final report

Project code: A.RBP.0015

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Date published: May 2014

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Occurrence of malignant neoplasia in adult cattle at slaughter

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Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

This project reports both gross and histopathological characteristics of 97 neoplastic lesions that have been submitted from five abattoirs across Eastern Australia. The lesions were obtained from 71 carcases and the number of lesions identified in each carcase ranged from one to more than five. Primary lesions were most commonly found in adrenals, followed by liver and then ovary, while secondary tumours were reported most commonly from lung and lymph nodes. Lesions varied in colour, consistency and size and while primary tumours were more likely to be larger than secondaries, colour and consistency was not reported to differ between primary and secondary classifications. However, it was reported that classification of lesions as benign or malignant was influenced by whether lesions were one of multiple single- ($p < 0.001$) or varying-sized ($p < 0.001$) lesions, the presence of tracking along vessels or lymph node involvement ($p < 0.001$) and the presence of necrosis ($p < 0.001$). On Plant Veterinarian disposition, whilst not perfect, was highly accurate for condemned carcases, and agreement between pathologists and between individual pathologists and a consensus for histopathological diagnosis was found to range between “substantial” and “almost perfect” (kappa = 0.647 – 0.826).

Executive Summary

This project reports both gross and histopathological characteristics of 97 lesions that have been submitted from five abattoirs across Eastern Australia. Lesions were obtained from 71 carcases and the number of lesions in each carcase ranged from one to more than five. Primary lesions were most commonly found in adrenals, followed by liver and then ovary and were also identified in almost all other tissues in the carcase on at least one occasion. It has been identified that there is a possible geographical clustering of adrenal tumours in Northern NSW / Queensland, based on the submissions and secondary telephone questionnaire, but this requires further exploration before confirmation. Secondary tumours were reported most commonly from lung and lymph nodes. Lesions varied in colour, consistency and size and while primary tumours were more likely to be larger than secondaries, colour and consistency was not reported to differ between primary and secondary classifications. However, it was reported that classification of lesions as benign or malignant was influenced by whether lesions were one of multiple single- ($p < 0.001$) or varying-sized ($p < 0.001$) masses, the presence of tracking along vessels or lymph node involvement ($p < 0.001$) and the presence of necrosis ($p < 0.001$). A telephone questionnaire of export abattoirs in Australia reported a throughput that ranged between 80 to 1300 head of cattle per day and a reported yearly period prevalence of condemnations due to non-ocular neoplasia between 0 – 0.1%, corresponding to 0 – 20 cases per 20,000 head slaughtered. Dispositions made by On Plant Veterinarians, whilst not perfect, was highly accurate for condemned carcases. Agreement between pathologists and between individual pathologists and a consensus for histopathological diagnosis was found to range between “substantial” and “almost perfect” (with associated kappa = 0.647 – 0.826).

Contents

1. Project background

The occurrence of malignant neoplasia in adult cattle at slaughter is the “leading cause of condemnation of the carcases of adult cattle” (MLA Terms of Reference, 2010). However, very little is known about the management of this issue and the diagnostic accuracy of the inspection process. Greater knowledge surrounding this area will potentially result in reduced condemnations and improved management of affected carcases.

Enhanced accuracy surrounding disposition subsequent to diagnosis of malignant neoplasia is important in order to optimize both economic return from the slaughter process, and to protect consumer public health and/or public health perception.

A review of the literature has found studies focusing on particular types of neoplasia and their associated characteristics. (Hamir, 1985; Herenda et al., 1990; Uma et al., 2011). Research investigating the characteristics of lesions in adult cattle at slaughter and the decision process leading to a disposition in Australia is currently not well represented in the literature.

2. Project objectives

The project consists of two parts, the aims of which, as outlined in the ToR (MLA, 2010), are outlined below:

Part A - Gross characteristics
The aim of this part of the project was to define the frequency and gross characteristics of various neoplasms causing condemnation of tissues or carcases at Australian export abattoirs (excluding ocular SCC).

Part B - Histological characteristics
The aim of Part B was to characterise the histological attributes of lesions identified by meat inspection as malignant neoplasia (“tumours” using the AS terminology) in the carcases of adult cattle at Australian export abattoirs (excluding ocular SCC).

3. Methodology

3.1 Human Research Ethics approval

The study proposal, information statement (Appendix 1), consent form (Appendix 3) and questionnaire (Appendix 5) were submitted to the Human Research Ethics Committee at Charles Sturt University. This research proposal was approved on 14/7/2011 (protocol number 416/2011/11).

A variation of this proposal, including addition of a telephone survey component was approved on 04/04/13. (protocol number 416/2011/11)

3.2 Data / sample collection

3.2.1 Part A - Gross characteristics

A paper-based questionnaire was developed in order to identify the characteristics of lesions associated with a classification of malignant neoplasia through meat inspection. Ocular lesions were excluded.

The questionnaire was developed using a standard and well documented structure, allowing the capture of all relevant pathological and epidemiological information (Dillman, 2007). A mixture of open and closed questions with categorical and likert scale responses were used (Appendix 5).

The questionnaire consisted of three sections:

- Section A: General Information – inspector name, date, plant name/location, carcase weight and condition, gender, age (estimated from incisor teeth), breed, NLIS/RFID number.

- Section B: Lesion Information - anatomic location(s), size, surface texture, colour, margins and consistency.

- Section C: Lesion Classification – gross post-mortem classification as benign, primary malignant or metastatic; factors that influenced the classification decision; suspected diagnosis.

Sections B and C included measures of confidence in classification using a categorical scale ranging from 1-minimum possible confidence to 10- maximum possible confidence.

The questionnaire was developed by the project team, led by Dr Jane Heller, with consultative iterative feedback from Dr David Jordan, the Scientific Coordinator of the project. After the questionnaire was fully developed and prior to implementation, feedback was sought from four members of the Scientific Panel of MLA. All feedback was subsequently incorporated into the questionnaire. Finally, the questionnaire was pretested on a small group of veterinarians, which included pathologists.

The questionnaire was initially modified for implementation through iPod touch™ technology, using iSURVEY, an online survey application. This application allows questionnaires to be completed and data stored on a device, after which they can be uploaded via Wi-Fi to the main iSURVEY site for subsequent download. However, after initial difficulties experienced by OPVs in uptake of this technology (discussed in Part 3.4.2) the questionnaire was re-distributed in paper format. Each questionnaire was labelled with a project identification containing the plant identifier and carcase number e.g. M1. Questionnaires were subsequently returned by mail and all responses were coded and entered into an excel spreadsheet by a single operator (Ms Lynne Hayes).

3.2.2 Part B – Histological Characteristics

Samples from lesions identified by meat inspection as malignant neoplasia were collected, with up to a maximum of four lesions sampled per carcase.

The guidelines for sampling were as follows:

a. If only one lesion detected grossly, one sample from that lesion was obtained;

b. If more than one lesion with similar gross characteristics in only one tissue detected the two largest lesions were sampled;

c. If lesions with similar gross characteristics were present in more than one tissue the two largest lesions in the most seriously affected tissue were sampled. In the other tissues, the largest lesions in each tissue to a maximum of two other tissues were sampled.

Collected samples were to be no more than 4 cm x 5 cm (or of equivalent area), and 5 to 10 mm in thickness. Samples were placed in at least 10 times their volume of formalin (10% NBF), in a plastic histology pot. Pots were sealed and labelled using a labelling protocol developed by the researchers.

An example of this follows:

e.g. sample “A 1 a”

“A”- Plant Identifier: A letter assigned to each plant.

“1” - Carcase number (1-50): Project specific identifier assigned to each carcase that is sampled.

“a” - Lesion identifier: Lower case a, b, c, d identifying up to four lesions per carcase

As discussed in 3.2.1, a corresponding label with the plant identifier and carcase number was attached to the questionnaire associated with the samples.

Samples were packaged according to provided instructions and stored in batches until sufficient numbers for cost effective transportation had been collected. (Appendix 7)

In addition, OPV's were instructed to photograph sampled lesions, using the camera feature of iPod touch™ to aid in the reporting and explanation of findings.

3.3 Participating plant recruitment

In the initial phase of recruitment, plants satisfying criteria for involvement in the project were identified by MLA with input from AQIS, industry and the research team. The aim was to have six plants, representing a geographical cross section of the industry and representing "Northern", "Central" and "Southern" regions. Although the project commenced in February 2011, complexities within AQIS, including unscheduled industrial action and the implementation of industry reforms, delayed the ability for MLA (through Julie Cassar) to discuss this project with AQIS (through Carol Sheridan) in order to gain collaboration with the OPVs. Following a lengthy delay in gaining approval from AQIS, contact was made in December 2011 through Area Technical Managers and/or directly with on plant veterinarians (OPVs) at five representative plants. Of the five plants initially identified, two agreed to participate in the project in its early phase.

The decision was taken to work with the two participating plants whilst simultaneously making attempts to secure the involvement of four additional plants, identified by MLA. Due to continuing difficulties in securing these additional plants (predominantly due to difficulty in obtaining response from AQIS, but also due to the added complexity of requiring approval from both participating OPV and management representative at each plant during a time of increased workload), the project was placed on hold by MLA from August 2012 and resumed in May 2013. The number of additional plants required was modified to three, with final recruitment to the project completed in November 2013. Signed consent from OPVs and plant management was obtained prior to the commencement of data/sample collection. (Appendices 1-4)

Table 1 Plant recruitment details

Commencement	Plant ID	Location of plant
April 2012	A	Victoria
April 2012	B	Victoria
May 2013	C	Victoria
June 2013	D	Queensland
Nov 2013	E	NSW

3.4 Project implementation

3.4.1 Pilot

A pilot study was commenced at Plant A and Plant B in April 2012. The broad aims of this process were to ensure that the questionnaire aligned with the project objectives, to evaluate the use of the iPod touch™ as an electronic method of data collection and to obtain feedback on any practical implications of the histological and cytological sampling with regards to workflows on site.

A member of the research team visited Plants A and B at this time to provide instruction on project procedures, including sampling, packaging and transportation, and the use of the iPod touch™ for the questionnaire and photography. In addition, a video, developed by the research team was shown, demonstrating appropriate tissue collection, the preparation and making of impression smears using glass slides and correct short-term storage. The video was stored on the iPod touch™ enabling the OPV to access it as required throughout the course of the project. Instruction sheets were also provided (Appendices 7-8).

At the completion of the pilot, minor adjustments were made to the questionnaire, in line with feedback from the two participating OPVs.

3.4.2 Project Suspension

Sample and data collection continued at Plants A and B until August 2012. After a period of suspension, as previously discussed, the project was recommenced in May 2013. During this time the research team re-evaluated the methodology of the project. Following feedback from the OPVs at the participating plants, the decision was taken to cease the use of the iPod touch™ as a tool for data collection. The physical manipulation of the device at the plant was difficult and resulted in errors in data entry. Although these errors were detected and resolved it was anticipated that this would be difficult to manage once the project achieved full implementation.

The cytology component of the study was also removed as feedback from the participating plants and those approached for participation reported that the OPVs had insufficient time to provide both histological and cytological samples. The investigating pathologists (Prof Shane Raidal, Dr Alan Kessell and Dr John Boulton) confirmed that, while cytological analysis would potentially provide helpful information for future diagnosis at the level of the abattoir, histological samples are appropriate for definitive characterisation of the lesions.

