

Two sampling techniques for game meat

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A study was conducted to compare the excision sampling technique used by the export market and the sampling technique preferred by European countries, namely the biotrace cattle and swine test. The measuring unit for the excision sampling was grams (g) and square centimetres (cm²) for the swabbing technique. The two techniques were compared after a pilot test was conducted on spiked approved beef carcasses ($n = 12$) that statistically proved the two measuring units correlated. The two sampling techniques were conducted on the same game carcasses ($n = 13$) and analyses performed for aerobic plate count (APC), *Escherichia coli* and *Staphylococcus aureus*, for both techniques. A more representative result was obtained by swabbing and no damage was caused to the carcass. Conversely, the excision technique yielded fewer organisms and caused minor damage to the carcass. The recovery ratio from the sampling technique improved 5.4 times for APC, 108.0 times for *E. coli* and 3.4 times for *S. aureus* over the results obtained from the excision technique. It was concluded that the sampling methods of excision and swabbing can be used to obtain bacterial profiles from both export and local carcasses and could be used to indicate whether game carcasses intended for the local market are possibly on par with game carcasses intended for the export market and therefore safe for human consumption.

Introduction

The *Meat Safety Act* (Act No. 40 of 2000) (Republic of South Africa 2000) stipulates that:

No person may export any meat from the Republic unless the essential National Standards in respect of the slaughtering of animals and the handling of meat, and such additional requirements as may be determined by the National Executive Officer have been complied with. (Republic of South Africa 2000)

The veterinary public notices (VPNs) are these essential standards in respect of safe game meat exportation from South Africa (Republic of South Africa 2010a). The VPN/15/-2010-01 is the standard for the microbiological monitoring of meat with which all exported meat must comply. This VPN makes provision for monitoring the microbiological status of meat that is used as an indicator of the adequacy of process interventions and process hygiene. The VPN further states that these monitoring programmes are only as valid as the competency and reliability of the laboratory performing the analyses (Republic of South Africa 2010b).

A laboratory approval programme was designed to provide a credible, independent system to verify that laboratories can competently conduct the required tests to verify hygiene during production (Capita, Prieto & Alonso-Calleja 2004). Laboratories performing microbiological analyses for establishments approved to export fresh meat from the Republic of South Africa must take part in the laboratory approval programme. This programme is managed by the ARC-Onderstepoort Veterinary Institute on behalf of the Directorate Veterinary Services. The management of the establishment approved to export must coordinate with the management of the laboratory performing their microbiological testing and arrange for regular inspections as well as training in relation to this standard. The strategy encompasses all aspects of a microbiological monitoring programme, including the development of standardised sampling plans, sampling and transportation procedures and analytical methods, and the verification of laboratory proficiency. The present study has focused on the comparison of two approved sampling techniques, one of which is used for carcasses intended for the export market (measuring unit grams [g]) and a second technique (measuring unit square centimetres [cm²]) to be used for the evaluation of game carcasses intended for the local market.

Materials and methods

Pilot project on spiked samples

A pilot study was conducted to:

- Verify whether the different measuring units in the two techniques could be compared in terms of the bacterial counts.

- Establish a comparable unit standard.
- Ensure that false positive results from the comparative study would not be obtained.

Pilot study methodology

According to the method of Andrin (M. Andrin 01 February 2008, lecture on spiking methods), brisket tissue samples were collected from 12 beef carcasses from a certified butchery and spiked with a purified *Klebsiella – Escherichia coli* strain. The suspension was homogenised in peptone water and spiked onto the beef tissue with a throat swab (long handled swab) using a template of $2.5\text{ cm}^2 \times 10.0\text{ cm}^2 = 25.0\text{ cm}^2$. A swab was taken from the tissue sample of each of the carcasses using the template as described. From the same tissue and cutting inside the same template, an excision sample was then taken. A measured 25 g of tissue from each of the carcasses was diluted 1:10 with 0.1% sterile peptone water (CM0009) and homogenised for 3 min at 3500 rpm. Samples were plated on Brilliance Chromogenic *E. coli* selective agar and incubated for 72 h at 30 °C (Bridson 2006). All samples were then analysed for the aerobic plate count (APC). The pilot study was conducted in the controlled sterile environment of a laboratory in Polokwane, Limpopo Province, that was approved by the South African National Accreditation System (SANAS).

