in the Northwest Province, South Africa

(https://www.google.co.za/maps/@-27.7458634,25.2993966,11z)

3.2. Target animals

Sixteen black wildebeest were sampled and divided into five groups, namely groups A, B, C, D and E, in order to obtain the best representative samples under the circumstances based on a time delay in evisceration. Group A consisted of one animal (A1) and was eviscerated roughly 1 hr and 30 min after exsanguination. Group B consisted of three animals (B1, B2, B3) and were eviscerated between 1 hr and 45 min up to 2 hrs after exsanguination. Group C consisted of three animals (C1, C2, C3) and were eviscerated 2 hrs and 45 min after exsanguination. Group D consisted of five animals (D1, D2, D3, D4, D5) and were eviscerated 3 hrs and 30 min after exsanguination. Group E that consisted of four animals were eviscerated 3 hrs (E5) and 5 hrs (E1, E3, E4) after exsanguination (Table 1).

Table 1 Delays in evisceration and animal identification

Delay in evisceration (hours)	Animal Number															
	A1	B1	B2	B3	C1	C2	C3	D1	D2	D3	D4	D5	E1	E3	E4	E5
1:30	•															
1:45-2:00		•	•	•												
2:45					•	•	•									
3:00																•
3:30								•	•	•	•	•				
5:00													•	•	•	

3.3. Sample collection

Each biopsy was cut with a new sterile Kruuse® 8 mm punch biopsy. All instruments used to remove the biopsy from the abdominal peritoneum and place it into a 2 ml Biofreeze vial were first sterilised in 70 % alcohol. Thereafter the instruments were flame-dried with a gas flame prior to taking the next biopsy. This was done in order to remove excess alcohol and water before removing the biopsy from the next carcass.

A biopsy sample was obtained through the following steps:

- At the time intended for delay in evisceration, the distended abdominal cavity was opened with the use of an “Auzzie Knife” (Figure 2) which prevented the accidental laceration of distended and pressurised intestines due to post mortem bloating.
- Once the abdominal organs were removed from the abdominal cavity while the carcass was in a hanging position, the right side of the abdominal wall was pulled open so that the abdominal peritoneum in the area of the right inguinal canal and rectus sheath was exposed.
- For each biopsy, a new sterile Kruuse® 8 mm biopsy punch was used to incise an 8 mm circular hole in the abdominal peritoneum, through the aponeurosis of the transverse abdominal muscle and into the rectus abdominal muscle layer (Figure 3).
- As previously described, a sterile scissor and forceps were used to remove the biopsy sample and place it in a sterile 2 ml Biofreeze vial for aerobic and anaerobic culture.
- The scissor and forceps used to remove the biopsy sample were sterilised in a 70 % alcohol mixture, after which it was flame-dried in a gas flame before removing the next biopsy sample.