3.4.3 Full implementation

The three additional plants Plant C, Plant D and Plant E received introductory visits from a member of the research team in May, June and November 2013. Paper based questionnaires and updated sampling methodology was introduced at all plants, and full implementation of the project was achieved in November 2013.

3.5 Additional components

3.5.1 Telephone questionnaire

A national telephone survey of export abattoirs not recruited to the study (n = 43) was commenced in May 2013. The addition of this aspect of the project was to enable the identification of the (perceived) prevalence of non-ocular neoplasia at all export abattoirs and to provide a baseline against which to compare the results of parts A and B above.

Phone calls were made to each export abattoir and an OPV or, where this was not possible, appropriate plant representative, were asked a series of short questions covering the following points:

- Geographical source of the herd (in order to identify geographical extent of source cattle)
- Description of cattle slaughtered at the plant (breed and age information)
- Reported rate of non-ocular neoplasias resulting in condemnation

All information was obtained by a single operator and information was recorded in an excel spreadsheet.

Throughput was calculated by changing all reported rates into both daily and yearly rates, based on an average of 250 culling days per year (50 weeks with 5 working days per week). Estimated rates of condemnation due to neoplasia were then calculated per year for each plant. Where a range was reported, the maximum value was used.

3.5.2 Agreement Study

A study to identify and report the agreement between histopathological diagnoses of non-ocular neoplastic lesions between pathologists was also performed. The inclusion of this component was to add value to the overall project outcomes by validating the diagnoses and allowing an overall assessment of accuracy of identification of neoplastic process at the level of the laboratory.

A number of histopathological samples collected as part of the main study (those collected by the time this study was performed) were assessed independently by four different pathologists. Each sample was randomly assigned a number between 1 and 77 and samples were reordered prior to assessment by each pathologist. Pathologists were asked to record the following information for each sample. (Appendix 9)

- Short Description- Malignant / Benign / Other
- Diagnosis- Round / Spindle / Epithelial / Other
- Specific diagnosis
- Confidence- (0-100%)
- Time taken- (min)

In addition, after each pathologist had assessed all samples independently, all four pathologists conferred on each sample and a consensus diagnosis was reached and recorded for each lesion. Immunohistochemistry was also performed on any of the 77 samples that were deemed by all pathologists to require it for a final diagnosis. Where lymphoid structure required assessment, CD3 and CD79a antigens were assessed. Synaptophysin was assessed where adrenal neoplasia was suspected and in combination with cytokeratin, vimentin and S100 to help differentiate sarcomas from carcinomas.

Assessments of agreement were made between each pair of pathologists and between each pathologist and the consensus after all assessments were completed.

3.6 Statistical analysis

Comparison between categorical data was performed using Chi square analysis, or Fisher's exact test where there were less than 5 observations in one or more cells. Where appropriate, agreement was measured between pairs using kappa, a measure of agreement beyond chance for categorical results. Analyses were performed in SPSS and significance was set at $p < 0.05$.

4. Results

4.1 Missing data

Questionnaires and formalin stored lesions were obtained from 71 carcases, distributed across the 5 plants as identified in Table 1. Any unmatched data ie samples received / no questionnaire (n=3) or questionnaire completed / no samples (n=2), were excluded.

Questions that resulted in poor responses on the questionnaire included weight (43 out of 71 did not respond) and breed (52 out of 71 did not respond). In addition, a single plant had a poor response for Q 5 ii) where information regarding influence on lesion classification was sought. All other questions elicited adequate responses.

4.2 Carcases sampled as a % of total condemnations

The numbers of carcases from which samples were obtained varied greatly between plants (ranging between 4 carcases over 15 months to 37 carcases over 8 months). Results obtained from the concurrent documentation of condemnations due to malignant neoplasia on the plants included in the study identify that the OPVs were able to sample up to 67% all carcases condemned due to malignant neoplasia during the period under study. Discussions with OPVs identified that the differentiation between numbers sampled and total condemned was due to staffing levels, varying shifts and the availability of the OPV to obtain samples due to other work demands (OPV Plant B, Plant D, pers comm.). A total of 97 samples have been collected and submitted from 71 carcases (Figures 1 and 2).

Table 2 Summary of number of samples obtained

Plant ID	Months involved in study	Carcases sampled	Total condemnations due to malignant neoplasia during the period of study*
Plant A	15	4	6†
Plant B	22	18	111‡
Plant C	9	8	34
Plant D	8	37	Information not provided
Plant E	4	4	15§

* Identified by condemnation certificates
†Period June 2012- June 2013
‡Period April 2012- Dec 2013
§Period December 2013 – March 2014

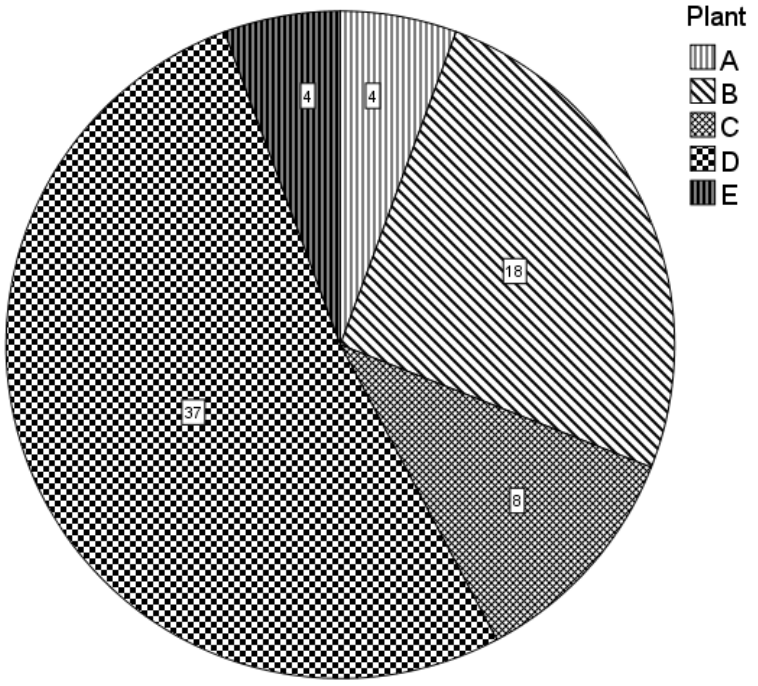

Figure 1 Number of carcases (n = 71) condemned for neoplasia sampled by plant

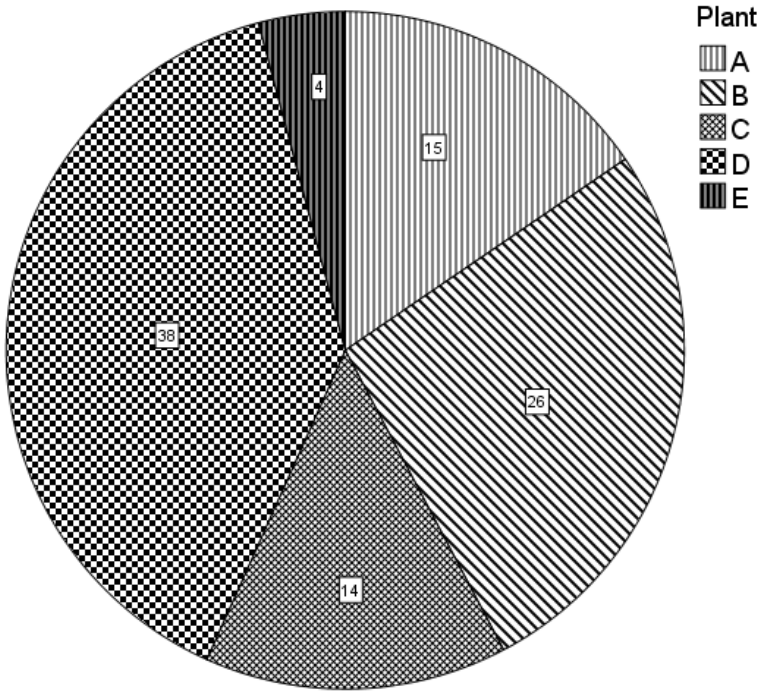

Figure 2 Number of lesions (n = 97) from carcases condemned for neoplasia collected by plant

Information on gender was provided for 62 carcases. Of these, 89% were female (n = 55), while 5% were entire male (n = 3) and 6% castrated male (n = 4). Age of the carcases ranged between 2 years and >6 years with >6 by far the most commonly represented age group (Figure 3). Condition scores of carcases (responses obtained for 67 carcases) ranged between 2 and 4, with 3 the most commonly reported condition score (Figure 4).