Comparative test on the two sampling techniques

A comparative study was conducted to verify the results of the excision versus the swabbing technique for the sampling of game carcasses. A total of 13 category B carcasses were sampled using both methods and the samples were submitted to the same laboratories for analysis. The two methods are explained in more detail in the following sections.

The excision sampling technique

Samples from carcasses harvested for the export market were collected within 30 min after secondary inspection in the abattoir. These carcasses were dressed in the abattoir after transportation for between 12 h and 72 h, depending on where the registered ranch for the harvesting was located.

For the APC and counts of the indicator organisms *Staphylococcus aureus* and *E. coli*, samples of 5 g each were excised from primal cuts (outside the hind leg) of five ($n = 5$) randomly selected carcasses. These five samples were combined into one composite sample and measure, to a total sample weight of 25 g. For the determination of *Salmonella*, samples of 5 g each were excised from the five ($n = 5$) carcasses and combined to form a composite sample with a total weight of 25 g. Subsequently, the inoculated pre-enrichment broths were tested for the presence of *Salmonella* spp. according to the standard method of the International Organisation for Standardisation (ISO 6579:1993) (Vanderzant & Splittstoesser 1992).

According to the relevant VPN (Republic of South Africa 2010b), microbiological meat tests must be conducted

annually and the results plotted on graphs each time. These results must be compared with the results of microbiological tests of the water supply and the equipment in the abattoir. An overall picture of the microbiological status of the establishment and its products must always be available.

To comply with the VPNs, the export abattoir must use good quality insulated cooler containers (polystyrene or similar) and samples must reach the laboratory prior to possible rises in temperature above 2 °C. Stomacher sample bags (80 mL or 400 mL) or other sterile plastic bags must be used. Furthermore, a jar with a wide mouth containing 70% ethanol, in which the excision 'heads' (or plug borer tips) are immersed, should be available during sampling. The excision apparatus (cork borer) should have an inner diameter of 25 mm and a surface area of 5 cm² to qualify as a standardised sampling tool. After the plastic bags with the samples are properly marked and folded several times to seal them properly, they are further secured in a tightly folded position using elastic bands. A scalpel with disposable blades is used to separate disks of meat excised by the cork borer and the blade should be sterilised between different sets of samples by immersion in 70% ethanol (Pepperell *et al.* 2005).

The following sample data were recorded: the exact time (hour and minute) of sampling, the date of sampling, the farm of origin, the temperature at the time of sampling and the nature of the product sampled. Environmental conditions (i.e. any condition that could have had an impact on the result of the sample) were also recorded.

The collection of the samples was undertaken with all the necessary aseptic precautions and the temperature of the samples on arrival and the time of arrival were recorded. According to the VPN, chilling the samples to temperatures lower than -15 °C could cause them harm and is estimated to kill off between 10% and 50% of the aerobic bacteria (Republic of South Africa 2010b). It is important to note that such low temperatures will cause less harm to spore-forming organisms (Pepperell *et al.* 2005). Therefore, the samples were transported to the laboratory at temperatures < 7 °C, but not below 0 °C.

The sample mass was noted in order to report the microbial count as the number of colony forming units (CFU) per gram. Most of the bacteria on or in the product are actively bonded to the tissues and therefore maceration in a stomacher (a total destruction technique) was used to separate the bacterial cells from the meat tissue. Serial decimal dilutions were made up to a 10⁻⁴ dilution in buffered peptone water (BPW) and were incubated at 37 °C for 16–20 h.