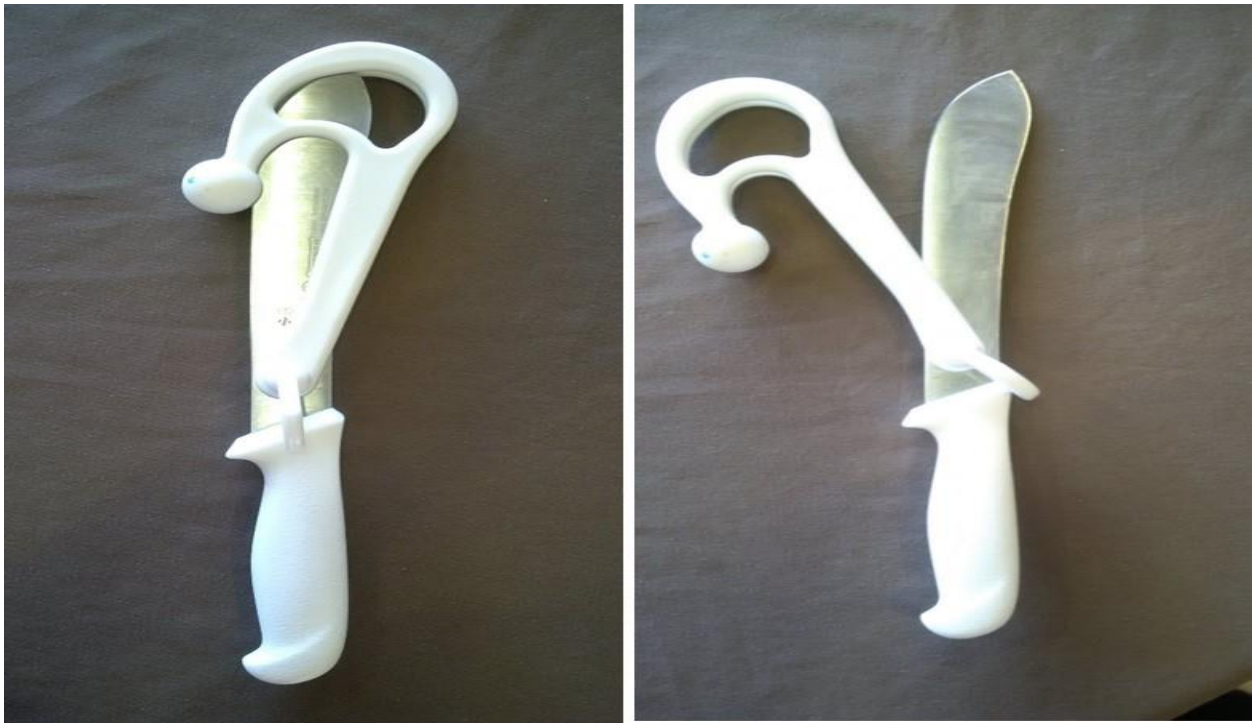

Figure 2 Auzzie Knife

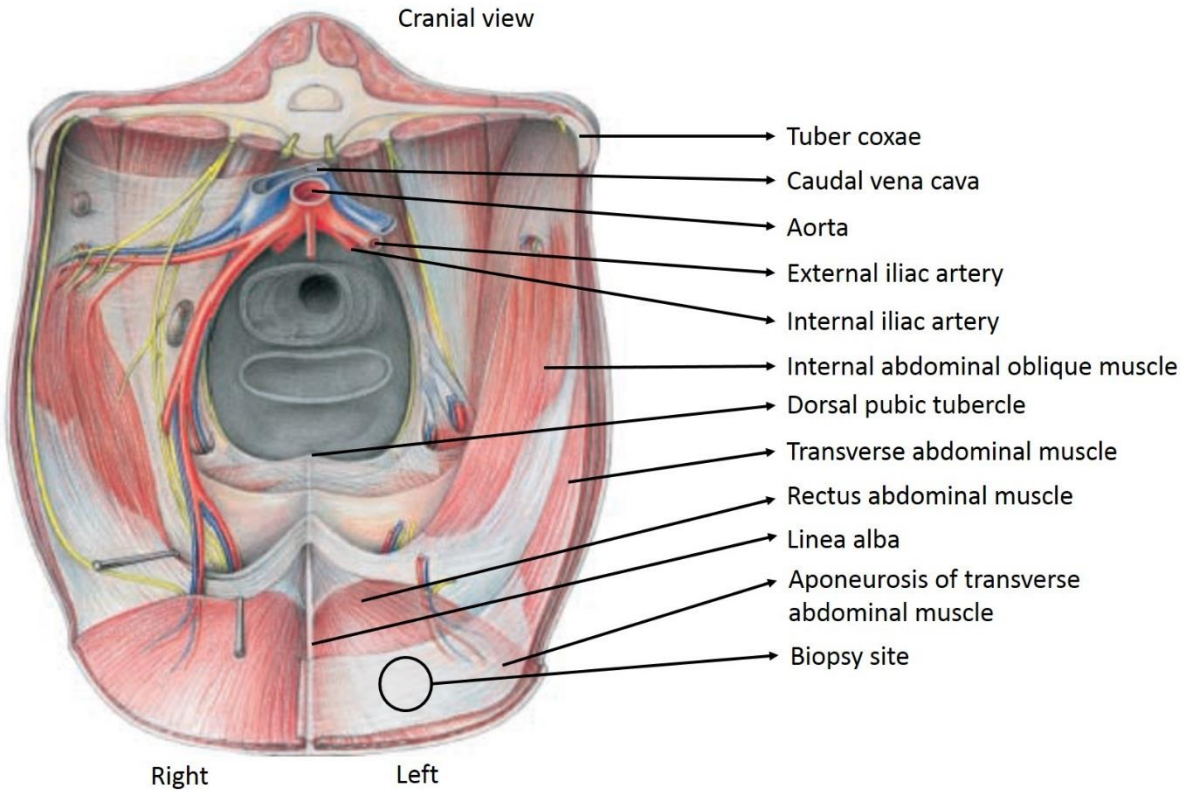

Figure 3 Cranial view which illustrates how the bovine (BUDRAS *et al*., 2003) aponeurosis of the transverse abdominal muscle layer terminates on the linea alba. The underlying rectus abdominal muscle layer together with the aponeurosis are the tissues that constituted the biopsy sample in each animal (Budras & Habel, 2003).