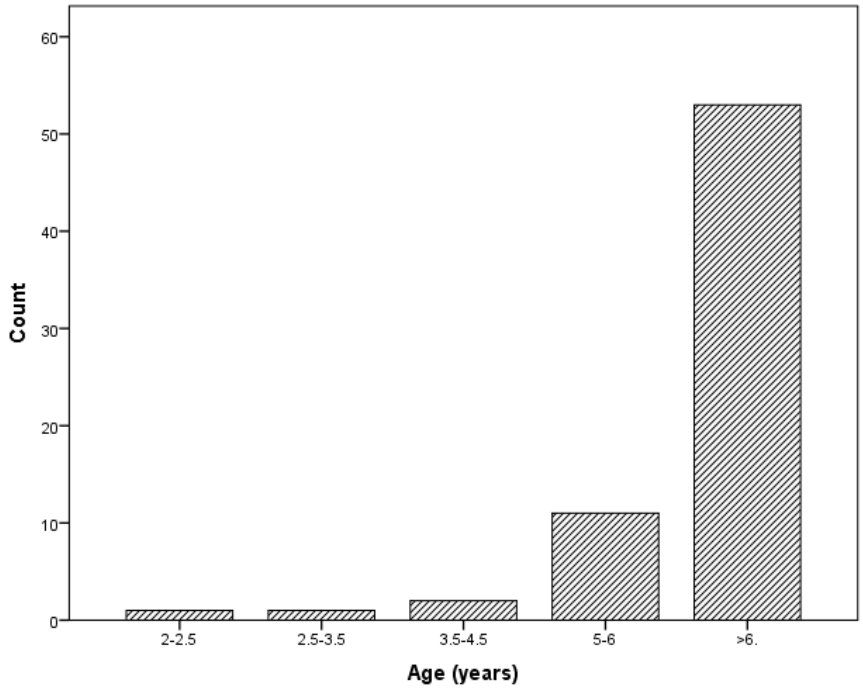

Figure 3 Age of carcases (n = 68) from which neoplastic samples were submitted

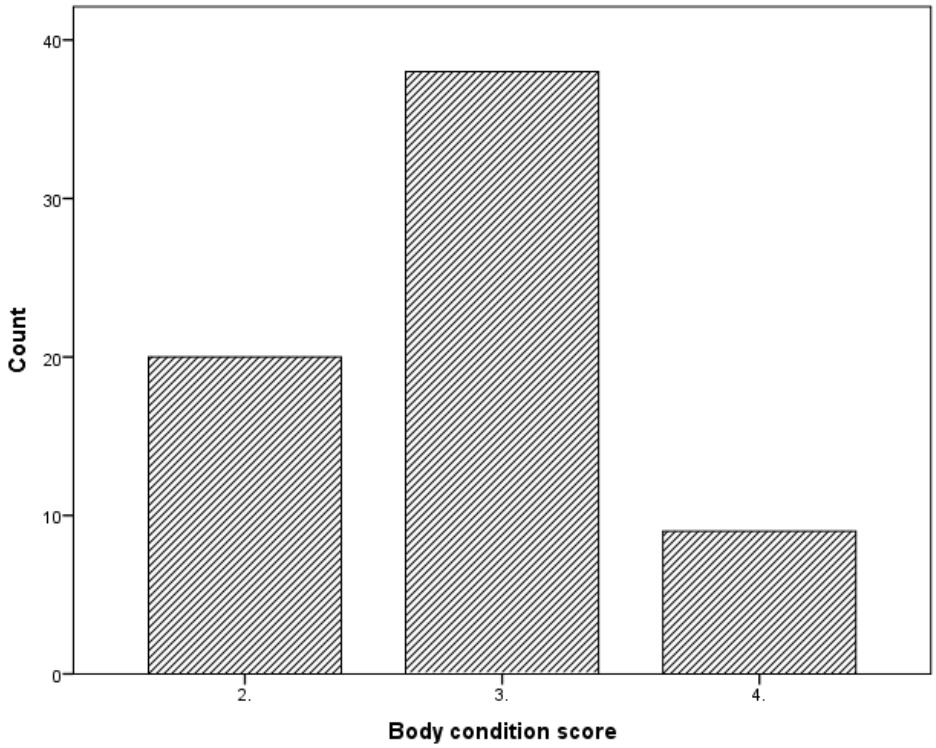

Figure 4 Condition score of carcases (n = 67) from which neoplastic samples were submitted

The number of lesions identified in carcases condemned due to neoplasia ranged between 1 and 5 (Figure 5) and these lesions were found in between 1 and 5 differing organs (Figure 6).
A single lesion was submitted for 55 carcases and, of these, 49 were identified as primary neoplastic lesions, three were identified as single metastases, one was identified as an inflammatory lesion and no classification was recorded for two lesions.

More than one lesion was submitted for analysis from 16 carcases. Nine of these carcases had a single lesion identified as the primary neoplasm with additional lesions identified as metastases, or in one case an additional inflammatory lesion. Six carcases were identified to have more than one metastasis and no primary lesions and one carcase was reported to have more than one (3) primary lesions and one metastasis.

The anatomical location of the collected lesions varied greatly. Figure 7 identifies that lesions from the adrenals are by far the most common lesions identified as primary neoplasias, followed by liver lesions. When the data are stratified by submitting plant (Figure 8), it is clear that there is a regional component to this variation in distribution, with a single plant (Plant D) responsible for the vast majority of tumours identified by OPVs as adrenal tumours. Lesions reported as metastatic most commonly occurred in lung and lymph node.

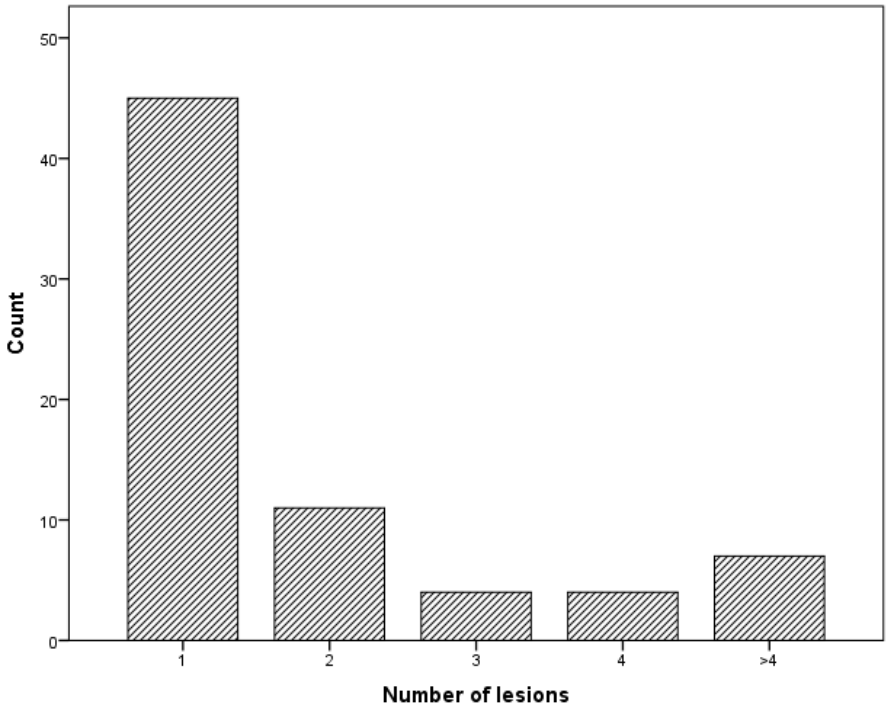

Figure 5 Number of neoplastic lesions identified in cattle carcases (n = 71) condemned due to non-ocular neoplasia

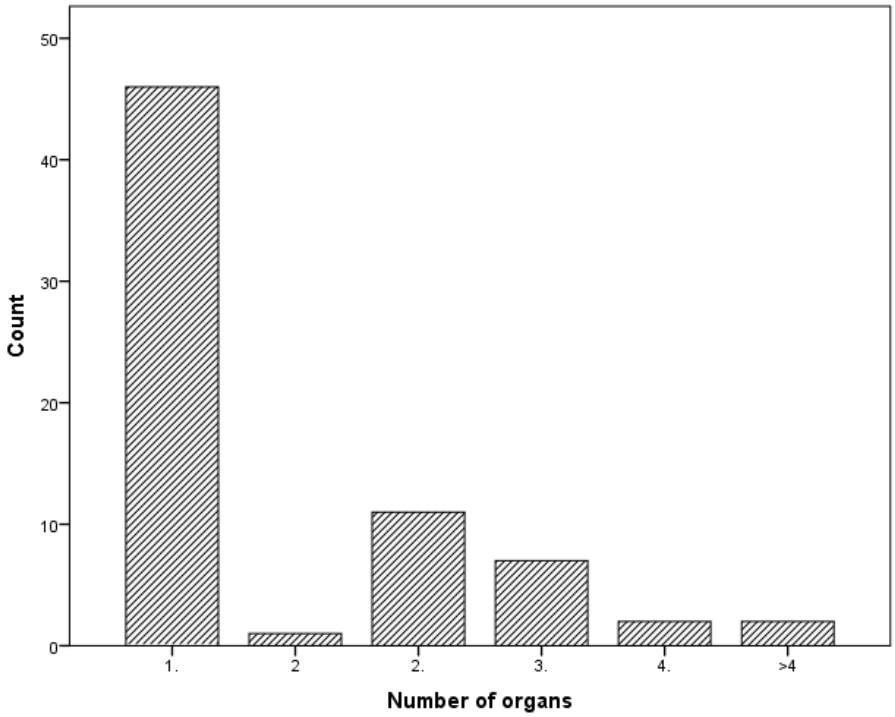

Figure 6. Number of organs in which lesions were found in cattle carcases (n = 69) condemned due to non-ocular neoplasia.

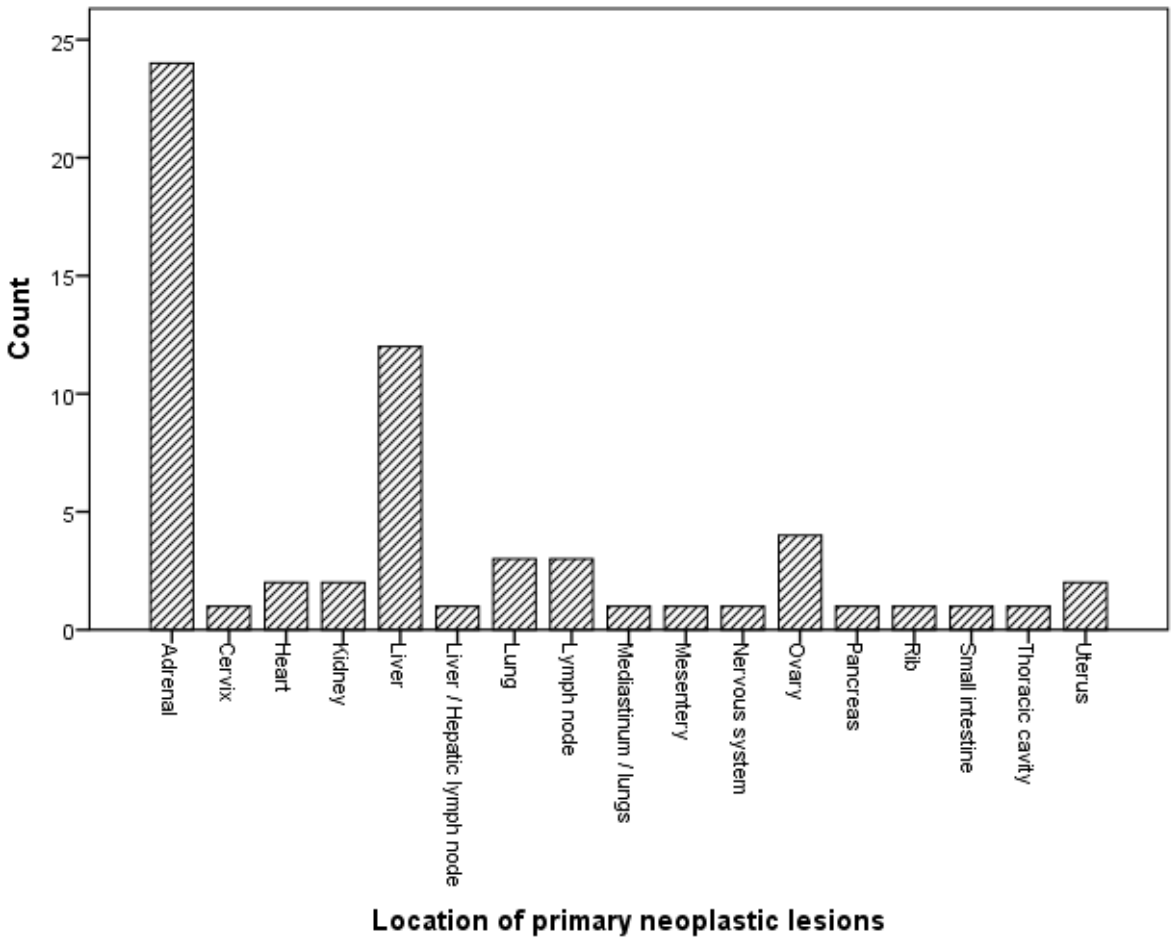

Figure 7 Anatomical location of lesions sampled and identified as primary neoplastic lesions (n = 61)