The laboratory in Claremont, Cape Town that does the bacteriological testing of all the samples of the export carcasses is accredited with the SANAS number T0050 and the facility complies with the general requirements of ISO/JEC 17025:2005 (Vanderzant & Splittstoesser 1992). It also participates in the abovementioned laboratory approval programme. The certificate of accreditation from SANAS

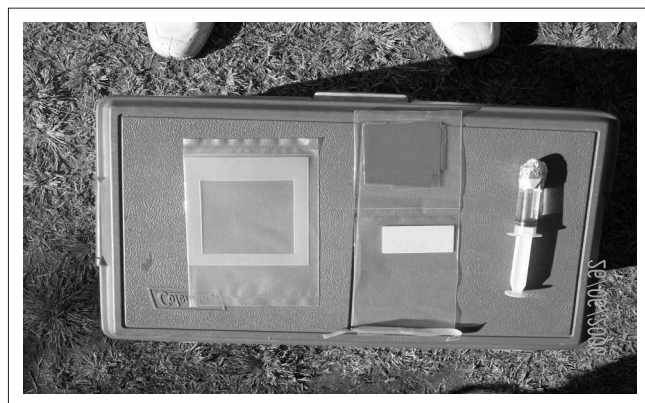
expires annually in September and is re-issued with proof of compliance. For the purpose of this study, the exact protocol used for export carcasses was followed, but with the game carcasses for the local market. These samples were then transported to the SANAS-accredited laboratory in Polokwane (Caprivet Veterinary Laboratories, 82 Hans Van Rensburg Street, Rondebosch Suite 4, Polokwane, South Africa) for testing.

The cattle and swine bio-trace swabbing technique

The surface sampling of all the carcasses intended for the local market (inclusive of trophy and biltong carcasses) was conducted in accordance with the method as prescribed by the United States Food and Drug Administration using the biotrace cattle and swine sampling equipment (Food and Agriculture Organization of the United Nations and World Health Organization 1997). Sampling was conducted on a surface area of 200 cm² on the external surface of the carcass prior to cooling. Figure 1 shows the sterilised pouch with the sponge, the template to standardise the sampling surface on each of the four quadrants of the carcass, the BPW diluents – 0.1% according to ISO 6887 (Vanderzant & Splittstoesser 1992) – and the sterilised gloves for aseptic sampling.

The surface sampling technique is generally preferred to the excision method because the sampling surface is statistically more attainable and target organisms are more effectively retrieved (Brodsky 1995; Brown *et al.* 2000). The present study focused on the aerobic plate count and counts of *E. coli*, *S. aureus* and *Salmonella* spp. as index and indicator organisms and the analytical methods are briefly discussed in the following paragraphs.

Firstly, the APC method was conducted according to the International Standard ISO 4833:1991. This technique is meant to be applied to blood and carcass swab samples. Blood samples were directly plated (undiluted) and surface swabs were diluted to provide a liquid matrix. Samples were plated on a non-selective medium (plate count agar), incubated in an aerobic atmosphere at 30 °C for 72 h to select for the mesophilic target group. Calculation of colonies was



Source: Van der Merwe, M., 2012, 'Investigating the concept of a game meat scheme to promote safe game meat on the South African market', D-Tech thesis, Tshwane University of Technology, South Africa

FIGURE 1: The sampling equipment for the biotrace cattle and swine test.

conducted on dishes containing between 15 and 300 colonies. The number N of micro-organisms per millilitre, per gram, or per square-centimetre, was calculated using the following equation (M. Andrin 01 February 2008, lecture on spiking methods) (Vanderzant & Splittstoesser 1992):

$$N = \Sigma C \div [n1(d)] \quad [\text{Eqn 1}]$$

Where,

- ΣC is the sum of colonies counted on all the dishes retained.
- $n1$ is the number of dishes retained in the countable dilution.
- d is the dilution factor corresponding to the counted dilution.

Secondly, the *E. coli* method was conducted according to Oxoid (Bridson 2006). The principle of this method is based on the direct counting of viable organisms within the coliform group, where differentiation between general coliforms and *E. coli* is based on the enzymes glucuronidase and galactosidase produced by the latter organism. A chromogen was incorporated in the medium to make the differentiation between these groups possible (M. Andrin 01 February 2008, lecture on spiking methods). Incubation was at 37 °C for 24 h. The number (N) of micro-organisms per gram or per square-centimetre was calculated using the same equation as for APC (M. Andrin 01 February 2008, lecture on spiking methods) (Vanderzant & Splittstoesser 1992).