3.4. Sample preservation and transport to laboratory

After securing the contents of the Biofreeze vial, it was placed in a liquid nitrogen flask for preservation and transportation to the bacteriology laboratory.

3.5. Bacterial isolation and identification

Aerobic and anaerobic bacterial culture of the liquid nitrogen preserved biopsies was done in the Bacteriology Laboratory, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, based on the microscopy, culture and identification of bacterial pathogens (Chapter 2), bacteriology of the streptococci and related cocci (Chapter 8) and the bacteriology of the *Enterobacteriaceae* (Chapter 17) (Markey *et al*., 2013).

The Department of Agriculture, Forestry and Fisheries (DAFF) approved the Bacteriology Laboratory and allocated approval number DAFF-07. Subsquenly the laboratory obtained accreditation from the South African National Accreditation System (SANAS) with Facility Accreditation Number V0027. The isolation and identification of the indicator bacteria were performed according to validated and SANAS accredited methods.

American Type Culture Collection (ATCC, Rockville, Maryland, USA) strains (Coudron *et al*., 2000) were used as positive controls for the identification of translocating bacteria. The laboratory reagents, Streptococcal Grouping Kit and API®10S standard identification system for the *Enterobacteriaceae* were tested against the following ATCC reference strains: *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 70063, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 35659 and *Enterococcus faecalis* ATCC 29212.

3.5.1 Aerobic Culture

For aerobic cultivaltion the biopsy samples were vortexed in sterile water to remove superficial surface bacteria after which it was inoculated on a blood tryptose agar plate supplemented with 5 % horse blood (Catalogue number 510110, Selecta Media™, +27 (0)11 792 7383, www.selecta-media.co.za) and a MacConkey agar plate without crystal violet (Catalogue number 510110, Selecta Media™, +27 (0)11 792 7383, www.selecta-media.co.za) and incubated for three days at 37°C. The bacterial identification was performed using a latex agglutination Oxoid™ Streptococcal Grouping Kit (Catalogue number DR0585A, Oxoid™ Ltd, www.oxoid.com/ifu) and a biochemical API®10S standard identification system for the *Enterobacteriaceae* and other non-fastidious Gram-negative rods (Catalogue number REF10100, bioMérieux® SA, www.biomerieux.com).

3.5.2 Anaerobic Culture

For anaerobic cultivation the vortexed biopsy samples were inoculated on blood agar plates and incubated for three days at 37°C in a Shellab Bactron II Anaerobic Environmental Chamber (Sheldon Manufacturing, Inc. 300N 26TH, Cornelius OR, 97113, USA).

Chaper 4: Results

4.1. Aerobic isolation and identification

Table 2 summarises the results of the aerobic isolation and identification as well as comments about each biopsy. Comments relate to bacterial growth, specific bacteria identified as well as the number of bacterial colony forming units (cfu) per cm².

Table 2 Results of aerobic isolation and identification

Specimen Number	Result of aerobic isolation and identification	Bacterial colony count (cfu per cm²)
A1	No growth obtained after 72 hrs of incubation	0
B1	No growth obtained after 72 hrs of incubation	0
B2	Non-significant growth was obtained	50
B3	Non-significant growth was obtained (fungi seen)	0
C1	Non-significant growth was obtained (fungi seen)	0
C2	Rough *Escherichia coli* was isolated	10
	Non-significant growth was obtained	80
C3	No growth obtained after 72 hrs of incubation	0
D1	No growth obtained after 72 hrs of incubation	0
D2	No growth obtained after 72 hrs of incubation	0
D3	Non-significant growth was obtained	30
D4	Non-significant growth was obtained	70
D5	*Enterococcus durans* was isolated	10
E1	Non-significant growth was obtained	10
E2	No growth obtained after 72 hrs of incubation	0
E3	Non-significant growth was obtained	50
E5	Non-significant growth was obtained	10

4.2. Anaerobic cultivation

Table 3 summarises the results of the anaerobic isolation and identification as well as comments about each biopsy. Comments relate to bacterial growth, specific bacteria identified as well as the number of bacterial colony forming units.