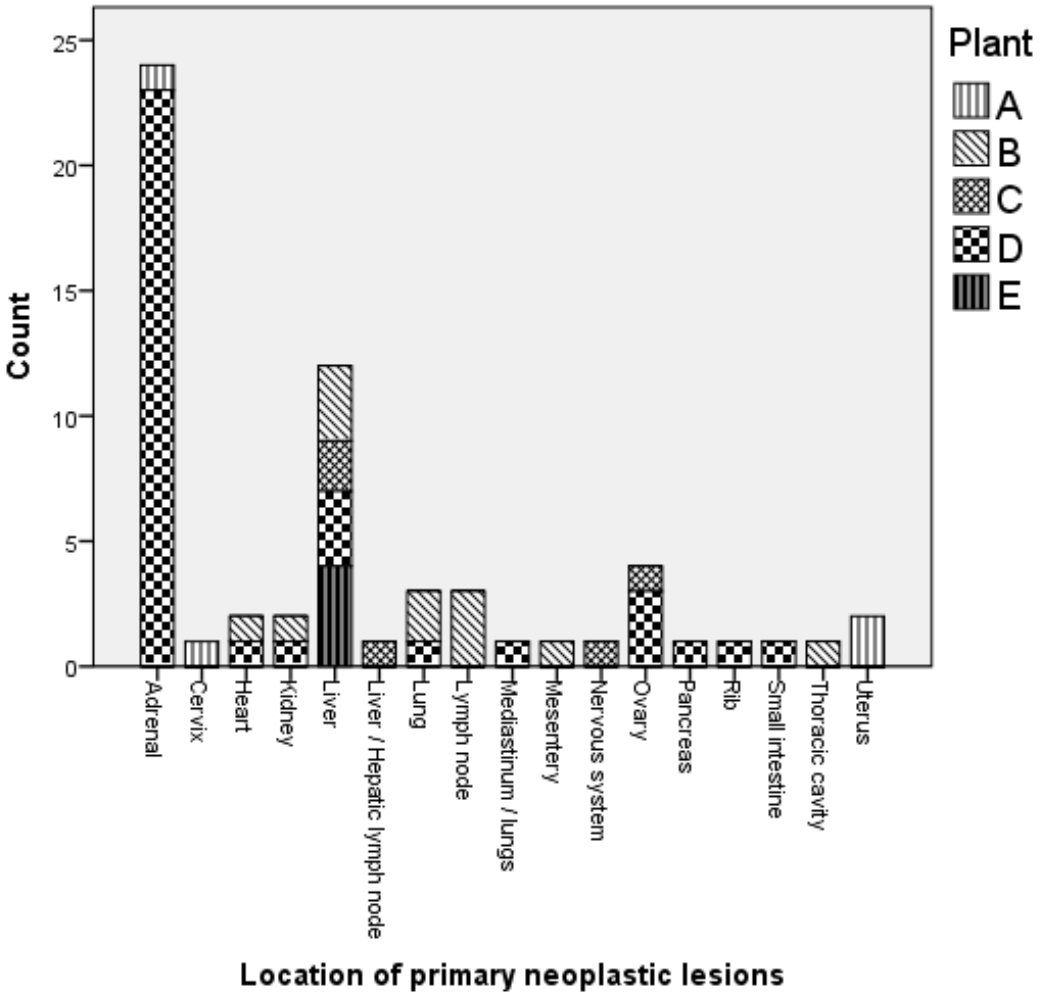

Figure 8 Anatomical location of reported primary neoplastic lesions classified by plant

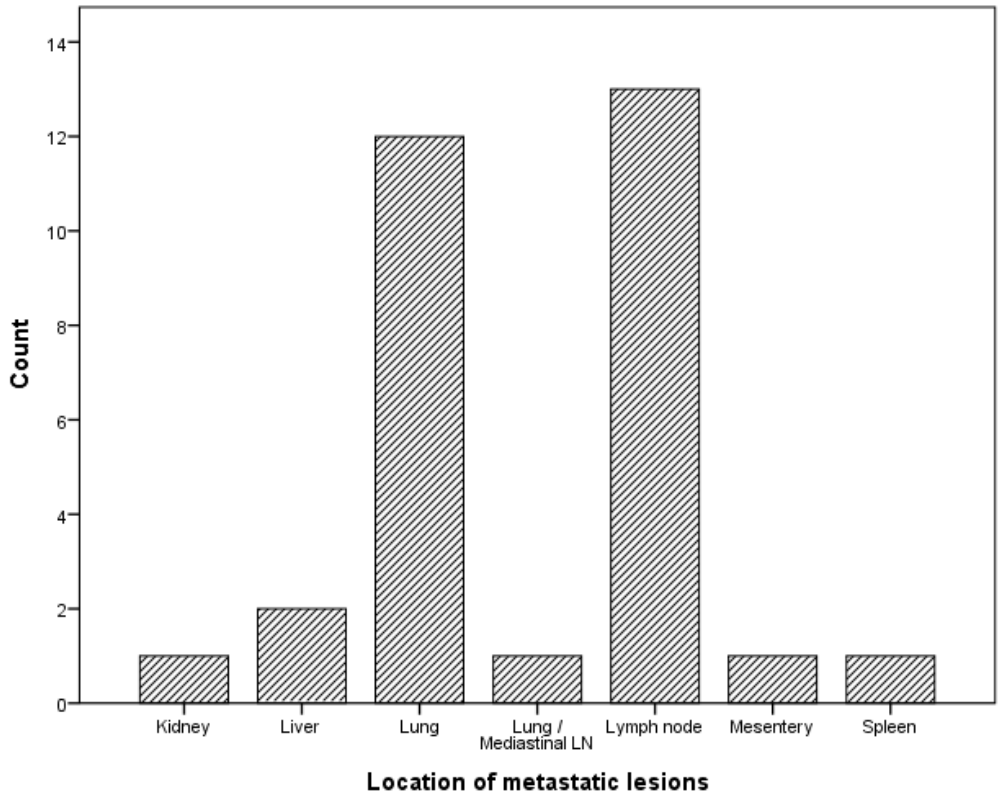

Figure 9 Anatomical location of lesions sampled and identified as metastatic lesions

Descriptions of the lesions varied.

A significant difference was identified between the reported size for primary and non-primary lesions ($p = 0.020$), with larger lesions associated more commonly with non-primary classification (Figure 10).

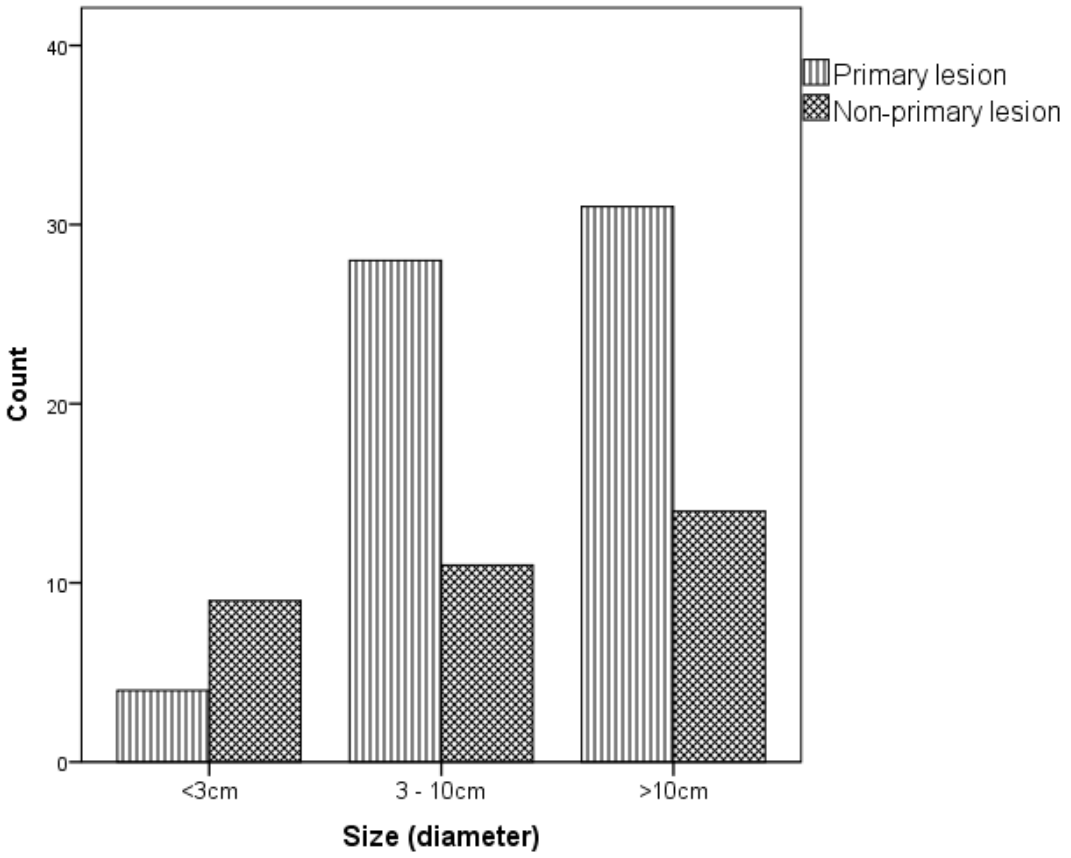

Figure 10. Size of lesions for those classified as primary or non-primary in carcases condemned for neoplasia.

The surface texture of the lesions was not found to be associated with classification as primary lesions or not (p = 0.257) and in total 56 of the lesions (58%) were identified to be smooth in texture while the remaining 42% (n = 41) were nodular/irregular.
The colour and consistency of all lesions varied between the five categories (from palest possible to darkest possible and from softest to firmest possible), with no significant difference identified between primary and secondary lesions for either of these descriptors (p = 0.498, p = 0.072 respectively) (Figure 11a and b).

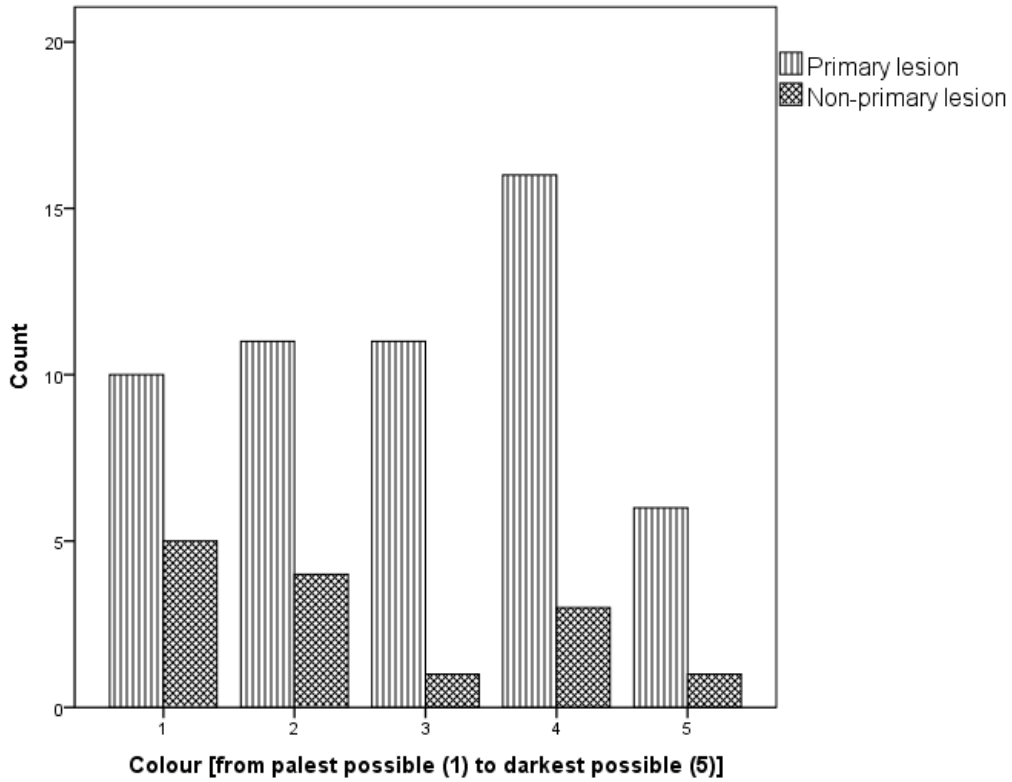

Figure 11a Colour of lesions for those classified as primary or non-primary in carcases condemned for neoplasia.