Thirdly, the method for *S. aureus* was conducted according to SANS 6888:1999 and ISO amendment 1 (Vanderzant & Splittstoesser 1992). The principle of this method is based on the primary selection of *S. aureus* organisms on Baird Parker egg yolk tellurite agar and the demonstration of coagulase positive *S. aureus* strains. The latter test was performed according to the staphylase agglutination procedure (M. Andrin 01 February 2008, lecture on spiking methods). The staphylase test demonstrates the ability of *S. aureus* to produce coagulase or clumping factor. Incubation was at 35 °C for 24–48 h.

The number (N) of micro-organisms per millilitre or per gram of product, the result of the number of CFU's (colony forming units) was measured per millilitre or per gram or per cm² of the product sampled. Depending on the case, was calculated using the following equation:

$$N = \Sigma C \div [n1 \times v \times d] \quad [\text{Eqn 2}]$$

Where,

- ΣC is the sum of colonies counted on all the dishes retained, all giving positive staphylase reactions.
- $n1$ is the number of dishes retained in the countable dilution.
- d is the dilution factor corresponding to the counted dilution.
- v is the volume spread over each dish.

Fourthly, the method for the detection of *Salmonella* spp. was performed according to SANS 6579:2003. The principle of this method is based on the recovery and multiplication of *Salmonella* present in the sample, in BPW as a primary enrichment mechanism. Secondary enrichment occurs in Muller-Kauffmann tetrathionate–novobiocin (MKN) and Rappaport-Vassiliadis medium with soya (RVS) cultures, followed by primary selection on *Salmonella*–*Shigella* (SS agar) and xylose lysine deoxycholate (XLD agar) media. Incubation was at 37 °C for 24 h. Identification was conducted using various carbohydrates and biochemical media such as, *inter alia*, oxidase, catalase, Gram's stain, Simmons citrate (scit), lysine, ornithine, Voges-Proskauer, Aesculin hydrolysis, lactose and xylose (Bridson 2006).

Sampling method

The surface swabbing of the carcasses was performed in the slaughter facility or abattoir on the farm after the carcass was eviscerated and dressed. The enviro-biotrace cattle and swine test kit consists of sterilised templates, gloves, resealable sachets with a 1.5 cm² × 3.0 cm² biocide-free, dry sponge and glass bottles containing 25 mL buffered peptone solution. The sponges were hydrated by adding 10 mL of BPW to the pouch. For convenience, the sponge was moved to the top of the sample pouch by shaking the pouch with a downward motion. By squeezing the bag behind the sponge, the sponge was then pushed up until it just protruded through the opening in the pouch.

The sterile glove was then aseptically removed from its holder and put on with care. Using the sterile gloved hand, the sponge was removed from the pouch. The inside of the bag was not touched to prevent contamination. The sponge was aseptically soaked in the peptone solution and, using the template (10 cm × 20 cm = 200 cm²) area, each of the areas on the four quadrants of the carcass (the shoulders and the outside surface of the hind legs) was firmly swabbed. The repetitive and abrasive swabbing technique ensured that most if not all bacteria on the surfaces were removed onto the sponge (Pepperell *et al.* 2005).

The collection of the swab samples was undertaken using all the necessary aseptic precautions and samples were kept on ice until delivered to the laboratory. Testing of the samples was carried out within 12 h of the samples being taken to ensure that the pathogens would not die off before testing. In the laboratory report, the microbial count was indicated as the number of colony forming units per 1 cm². The swabbing of the carcass surface is demonstrated in Figure 2.

The sponges are durable, withstand scrubbing, are biocide-free to maintain organism viability, have a long shelf-life and are Gamma irradiated to guarantee sterility. After swabbing, the sponges were placed in the 510 g leak free plastic (Stomacher) pouch and the remaining 15 mL of the buffer was added. The top of the pouch was folded down and wire tabs were used to secure the pouch before placing it in the cooler. The sample was identified by using a waterproof marker pen

to write on the allocated space on the pouch. For the purpose of this report, the same 13 carcasses were sampled with this technique and the excision technique and the samples were transported to the same laboratory in Polokwane.