Table 3 Results of anaerobic isolation and identification

Specimen Number	Result of anaerobic isolation and identification	Bacterial colony count (cfu per cm²)
A1	No growth obtained after 72 hrs of incubation	0
B1	No growth obtained after 72 hrs of incubation	0
B2	No growth obtained after 72 hrs of incubation	0
B3	No growth obtained after 72 hrs of incubation	0
C1	No growth obtained after 72 hrs of incubation	0
C2	No growth obtained after 72 hrs of incubation	0
C3	No growth obtained after 72 hrs of incubation	0
D1	No growth obtained after 72 hrs of incubation	0
D2	No growth obtained after 72 hrs of incubation	0
D3	No growth obtained after 72 hrs of incubation	0
D4	No growth obtained after 72 hrs of incubation	0
D5	No growth obtained after 72 hrs of incubation	0
E1	No growth obtained after 72 hrs of incubation	0
E2	No growth obtained after 72 hrs of incubation	0
E3	No growth obtained after 72 hrs of incubation	0
E5	No growth obtained after 72 hrs of incubation	0

4.3. Recording of the environmental temperatures

The environmental temperature was taken from the time when killed and exsanguinated animals arrived at the temporary slaughter facility, through to when the last shot and exsanguinated animal was exposed to a delay in evisceration. Most of the animals were eviscerated after 20:30 on the specific evening (Table 4). Figure 4 illustrates a temporal decrease in environmental temperature during delayed evisceration based on minutes post arrival of the first killed and exsanguinated animal (Table 4).

Table 4 Environmental temperature over time when delayed evisceration was performed

Time	Environmental Temperature (˚Celcius)	Time in minutes after arrival of the first killed and exsanguinated animal
18:20:00	18.4	0
18:45:00	15.1	25
19:25:00	14.2	65
20:45:00	12.3	145
22:00:00	10.0	220

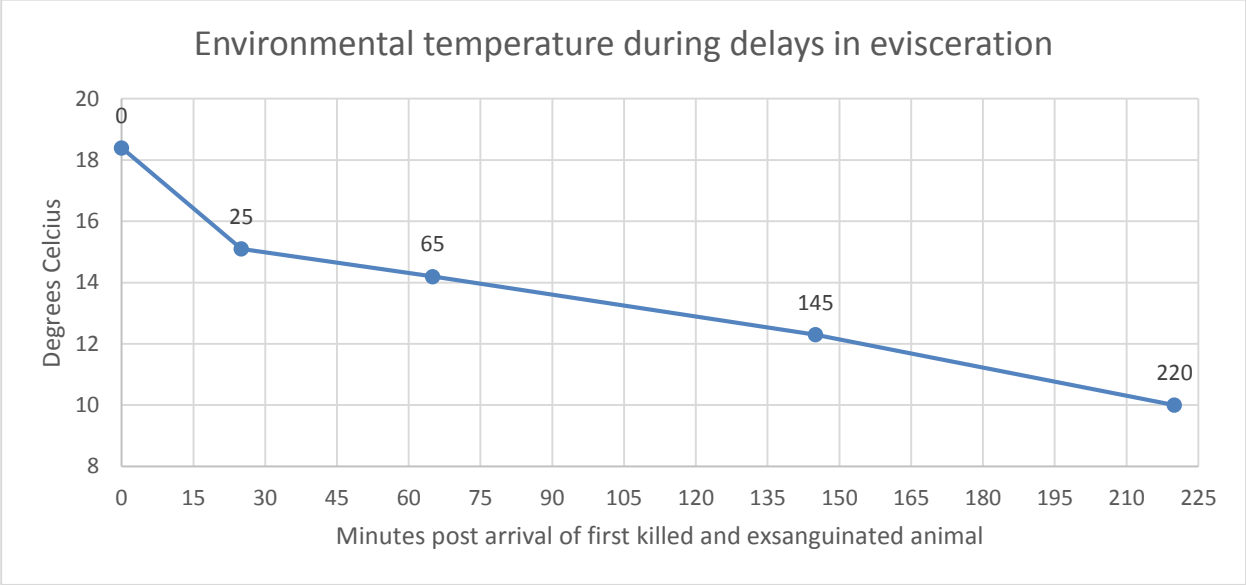

Figure 4 Graph illustrating environmental temperature during delays in evisceration

Chapter 5: Discussion

The aim of this research project was to determine whether there is significant bacterial translocation across the intestinal barrier due to the effects of delayed evisceration and post mortem bloating in harvested black wildebeest (*Connochaetes gnou*). In other words, was there a corresponding increase in bacterial numbers translocating across the intestinal barrier into the abdominal cavity as the delay in evisceration for each group of animals sampled increased temporally?