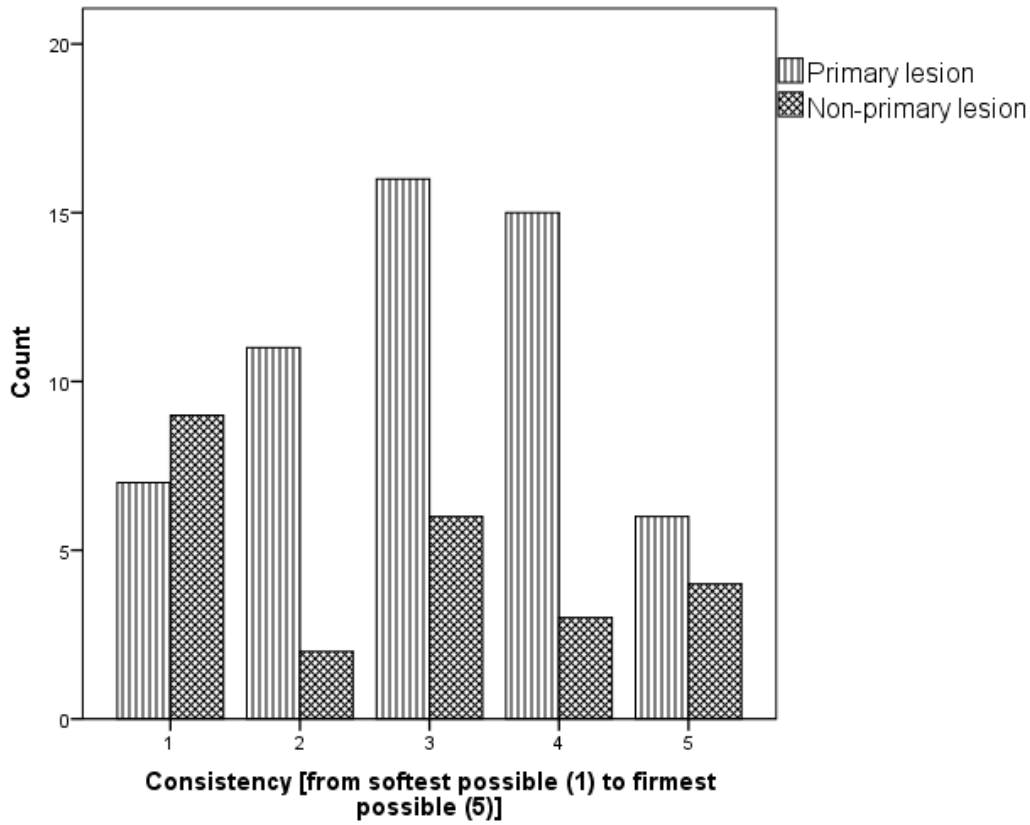

Figure 11b Consistency of lesions for those classified as primary or non-primary in carcases condemned for neoplasia.

The margins of each lesion (clearly demarcated vs diffuse) did not vary between those classified as primary or non-primary (p = 0.795). Overall, of the 94 lesions that were classified, 62 (64%) were identified to have clearly demarcated margins and 32 (33%) had diffuse margins.

Classification of lesions as benign or malignant was identified to be influenced by whether or not there were multiple lesions of similar size (p < 0.001), multiple lesions of differing size (p < 0.001), presence or absence of tracking nodes (p < 0.001) and presence or absence of necrosis (p < 0.001) (Figures 12a, b, c and d). Presence or absence of homogeneous colour and whether or not the lesion was single were not found to be influential in decision making (p = 0.183 and p = 0.0121 respectively).

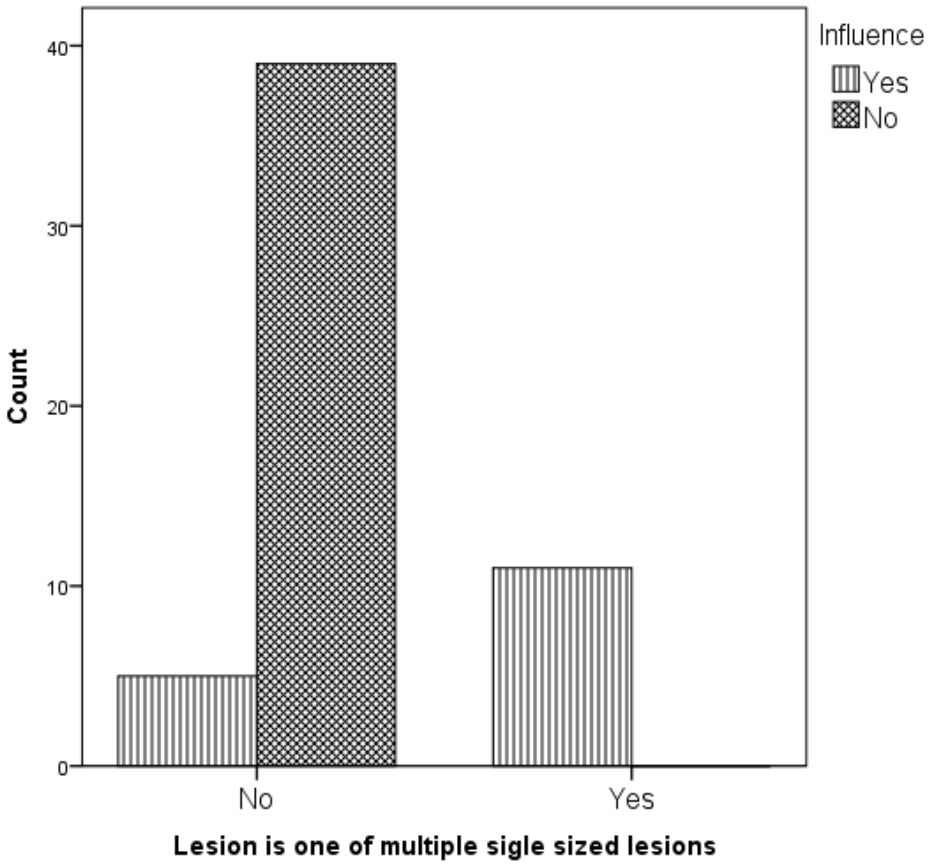

Figure 12a Influence, as identified in the question “Please identify whether the presence or absence of multiple single sized lesions influenced your decision to classify the lesion as benign or malignant”, in carcases condemned for neoplasia.

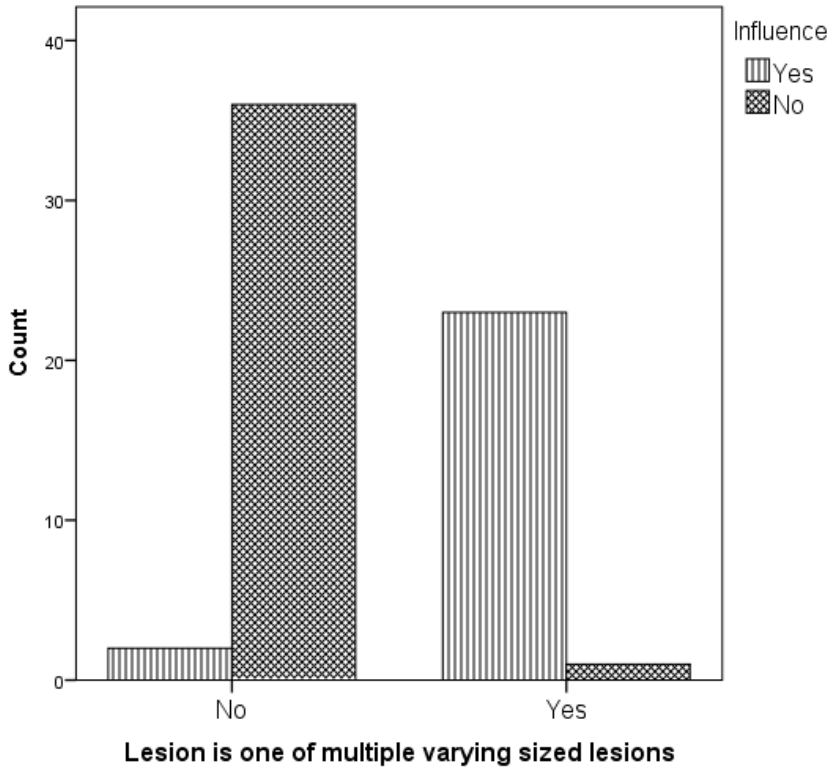

Figure 12b Influence, as identified in the question “Please identify whether the presence or absence of multiple varying sized lesions influenced your decision to classify the lesion as benign or malignant”, in carcases condemned for neoplasia.

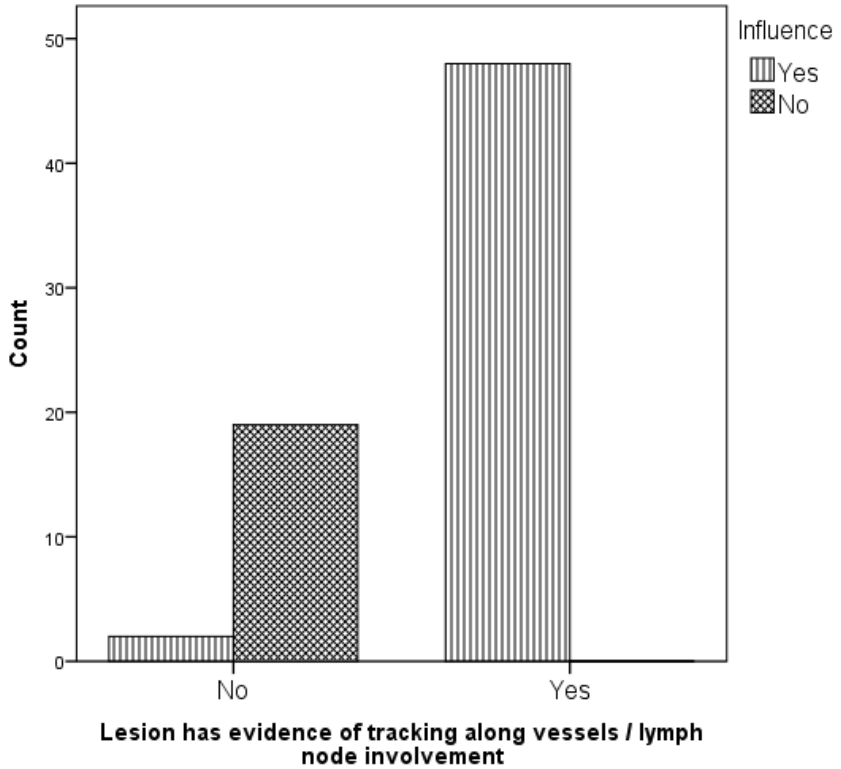

Figure 12c Influence, as identified in the question “Please identify whether the presence or absence of tracking along vessels / lymph node involvement influenced your decision to classify the lesion as benign or malignant”, in carcases condemned for neoplasia.