Statistical procedures

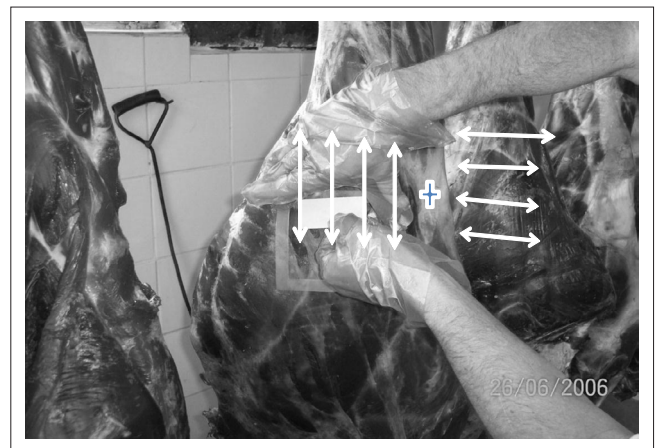
The statistical analyses were performed using SAS statistical software version 9.2 (2008). Shapiro–Wilk's test was performed on the standardised residuals to test for deviation from normality (Shapiro & Wilk 1965). Data were analysed using the paired *t*-test for both methods on the same carcasses (*n* = 13) for the comparative test between the biotrace swabbing method and excision. The data for the pilot spiking (unit correlation) study (*n* = 12) was analysed using the paired *t*-test for both units. Generally, a correlation coefficient (*r*) of about ± 0.7 or more is regarded as indicating fairly strong correlation and ± 0.9 indicates a very strong correlation. The correlation is considered moderate if *r* = ±0.5, whereas if *r* is in the range -0.3 to +0.3, the correlation is weak (Rayner 1969).

Ethical considerations

Dr W.A. Hoffman, chairperson of the Research Ethics Committee of the Tshwane University of Technology reviewed the study proposal at a meeting held on 18 May 2009 and approval for the proposal was granted. Reference number: 2009 05 002 VanDerMerweM.

Results

The results of the pilot spiking project conducted for APC with different measuring units showed a statistically significant (*p* < 0.01) correlation, *p* = 0.9, between gram and square-centimetre units (Figure 3). The different sampling techniques used for the export and local market that measure in grams and square-centimetres, respectively, could therefore be compared to obtain results for this study. It should be noted that the template used for the swabbing technique was 25 cm² and was compared with the 25 g



Source: Van der Merwe, M., 2012, 'Investigating the concept of a game meat scheme to promote safe game meat on the South African market', D-Tech thesis, Tshwane University of Technology, South Africa

FIGURE 2: The biotrace swabbing method.

(as specified by VPN 15) used for the excision technique (Republic of South Africa 2010b).

The APC counts were selected and used for the correlation test as these was intended to determine whether the organisms used in the study, as well as the recovery rate on spiked samples for indicator organisms, are effective and viable (M. Andrin 01 February 2008, lecture on spiking methods).

Salmonella, *S. aureus* and *E. coli* reside within the APC group and a good recovery on APC will indicate the same for these indicator organisms (Bridson 2006). However, according to M. Andrin (01 February 2008, lecture on spiking methods) the recovery of *E. coli* and *S. aureus* from spiked meat tissue is low and they were therefore not included in the pilot study. It should be noted that meat is always contaminated and although an initial bacterial count was not conducted for the pilot study, the intention of the study was to obtain a correlation between the measuring units and not the bacterial counts. Figure 3 shows the distribution of data in a specific pattern with a positive incline of > 7% to confirm the correlation of the different units (Snedecor & Cochran 1967).

The excision sampling technique is motivated by the argument that bacteria migrate deeper into the tissues to more favourable conditions when the exposed, drying meat surface restricts bacterial growth. This, however, has been researched and refuted (Capita *et al.* 2004; Hutchison *et al.* 2005; Pepperell *et al.* 2005). The argument in terms of game meat possessing a dryer surface than meat from domesticated animals (Hoffman & Dicks 2011) motivated the use of the EU-approved method (Zweifel, Baltzer & Stephan 2005) of swabbing in this study. A more representative result is obtained by swabbing and no damage is done to the carcass. The results in this report (see Table 1) clearly show a better bacterial recovery rate using the swabbing method in comparison with the excision techniques. The ratio for APC improved 5.4 times, for *E. coli* 108.0 times and for *S. aureus* 3.4 times over the results obtained from the excision technique.