Originally it was planned that there will be five groups of animals consisting of five animals per group, totalling 25 animals being sampled. Due to the natural and extensive terrain conditions under which these animals roamed freely, the logistical arrangments and lighting conditions did not allow sampling of 25 animals. The small sample size (16) was limited by the availability of carcasses during one night of harvesting. The duration of the entire commercial harvesting was one night. Sampling occurred at different times based on the amount of time that elapsed after an animal was shot in the field and exsanguinated while being loaded onto the vehicle transporting the shooter.

It is acknowledged that one animal in a group (A1) does not constitute a meaningfull study group, however logistical contraints at the start of the trail made it impossible to include additional animals. Nevertheless, it was decided to include this one animal as possible carcass contamination was anticipated in subsequent groups.

The sampling methodology, which involved an 8mm punch biopsy into the abdominal peritoneum through the aponeurosis of the transverse abdominal muscle into the rectus abdominal muscle layer, was not based on a referenced method but on personal communication with experts working in the field. Therefore, the possibility of false negative results due to the size of the biopsy sample, as well as the lack of statistical representativity due to the sample size of this research project are putative.

Harvesting of game in South Africa normally take splace in mid-winter. The implication is that the results of this study are only valid under South African winter harvesting conditions at environmental temperatures below 18˚C. No conlusions are binding above 18˚C.

The non-significant growth (based on colony forming units per square centimetre) for samples B2, B3, C1, C2, D3, D4, E1, E3 and E5 can be ascribed to environmental contamination during sample collection. The fungal organisms visualised on samples B3 and C1 can also be ascribed to environmental contamination during sample collection. The rough *Escherichia coli* isolated from sample C2 quantified at 10 cfu/cm² per sample was likely a commensal enteric bacterium indicating bacterial translocation across the intestinal barrier. The *Enterococcus durans* isolated from sample D5 quantified at 10 cfu/cm² per sample is a commensal enterococcal bacterium (Franz *et al*., 2003), its presence being indicative of bacterial translocation across the intestinal barrier.

Species of the family *Enterobacteriaceae* and genus *Enterococcus* which translocates with greatest efficiency across the intestinal barrier include *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and members of the *Enterococcus* genus, e.g. *Enterococcus faecium* and *Enterococcus fecalis* (Berg, 1995). However, none of these strains were isolated from the samples obtained from animals exposed to delays in evisceration, except samples C2 and D5. The bacterial isolation results did not reveal any significant quantifiable increases as the periods of delayed evisceration increased respectively up to 5 hrs. The bacterial species targeted for isolation are indicator bacteria for movement across the intestinal barrier (Berg, 1995). Their quantifiable absence from the samples that were subjected to bacterial isolation in this study indicates that bacterial translocation across the intestinal barrier was negligible in black wildebeest (*Connochaetes gnou*) exposed to delays in evisceration.

It must be borne in mind that the samples from the animals exposed to delays in evisceration were not serially diluted with sterile water before cultivation to obtain a quantitative result (colony-forming units). The fact that the samples were not diluted emphasizes that there was very little, if any, bacterial translocation across the intestinal barrier into the abdominal cavity in the animals exposed to delays in evisceration. The assumption that post mortem bloating and delay in evisceration will lead to bacterial contamination of carcass meat and tissues as time after death increases, has therefore

not been confirmed in black wildebeest (*Connochaetes gnou*) during this research project.

The results of this study correlates with the findings of Saegeman *et al*. (2009) who performed blood cultures on 100 human tissue donors both within and after 24 hrs of death. They concluded that inherent characteristics (cause of death being cerebral, cardiac or other) in the individual cadavers dictated the extent of post mortem bacterial migration rather than the interval between death and culture. This can be better explained through the effects of a gastro-intestinal ulcer which predisposes and will rapidly augment bacterial translocation to other tissues ante mortem, compared to an intact intestinal barrier where the cause of death was cerebral or cardiac. The findings of Saegeman *et al*. (2009) are not supportive of the theory explaining bacterial translocation in healthy immunocompetent individuals. It is however, important to remember that their results are similar to those of this project, bearing in mind that each study has its own context and variables indicating what an intact intestinal barrier can achieve.