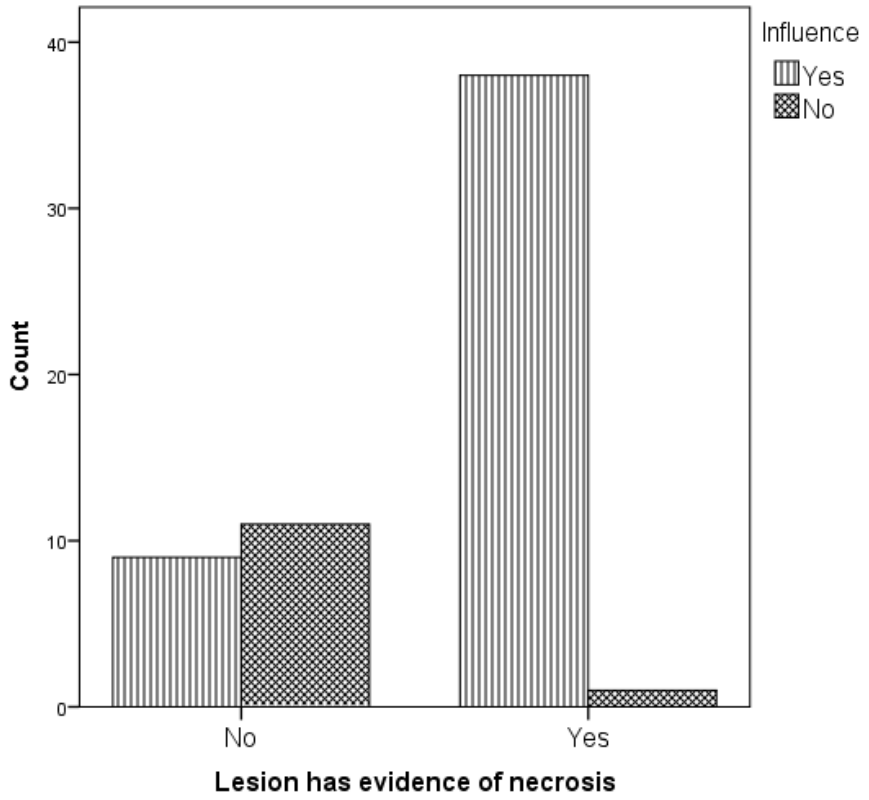

Figure 12d Influence, as identified in the question "Please identify whether the presence or absence of necrosis influenced your decision to classify the lesion as benign or malignant", in carcases condemned for neoplasia.

4.3 Accuracy of diagnoses

An assumption was made that the disposition was based on the primary malignant tumour (where present) or the presence of multiple metastatic tumours, as identified in AS 4696:2007. Thus all measures of accuracy were made on tumours that satisfied these criteria, as follows:

- All lesions were assessed for accuracy where only one lesion was submitted per carcase.

Where more than one lesion was submitted per carcase, accuracy was measured for the single lesion identified as primary neoplastic, or all lesions where no primary lesion was identified (and thus condemnation was based on the presence of multiple metastatic lesions).

In accordance, the diagnoses of 88 lesions were assessed for accuracy in their categorisation as either malignant or benign (i.e. the appropriateness of disposition based on the presence of neoplasia as per AS 4696:2007). It was found that discrepancies between OPV and pathologist classification of lesions occurred in 7 cases (8.0%) across three plants. The misclassifications are identified in Table 3.

Table 3. Discrepancies between OPV and pathologist classification for 7 lesions

Organ*	OPV classification	Confidence[#] (%)	Pathologist classification	Confidence[#] (%)
Liver	Carcinoma**	30	Hepatoma	50
Adrenal	Carcinoma	60	Reactive lymph node	95
Mediastinum / lung	Carcinoma	70	Chronic pneumonia	100
Liver	Carcinoma^	80	Chronic fibrosing hepatopathy	100
Lung	Carcinoma	80	Actinobacillus infection	100
Liver	Carcinoma	80	Hepatoma/telangiectasis	60
Lung	Benign	NA	Lymphoma	60

* As classified by the OPV on sample submission

[#] 'Confidence' relates to the OPV or pathologist confidence in classification, rated between 1 – 10 (1 is minimum possible and 10 maximum possible) and converted to percentage.

** A second lesion was visualized in the liver in this case but unable to be collected

^ Extensive damage to the liver, with virtually no recognizable liver tissue was identified by OPV

4.4 Telephone questionnaire

After numerous attempts, contact was able to be made via telephone with 31 export abattoirs (77.5%). Information on prevalence was obtained from 29 of these plants.

The throughput of plants was reported to range from approximately 80 to 1300 head of cattle per day. Cattle were reported to be sourced from multiple locations including local, state and interstate and the age of cattle slaughtered ranged from 0 to 8 tooth, corresponding to up to and over 5 years of age.

The reported yearly (period) prevalence of condemnation due to non-ocular neoplasia ranged from 0 to 0.1% (that is, between 0 and 20 cases per 20,000 animals slaughtered), with a median yearly prevalence of 0.00004 (just less than 1 case per 20,000 animals). Full reporting is presented in Table 4.

Table 4. Throughput - rates of condemnations of cattle due to non-ocular neoplasia and comments reported from 29 export abattoirs in Australia.

Abattoir identifier	Approximate daily plant throughput (head of cattle)	Cases of non-ocular neoplasia reported per 20,000 animals slaughtered	Comments from abattoir representative
1	340	1.4	
2	360	0.96	
3	1000	20	See very few. Those seen are sarcomas, skin and uterus.
4	475	Unable to estimate	Very low numbers – we had 2 in one month but otherwise uncommon. Most common is melanoma.
5	145	20	
6	775	9	1-2 metastatic melanoma per week, 2 adrenal tumours per month, 5-6 ovarian tumours per year, 5-6 other cancers per year.
7	850	2.4	Mainly melanomas
8	1300	0.5	
9	575	0.8	Most common ovarian / uterine
10	950	84	
11	360	0	Can not recall the last one seen
12	950	0.2	Very rare. Lymphosarcoma and melanoma.
13	900	8.9	
14	450	0.4	Very rare. A couple of melanomas only.
15	200	9.5	In 9 months, 5 adrenal, 5 lung, 2 neurofibroma, 1 ovarian and 1 renal neoplasias
16	1000	Unsure	Very few – couldn't put a figure on it
17	700	0.6	
18	1145	7.5	
19	1300	9.2	Main malignancies are adrenal and advanced ovarian. Very occasional melanoma and others are metastases.
20	735	0.5	Very rare.
21	360	0.2	Very rare.
22	1025	0.6	
23	700	0.3	
24	365	4.2	In one year, 4 disseminated/multicentric neurofibroma, one metastatic melanoma.
25	400	5.6	In 6 months, 2 neurofibromas, 2 lymph node tumours, one gastrointestinal and 9 that were a combination of lung or liver and lung.
26	100	2.4	
27	860	1.6	
28	80	0	Have not had any in a year
29	450	4.6	

4.5 Agreement between pathologists

Seventy seven lesions were assessed independently by four pathologists and by all pathologists together to reach a consensus diagnosis. Absolute agreement between all assessors (including the consensus) occurred for 58 of the 77 lesions (75%) for the short description (malignant vs benign vs non-neoplastic vs unsure) and in 56 of the 77 lesions (73%) for the specific diagnosis (round cell vs spindle cell vs epithelial vs other).

Estimates of kappa ranged between 0.321 and 0.653 for pairwise agreement between each individual pathologist and the consensus diagnosis and between 0.310 and 0.579 between all pairs of pathologists for the short description. Agreement was greater for the specific diagnosis, ranging between 0.665 and 0.826 between each pathologist and the consensus and 0.647 and 0.753 between all pairs of pathologists.

When agreement was assessed using the combined results of the IHC stains, it was found that agreement between the consensus diagnosis and IHC diagnosis was 0.620. All but one discrepancies were between classification as either a ‘sarcoma’ (spindle cell origin) or ‘carcinoma’ (epithelial origin), with 11 of 74 lesions with diagnoses identified by consensus as carcinomas but by stains as sarcomas. The final discrepancy was between consensus diagnosis of lymphoma and IHC diagnosis of reactive lymphoid.

5. Discussion

This project reports on the occurrence of malignant neoplasia in cattle at slaughter in Australia. Five abattoirs were recruited submitting samples from 97 lesions obtained from 71 carcases.

Despite the logistical issues that hampered this project, with respect to recruitment and ongoing communication with plants, good relationships have been formed with the five participating plants and the flow of information and material has been effective. Collection of material varied between plants, with the ability to effectively sample carcases for this study reported to be affected by staffing and workload issues. This resulted in material collected from condemned carcases to range between 16% and 67% of total condemnations due to malignant neoplasia. The assumption is made that there is no bias associated with this and that issues that affected sample collection are likely to be independent of issues associated with variables of interest within this study.

The age of cattle from which the samples were collected was predominantly >6 years old. As we do not have access to information from the plants from which these cattle were sourced or information on the age of condemnations due to malignant neoplasia and other dispositions from all abattoirs at this stage, no comparisons can be made. Despite the efforts of Long Huynh (MLA) we were unable to obtain access to the EPACS database. We had hoped, through EPACS, to obtain more information on neoplasia condemation that would allow comparison of reported condemnation rates within this study with collated data. Additional signalment data that was obtained is as might be expected. Most commonly a single lesion was identified to be present in each carcase, but up to and including five or more lesions were also identified. Similarly, lesions were

identified in a single organ most commonly, but again, up to and including five or more organs were identified to be affected in some animals, in line with malignant or disseminated disease.

An overrepresentation of adrenal tumours was present in this data set, with the adrenals identified as the location for a third of the primary lesions reported. When information on the location of the abattoir from which this information was obtained was overlayed it was identified that a single plant was responsible for almost all of the adrenal tumour submissions. This plant was the only Northern plant in the study. Comparison with the prevalence data obtained from the telephone survey is difficult as information on lesion types were not reliably obtained. However, it is interesting that the three abattoirs that volunteered information about adrenal tumours in the telephone survey (identified as abattoirs 6, 15 and 19 in Table 4) are also located in Northern NSW/Queensland region and all four abattoirs are within a maximum distance of 280km from each other. These data must be interpreted with caution as it is possible and likely that adrenal tumours are present elsewhere in Australia (a single adrenal tumour was also submitted from a Victorian plant) but further exploration of this putative geographical clustering may be warranted.

The location of lesions identified as metastatic (Figure 9) was predominantly reported to be lung and lymph node, which is in line with expected regions for secondary lesion occurrence.

Classification of lesions as primary or non-primary was found to be associated with size of the lesion (with smaller lesions more likely to be identified as non-primary), although size was not identified by the OPVs as influential in their differentiation between benign and metastatic lesions. Differentiation between primary and non-primary was not associated with either colour or consistency of the lesion, or with the presence of clearly demarcated or diffuse margins.