The normality test on standardised residuals indicated no deviation from normality (see Shapiro–Wilk results in Table 1). Non-significant differences ($p < 0.001$) were found when comparing the excision and swabbing techniques. This confirmed the desirability of applying the biotrace cattle and swine test as a sampling technique in this study.

Discussion

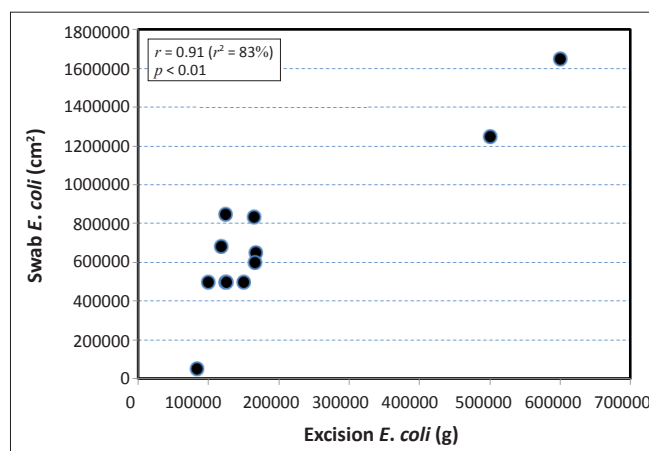
This study compared two approved sampling techniques, one used for carcasses intended for the export market (measuring unit grams) and a second technique (measuring unit square centimetres) used on game carcasses intended for the local market. The pilot study on the spiked beef tissue confirmed, through very good correlation results, the applicability of the two techniques. Furthermore, a good correlation was obtained for the different measuring units in this study.

It can therefore be concluded that for the purpose of this study both the excision and the swabbing techniques can

be used to obtain bacterial profiles from both export and local carcasses and to compare these results. However, the swabbing technique could prove even more effective than the excision method in terms of recovery rate. This can be explained by the larger, more representative sampling area but also by the sampling method used during the biotrace cattle and swine test (the standard template surface used, i.e. 200 cm²) (Van der Merwe, Jooste & Hoffman 2011). It can be argued that the latter test can have an unfair advantage in terms of the recovery rate of micro-organisms and that the game carcasses intended for the local market may present with higher bacterial counts than the game carcasses intended for the export market which were sampled with the excision technique (Van der Merwe, Jooste, Hoffman & Calitz, in press).

Conclusion

The study described above was applied on both game carcasses for export and local markets but could be repeated using only one of the two to further compare possible differences in results. The findings of this study could therefore possibly be applied to change the current sampling technique of excision for game carcasses intended for the export market to the swabbing technique, which causes less damage to game carcasses and which may portray more representative bacterial profiles of such carcasses.



E. coli, *Escherichia coli*.

FIGURE 3: Correlation and scatter plot between the two sampling techniques with different measuring units from the spiked aerobic plate count.

TABLE 1: Means of the excision and swab methods.

Method	APC	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Excision method	^a 0.5000/g	1.6400/g	167261.4000/g
Swab method	^b 27.7100/cm ²	173.6400/cm ²	638850.1000/cm ²
^c Probability (<i>p</i>) of difference	0.0015	0.0044	0.0037
^d Shapiro–Wilk (<i>p</i> < <i>W</i>)	0.5810	0.7127	0.0449
Ratio	5.4000	108.0000	3.4330

APC, aerobic plate count.

^a, The measuring unit for the excision sampling method: gram (g).

^b, The measuring unit in the biotrace cattle and swine test: square-centimetres (cm²).

^c, $p < 0.001$ indicates significant differences; $p < 0.05$ indicates significant differences at the 5% level.

^d, Shapiro–Wilk's ($p < W$) probability test the H_0 hypothesis for normality, thus for a probability > 0.01 there is not enough evidence of non-normality.

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Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors' contributions

P.J.J. (Tshwane University of Technology) was the project leader, whilst M.v.d.M. (Tshwane University of Technology) performed most of the experiments and wrote the manuscript. L.C.H. (University of Stellenbosch) made conceptual contributions and statistical interpretations were performed by F.J.C. (Agricultural Research Council Biometry Unit).

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