An intact intestinal barrier (not primed or affected by inflammatory mediators nor being ischeamic) limits the numbers of bacteria that do manage to translocate across the intestinal barrier to an absolute minimum. Simultaneously the intact mesenteric lymph nodes containing inflammatory mediators and phagocytic immune mediator cells are ready to eliminate the remaining enteric bacteria that were able to translocate across the intestinal barrier. Saegeman's study contained healthy (cause of death being cerebral) and compromised individuals whereas this project included only healthy animals (cause of death being "cerebral" on the basis of being shot). The results obtained from this study do not support the theory of bacterial translocation in healthy immunocompetent black wildebeest (*Connochaetes gnou*).

The animals exposed to the longest delay in evisceration in this study (E1, E3, E4) were not found to be contaminated and were not discarded or condemned based on the grounds of bacterial contamination when they reached the processing facility four days later (H. Beukes, unpublished results).

Chapter 6: Conclusion

The results obtained from this study during normal winter harvesting conditions in South Africa indicates that there does not seem to be significant trans-enteric bacterial translocation to extra-intestinal tissues in the first few hours after death in black wildebeest. Under normal winter harvesting conditions in the field, logistical or other challenges occuring during retrieval of a shot animal might lead to bloating and a temporal delay in evisceration. Such an animal can be safely dressed with the correct equipment to prevent accidental rupture of bloated intestines at the temporary slaughter facility. Despite bloating and a temporal delay in evisceration, meat from such a carcass will still be safe for human consumption. Evidence indicating that black wildebeest (*Connochaetes gnou*) meat exposed to bloating and a delay in evisceration is not safe for human consumption due to bacterial translocation and contamination is therefore lacking.

Further research to obtain immediate post mortem blood culture results during harvesting may be valuable. After the harvested animals have been running for hours during harvesting which causes stress, the animals shot first will possibly have different blood culture results compared to animals shot last which have been stressed for longer. The animals shot last will possibly have an increased bacterial blood culture load primarily due to the effect of prolonged gut ischeamia, leading to intestinal mucosal injury/death which culminates in bacteria translocating to the liver/spleen via the portal circulation or the systemic circulation via thoracic duct. If there are aggravated circumstances where a specific animal was incorrectly shot and running around injured and dehydrated/ hypovolaemic, the immune system's ability to eliminate translocating bacteria in the thoracic duct/central blood will be less effective due to stress and the effects of adrenaline. This situation will lead to the final stages of translocation as mentioned in Berg's review where bacteria will be able to spread to extra-intestinal sites such as the abdominal cavity, which was monitored during this study.

Post mortem blood culture results could possibly help in distinguishing animals with a normally perfused gastrointestinal barrier that are able to keep bacterial translocation at or below normal levels from the animals with an ischeamic gastrointestinal barrier which are unable to keep translocating bacterial numbers to an absolute minimum.

It may also confirm low grade bacteraemia in central blood and indicate at what point the immune system's defences are overwhelmed. At that stage the mesenteric lymph node complex and the associated immune system will not be able to eliminate the bacteria that do manage to translocate (in low numbers in a physiologically normal animal). This might occur due to their increasing numbers which exceed the capacity of the intestinal immune system, terminating in the final stage of intestinal bacterial translocation to other extraintestinal sites such as the abdominal cavity or muscle tissue leading to bacterial contamination of game meat.

In addition, what combination of predisposing factors (environmental temperature, amount of time elapsed since start of harvesting, dehydration and splanchnic hypoperfusion) will contribute to overwhelming the immune system's innate ability to prevent distant bacterial contamination of game meat.

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Animal Ethics Committee approval certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
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Animal Ethics Committee

PROJECT TITLE	**Effect of delayed evisceration and its effect on the microbiological safety of game meat**
PROJECT NUMBER	**V080-13**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. CV van Heerden**

STUDENT NUMBER (where applicable)	**243 315 80**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Black wildebeest *(Connochaetus gnou)***	**Blue wildebeest *(Connochaetes taurinus)***
NUMBER OF ANIMALS	**25**	**25**
Approval period to use animals for research/testing purposes		**January 2014-August 2014**
SUPERVISOR	**Prof. M van Vuuren**	

KINDLY NOTE:

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 25 November 2013
CHAIRMAN: UP Animal Ethics Committee	Signature