The identified factors that influenced OPVs in their decision to classify lesions as either benign or malignant were reported to be the presence of a lesion as one of multiple single OR varying sized lesions, evidence of tracking and evidence of necrosis.

The accuracy of OPV disposition was high. While discrepancies between OPV and pathologist classification occurred in 7 cases (8.0%), according to AS 4696:2007, these misclassifications would have resulted in incorrect condemnation of the entire carcase (instead of a single organ) with certainty in only two cases. In addition, it must be noted that, after the use of IHC stains, one of these cases was noted to be correctly classified by the OPV and this highlights the difficulty surrounding disposition in some situations. Use of quantitative estimates of agreement are not appropriate here as the subtleties of level of confidence will be lost through the use of measures such as kappa, resulting in an underestimation of OPV accuracy.

While the pairwise agreement for the pathological identification of a lesion as either malignant or benign was low (kappa = 0.310 – 0.653), it must be acknowledged that this measure is flawed for use with these data due to the inclusion of categories titled “other” and “unsure”, which will have resulted in a lower estimate of agreement than is likely to

truly be present. The estimate of agreement for the specific diagnosis (categories of round, spindle, epithelial and other) is more reliable and resulted in estimates of kappa between 0.647 and 0.826. This is supportive of “substantial” to “almost perfect” agreement between pairs of pathologists and between each pathologist and the consensus. Thus, while differences do exist between operators, we should be confident in the accuracy of histopathological diagnosis to the level of ‘type’ of tumour. Notwithstanding this, the finding that agreement between pathologists is imperfect in some situations supports the fact that diagnosis of pathological conditions, or classification as malignant or benign through visual inspection or indeed through histopathological examination is unlikely to be perfect using any diagnostic method.

This project has reported both gross and histopathological characteristics of the 97 lesions that have been submitted from five abattoirs across Eastern Australia. It has been identified that there is a possible geographical clustering of adrenal tumours in Northern NSW / Queensland, but this requires further exploration before confirmation. OPV disposition, whilst not perfect, can be classed as highly accurate for those carcases that have been condemned but of course we are unable to make any assessment of the accuracy of those carcases that were not condemned (i.e. possible false negatives) as this was beyond the scope of the current study.

6. Acknowledgements

The authors acknowledge the contribution of the participating on plant veterinarians and plant management to this research.

7. Appendices

7.1 Appendix 1- Information Statement

Charles Sturt University

FACULTY OF SCIENCE
School of Animal and Veterinary Sciences

Boorooma Street
Locked Bag 588
Wagga Wagga NSW 2678
Australia

Tel & Fax: +61 2 6933 4107
Email: nsangster@csu.edu.au
Web: www.csu.edu.au/vet/

Information Statement

Project title: Condemnation of cattle carcases at meat inspection due to malignant neoplasia

Principal Investigators:

Dr Jane Heller (Senior Lecturer in Veterinary Epidemiology and Public Health)
Phone: 02 69332839 Email: jheller@csu.edu.au

A/Professor Shane Raidal (Associate Professor in Veterinary Pathobiology)
Phone: 02 69334450 Email: Shraidal@csu.edu.au

This questionnaire is funded by Meat and Livestock Australia (MLA) and aims to define the frequency and gross characteristics of various neoplasms causing condemnation of adult cattle tissues or carcases at Australian export abattoirs.

The questionnaire will be implemented 'carcase-side', for each cattle carcase that is condemned due to neoplasia. In addition, tissue from each of the neoplastic lesions will be collected and forwarded to the investigators at Charles Sturt University (CSU) for analysis.

Please refer to the laminated instruction sheet for full instructions for this part of the project.

A consent form is required to be completed at the commencement of the study. Your name will not be used within the publication of this data and confidentiality and anonymity will be strictly maintained. Participation is voluntary and non-participation or withdrawal will not result in penalty in any form. Information of a personal nature will not be obtained throughout this research project and I do not foresee any risks associated with participation.

Please do not hesitate to contact Jane Heller, Shane Raidal or Lynne Hayes (ph 69332802; lhayes@csu.edu.au), for further information at any stage during this project.

NOTE: The School of Veterinary and Animal Science Ethics Committee has approved this project. If you have any complaints or reservations about the ethical conduct of this project, you may contact the Committee through the Executive Officer:

Dr Raf Freire
School of Animal and Veterinary Sciences
Charles Sturt University
Tel: (02) 69334451

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome.

7.2 Appendix 2- Letter to plant

MLA has initiated a research program to support changes in meat inspection in the red-meat sector. The program focuses on the use of scientific evidence to identify and manage food safety risks related to meat inspection. The program is overseen by a steering group which includes experts from industry (AMIC), government (DAFF Biosecurity) and the research community.

One of the key areas identified by the steering group is the very high rate of adult cattle carcases condemned due to malignant neoplasia. A study on this topic is being conducted by Charles Sturt University with the aim of describing the number and types of malignant neoplasia in adult cattle condemned at slaughter. The project leaders (Dr. Jane Heller/A/Professor Shane Raidal) have developed a questionnaire to collect information such as: number of lesions per carcass, location of lesions, gross characteristics etc. Specimens eventually taken from carcasses will allow histological classification. This data will help identify opportunities for reducing the losses due to malignant neoplasia.

MLA, DAFF Biosecurity and the research team would greatly appreciate your assistance in collecting specimens and data for this project. Your cooperation will help develop a more scientific and "risk based" approach to meat inspection aimed at increasing the yield and safety of red-meat products.

Importantly, the day-to-day management of neoplasia in carcases will not change while this project is running. Cooperating on-plant veterinarians will continue to make dispositions as per their normal practice.

Please find attached the questionnaire which requires your attention. If you have any technical questions regarding the project please feel free to contact Shane Raidal.

Kind Regards

A/Professor Shane Raidal (Associate Professor in Veterinary Pathobiology)
Shraidal@csu.edu.au
Phone: 02 69334450

7.3 Appendix 3- Consent On Plant Veterinarian

Charles Sturt University

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Australia

Tel & Fax: +61 2 6933 4107
Email: nsangster@csu.edu.au
Web: www.csu.edu.au/vet/

Consent form – On Plant Veterinarians

Project title: Condemnation of cattle carcases at meat inspection due to malignant neoplasia

Principal Investigators:

Dr Jane Heller (Senior Lecturer in Veterinary Epidemiology and Public Health)
Phone: 02 69332839 Email: jheller@csu.edu.au

A/Professor Shane Raidal (Associate Professor in Veterinary Pathobiology)
Phone: 02 69334450 Email: Shraidal@csu.edu.au

I consent to participating in the project entitled "Condemnation of cattle carcases at meat inspection due to malignant neoplasia".

I understand that I am free to withdraw my participation in the research at any time, and that if I do I will not be subjected to any penalty or discriminatory treatment.

The purpose of the research has been explained to me and (I have read and understood the information sheet given to me). I understand that any information or personal details gathered in the course of this research about me are confidential and that neither my name nor any other identifying information will be used or published without my written permission.

The School of Animal and Veterinary Sciences Ethics Committee has approved this study.

I understand that if I have any complaints or concerns about this research I can contact:

Dr Raf Freire
School of Animal and Veterinary Sciences
Charles Sturt University
Tel: (02) 69334451

Signed by: .....

Name and Role:.....

Date: .....

7.4 Appendix 4- Consent Plant Management

Charles Sturt University

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Email: nsangster@csu.edu.au
Web: www.csu.edu.au/vet/

Consent form – On Plant Management

Project title: Condemnation of cattle carcases at meat inspection due to malignant neoplasia

Principal Investigators:

Dr Jane Heller (Senior Lecturer in Veterinary Epidemiology and Public Health)
Phone: 02 69332839 Email: jheller@csu.edu.au

A/Professor Shane Raidal (Associate Professor in Veterinary Pathobiology)
Phone: 02 69334450 Email: Shraidal@csu.edu.au

I consent to participating in the project entitled "Condemnation of cattle carcases at meat inspection due to malignant neoplasia".

I understand that I am free to withdraw my participation in the research at any time, and that if I do I will not be subjected to any penalty or discriminatory treatment.

The purpose of the research has been explained to me and (I have read and understood the information sheet given to me). I understand that any information or personal details gathered in the course of this research about me are confidential and that neither my name nor any other identifying information will be used or published without my written permission.

The School of Animal and Veterinary Sciences Ethics Committee has approved this study.

I understand that if I have any complaints or concerns about this research I can contact:

Dr Raf Freire
School of Animal and Veterinary Sciences
Charles Sturt University
Tel: (02) 69334451

Signed by: .....

Name and Role:.....

Date: .....

7.5 Appendix 5 - Questionnaire

Sample Number (Please attach sticker)

A STUDY OF CARCASE CONDEMNATION DUE TO MALIGNANT NEOPLASIA

Dr Jane Heller, A/Professor Shane Raidal, Dr Allan Kessell and
A/Professor John Boulton

PLEASE DO NOT SAMPLE OCULAR LESIONS

For each carcase condemned for malignancy, please sample the lesions within the carcase as follows:

a) If ONLY ONE lesion is detected

- obtain **one** sample from that lesion **(identify as "Lesion A")**

b) If MORE THAN ONE lesion is detected but they are confined to one tissue and they are all with similar gross characteristics

- sample the **two** largest lesions **(identify as "Lesion A" and "Lesion B")**

c) If MORE THAN ONE lesion is detected with lesions present in MORE THAN ONE tissue OR lesions with dissimilar characteristics

- sample the **two** largest lesions in the most seriously affected tissue **(identify as "Lesion A" and "Lesion B")**

AND

- in the other tissues, sample the largest lesion in each tissue to a maximum of **two** other tissues (i.e. max four lesions sampled)
(identify as "Lesion C" and "Lesion D")

- **Please follow the labelling protocol as described in Instruction Sheet 2.**

Section A: General Information

Plant Information:
Date (dd/mm/yy): _____
Plant Name: _____
OPV Name: _____

Carcase Information:
NLIS number: _____
Breed: _____

Gender (please circle):

Male Entire Male Castrate Female

Weight:

_____ kg

Condition score (please circle):

1 2 3 4 5

Age (estimated from incisors): <2years 2 – 2 ½ years 2 ½ - 3 ½ years 3 ½ - 4 ½ years 5 - 6years >6years

Section B: Lesion Information

Q1a. How many neoplastic lesions have been detected in this carcase? (please circle):

1 2 3 4 > 4

Q1b. How many distinct organs are affected?

1 2 3 4 > 4

From here in, the sampled lesions will be referred to as "Lesion A", "Lesion B" and so on, as identified on the title page.

Q2. Where is each neoplastic lesion located?

	Lesion A	Lesion B	Lesion C	Lesion D
Location	_____	_____	_____	_____

Comments:

Q 3. Characteristics of lesions (please respond for each lesion)

	Lesion A	Lesion B	Lesion C	Lesion D
a) What is the **size** of each lesion (diameter) at its greatest width?				
- < 3 cm	☐	☐	☐	☐
- 3 – 10 cm	☐	☐	☐	☐
- >10 cm	☐	☐	☐	☐
b) What best describes the **surface texture** of each lesion?				
- Smooth	☐	☐	☐	☐
- Nodular / Irregular	☐	☐	☐	☐
c) What is the depth of **colour** of each lesion?				
- Please use a **scale of 1 to 5**, where 1 is the palest	_____	_____	_____	_____

possible, 5 is the darkest possible colour within normal tissue.				
d) What is the **consistency** of each lesion?				
- Please use a **scale of 1 to 5**, where 1 is the softest possible, 5 is the firmest possible consistency.	_____	_____	_____	_____
e) What are the **margins** of each lesion?				
- Clearly demarcated	☐	☐	☐	☐
- Diffuse	☐	☐	☐	☐

Section C: Lesion Classification

Q4. Malignancy (please respond for each lesion):

	Lesion A	**Lesion B**	**Lesion C**	**Lesion D**
a) Please identify whether you would classify each lesion as **benign, primary malignant or metastasis**				
- Benign	☐	☐	☐	☐
- Primary malignant	☐	☐	☐	☐
- Metastasis	☐	☐	☐	☐
b) How **confident** are you in the above classification				
Please use a **scale of 1 to 10** where 1 is minimum possible and 10 is maximum possible confidence	_____	_____	_____	_____

Comments

i) Q5. i) For each lesion, please identify;

a) Whether each feature is present or absent

b) Whether the presence or absence of each feature influenced your decision to classify the lesion as benign or malignant.

	Lesion A		**Lesion B**		**Lesion C**		**Lesion D**	
	Feature present?	**Influenced decision?**	**Feature present?**	**Influenced decision?**	**Feature present?**	**Influenced decision?**	**Feature present?**	**Influenced decision?**
The lesion is a single lesion	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
The lesion is one of multiple lesions of similar size	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
The lesion is one of multiple lesions of varying size	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
The lesion had evidence of tracking along vessels / lymph node involvement	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
The lesion is homogeneous in colour	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
The colour of the lesion was the same as surrounding normal tissue	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
The lesion has evidence of necrosis	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N

ii) For each lesion, please identify;

	Lesion A	**Lesion B**	**Lesion C**	**Lesion D**
Did the characteristics of the margins of this lesion influence your decision to classify it as benign or malignant?	☐**Yes** **No**☐	☐**Yes** **No**☐	☐**Yes** **No**☐	☐**Yes** **No**☐
Did the location of this lesion influence your decision to classify is as benign or malignant?	☐**Yes** **No**☐	☐**Yes** **No**☐	☐**Yes** **No**☐	☐**Yes** **No**☐
Did the size of this lesion influence your decision to classify it as benign or malignant?	☐**Yes** **No**☐	☐**Yes** **No**☐	☐**Yes** **No**☐	☐**Yes** **No**☐
Other, please describe_____	☐**Yes** **No**☐	☐**Yes** **No**☐	☐**Yes** **No**☐	☐**Yes** **No**☐

Q6. Diagnosis (please respond for each lesion):

	Lesion A	Lesion B	Lesion C	Lesion D
a) Please identify your suspected **diagnosis** for each lesion.	_____	_____	_____	_____
b) How **confident** are you in the above classification? -Please use a **scale of 1 to 10** where 1 is minimum possible and 10 is maximum possible confidence	_____	_____	_____	_____

Comments:

Please include any extra information that may not have been captured by the above questions regarding each of the lesions:

Lesion A

Lesion B

Lesion C

Lesion D

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7.6 Appendix 6- Prevalence Tally Sheet

Disposition summary

Date (dd/mm/yy)	NLIS	Age	Neoplasia		Sampled ☑
			Organ	Type	
					☐
					☐
					☐
					☐
					☐
					☐
					☐
					☐
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					☐
					☐
					☐

7.7 Appendix 7- Plant Instructions

Instruction Sheet 1: Sampling

You have been provided with a video demonstration of techniques for Lesion Sampling. This can be accessed on the iPod by opening the Video  on the screen. (Note that the video also contains demonstrations of Fine Needle Aspiration and Impression Smears which are not required)

PLEASE DO NOT SAMPLE OCULAR LESIONS

For each carcase condemned for malignancy, please sample the lesions within the carcase as follows:

d) If ONLY ONE lesion is detected

- obtain one sample from that lesion (identify as “Lesion A”)

e) If MORE THAN ONE lesion is detected but they are confined to one tissue and they are all with similar gross characteristics

- sample the two largest lesions (identify as “Lesion A” and “Lesion B”)

f) If MORE THAN ONE lesion is detected with lesions present in MORE THAN ONE tissue OR lesions with dissimilar characteristics

- sample the two largest lesions in the most seriously affected tissue (identify as “Lesion A” and “Lesion B”)

  AND

- in the other tissues, sample the largest lesion in each tissue to a maximum of two other tissues (i.e. max four lesions sampled) (identify as “Lesion C” and “Lesion D”)

Cut a representative slice of the lesion. The slice should be no more than 4 cm x 5 cm (or of equivalent area), and 5 to 10 mm thick but no thicker.

Place the slice in at least 10-times its volume of formalin, in a plastic pot that has been provided.

Seal the pot, then agitate it to ensure that the slice is not stuck to the plastic.

Ensure that all samples have at least 2 full days to fix in the formalin.

Instruction sheet 2: Labelling, photographing, packaging and transporting of samples

Sample ID e.g. M1a
Plant Identifier: An assigned letter eg M
Carcase number: Project specific identifier that is assigned to each carcase that is sampled. i.e. 1-50.
Lesion identifier: Up to 4 lesions can be sampled for each carcase. Lower case a, b, c, d. (refer to Instruction Sheet 1 ; Sampling for further information)

Labelling

Using the labels provided, place a label on each sample pot indicating Plant Identifier, Carcase Number, Lesion Identifier. The example below shows the series of labels for one carcase with up to 4 lesions sampled. If only one lesion is sampled, you will only need to use 1 label etc.

M1a	M1b	M1c	M1d

Photographing lesion:

Place the corresponding labelled empty pot adjacent to the entire lesion prior to sampling. Using the iPod camera  take a photo of the lesion ensuring that the sample ID is clearly visible. The photos will be downloaded at the completion of the project.

Sampling and Packaging

- When sufficient samples have been collected for transportation, pour the formalin from each pot into a chemical container. Please wear appropriate personal protective equipment when working with formalin.
- Using forceps that have been provided remove the slice from the pot and wrap it in gauze. Replace it in the pot.
- Pour sufficient formalin into the pot to just soak the gauze. Add more dry gauze to the pot to absorb all the formalin.
- Seal each pot firmly, ensuring that the seal is taped with the black tape provided.
- Place each pot into a press-seal plastic bag, exclude air, and ensure that the seal is pressed tightly.
- Place all pots into a large press-seal plastic bag.
- Place the large bag in the cardboard box. Place a layer of shredded paper in the bottom of the box. Attach THIS SIDE UP labels at the appropriate location. Pack the bag with sufficient shredded paper to absorb leakage and cushion the pots. Exclude air from the bag and press its seal tightly.

At the completion of the project I will make arrangements for the safe disposal of the formalin.

Transporting

You have initially been provided with 2 TOLL Priority Consignment notes. The return address details have been completed but we have left the senders details and size and item description sections for you to complete as these may vary depending on the content of the package.

Instruction sheet 3 Questionnaire and prevalence information

Questionnaire

For each carcase sampled please complete one questionnaire. Affix a carcase ID label (example below) on the front of the questionnaire; ensuring that this ID matches the carcase number matches the number on the Sample ID. In this example it is carcase 1. The questionnaire has provision for information on 4 lesions per carcase to be entered.

M1

At the completion of the questionnaire, please place into provided pre addressed and pre paid envelope and post to:

XXX
School of Animal and Veterinary Sciences
Charles Sturt University
Locked Bag 588
Wagga Wagga NSW 2678

Prevalence information

Complete the disposition summary and return in envelope with questionnaires when page complete.

7.8 Appendix 8 - iSURVEY instructions

How to complete the questionnaire on iPod

1. Locate the iSURVEY app  on the iPod screen

2. Press on the iSURVEY app to open.

3. Press “Start” to commence questionnaire.

NOTE- The next group of questions must be answered and the questionnaire will not move to the next page if they are not answered.
NOTE- you must press the next button at top of screen to move to the next page of the questionnaire.

4. “Select date” will display. Gently roll calendar to today’s date. Press next button at top. If the next page does not display, gently roll the date again and then press the next button.

5. The “Plant Identifier” is the first letter of the location of the plant; eg T (for Tongala) would be entered.

6. The Carcase Number is the project identifier that is assigned to each carcase that has been sampled. i.e. 1-50. This will link the survey to the pathology samples.

7. The “OPV initials” is the first and surname initials of the OPV conducting the sampling/questionnaire; eg BN or the initials of other OPV on site conducting sampling would be entered.

   NOTE- if there are two OPV’s with identical initials, the first, middle and surname initials will need to be entered. i.e. BHN

This completes the “must be answered “group of questions.

8. Continue through the questions until you complete the questionnaire for each of the lesions that have been sampled (up to 4 lesions).

   NOTE- The question asking for a Benign or Malignant classification must be answered as the questionnaire moves to different sections depending on the response entered.
   NOTE - If you have to leave the questionnaire unfinished for any reason (eg between samples within the one carcase) you will be prompted on return to complete the unfinished questionnaire. Tap Yes to resume the questionnaire. If you tap No at this point, any data previously entered will be deleted. This function will still work if you turn the iPod off in the middle of completing a questionnaire.

9. When you have completed the questionnaire the screen will ask you to “Touch to finish”. This concludes the questionnaire.

How to Upload Results
The iPod will automatically upload the completed questionnaires. To do this, the iPod needs to have a Wi-Fi connection.

Checking for Wi-Fi

1. To check if there is a connection, go back to the start screen of the iPod and press  on the settings icon.

2. Check to see if the network/Wi-Fi is available by tapping the  Wi-Fi icon. Ensure that it is “ON”. If “OFF” is displayed, tap it until it changes to “ON”.

3. Choose from the available networks listed there. Note: It may take a few seconds for the Wi-Fi network name to appear if there. You may be asked to enter a password to join the network. There will be a tick next to the network that you are connected to.

NOTE: if there are no available Wi-Fi networks you will not be able to upload the results. If this is the case, we will upload all of the questionnaires when the iPod is returned to us at the completion of the project.

7.9 Appendix 9- Agreement Study response template

Case No.	Short Description (please circle)	Diagnosis (please circle)	Confidence (0 – 100%)	Time taken (min)
	Malignant / Benign Other (please identify) ______________________ ______________________	Round / Spindle / Epithelial Other (please identify) ______________________ **Specific diagnosis** ______________________		
	Malignant / Benign Other (please identify) ______________________ ______________________ ______________________	Round / Spindle / Epithelial Other (please identify) ______________________ **Specific diagnosis** ______________________		
	Malignant / Benign Other (please identify) ______________________ ______________________ ______________________	Round / Spindle / Epithelial Other (please identify) ______________________ **Specific diagnosis** ______________________		
	Malignant / Benign Other (please identify) ______________________ ______________________ ______________________	Round / Spindle / Epithelial Other (please identify) ______________________ **Specific diagnosis** ______________________		
	Malignant / Benign Other (please identify) ______________________ ______________________ ______________________	Round / Spindle / Epithelial Other (please identify) ______________________ **Specific diagnosis** ______________________		

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