Effects of Repeated Use and Resterilization on Structural and Functional Integrity of Microwave Ablation Antennas.

&

Development of a Protocol for Microwave Ablation of Normal Canine Long Bones and Bones with Osteosarcoma

By

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A Thesis
Presented to
The University of Guelph

In partial fulfilment of requirements for the degree of Doctor of Veterinary Science in Clinical Studies

Guelph, Ontario, Canada

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ABSTRACT

Effects of Repeated Use and Resterilization on Structural and Functional Integrity of Microwave Ablation Antennas & Development of a Protocol for Microwave Ablation of Normal Canine Long Bones and Bones with Osteosarcoma

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Microwave ablation is a palliative or curative treatment for neoplasms in various organs in human medicine. This thesis is an investigation of the use of microwave ablation in veterinary medicine. In the first phase of the project, the goal was to determine effects of repeated use and resterilization on the structural and functional integrity of microwave ablation (MWA) antennas. Ablations were performed in livers of bovine cadavers at the maximum recommended settings. Antennas were cleaned, sterilized in hydrogen peroxide plasma, and the process was repeated (reprocessing cycle; n = 6). Aerobic and anaerobic bacterial cultures were performed, and antennas were assessed for damage with microscopy. The results showed that the structural and functional integrity of the microwave antennas remained acceptable during repeated use and reprocessing for up to 4 cycles. However, there was a decrease in functional integrity at cycles 5 and 6. It is
suggested that these microwave antennas be subjected to \( \leq 3 \) reprocessing cycles. This will decrease the cost of the technique in veterinary medicine. Antennas should be carefully examined before reuse.

The objectives of the second phase of the project were to describe a technique to perform MWA in long bones of normal dogs, and develop a protocol for MWA zone sizes in normal canine bone at different settings. An additional goal was to compare MWA zones in normal bones to those with osteosarcoma (OSA). MWA at 4 different settings were randomized in the proximal and distal metaphyses of femurs and tibias from normal dog cadavers. In 3 cadaver OSA bones, ablations were performed at the maximum previous setting. A MWA technique was successfully described in normal canine long bones. For the settings tested, it was not possible to build a rigorous chart of ablation zone sizes. This highlights the importance of monitoring the ablation with diagnostic imaging techniques such as computed tomography (CT). The MWA technique was applied to bones with OSA. Some ablations were difficult to delineate; overall the ablation zone sizes were slightly larger than in normal bones. Further work is needed to fully compare the ablation zone sizes obtained to normal bone.
Acknowledgements

I would like to express my deepest gratitude to Dr zur Linden for his continual support, advice and guidance over the course of this project, the completion of which would not have been possible without his patience and dedication. I thank him for his invaluable mentorship throughout my residency and count myself lucky to have had him as my advisor.

Many thanks to my co-advisor, Dr Nykamp, and committee members, Dr Foster and Dr Singh, for all their pertinent recommendations on this project, insightful guidance and valuable corrections.

I would like to thank Dr Culp who accepted the responsibilities of external examiner on the committee at my DVSc defense as well as Dr Oblak for the additional help as member of the examination committee.

I wish to thank Andrew Moore for graciously accepting our use of the stereo light microscopy in the Laboratory Services Division of the Agriculture and Food Laboratory at the University of Guelph, for always being approachable, and for making himself available on the multiple occasions required to complete the project. I would also like to thank the technician staff of the Diagnostic Imaging Department at OVC for their availability and help. Additionally, I thank Anthony Arrigo from Spectra Medical Devices (Wilmington, MA, USA) for the generous donation of the trocars used in the second phase of this project.
This work would not have been possible without the financial support of the Ontario Veterinary College Pet Trust.

Finally, I would like to thank my family wholeheartedly for their uninterrupted support throughout my education, never fading in spite of the geographic distance, as well as Jordyn for his patience and encouragements through the busiest of times.
Declaration of work performed

I declare that all the work presented in this thesis was performed by myself, except for that acknowledged below.

Dr Alex zur Linden helped perform the experiments of ablation in bovine cadaver livers, and of ablation in normal canine bones and those with osteosarcoma.

Dr Durda Slavic was responsible for the interpretation of the cultures performed during the first phase of the project. The culture samples were processed by the technicians at the Animal Health Laboratory at the University of Guelph.

Dr Robert Foster performed the histopathology studies and interpretations of the canine osteosarcoma bones used in the second phase of the project.

Bruce Cornfield performed the sawing of the canine bones in the post-mortem room at the Animal Health Laboratory, University of Guelph.

William Sears, statistician at the Ontario Veterinary College, University of Guelph, performed the statistical modeling and analyses.
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List of abbreviations

CI – Confidence interval
CFU – Colony-forming unit
CT – Computed tomography
MRI – Magnetic resonance imaging
MWA - Microwave ablation
OSA – Osteosarcoma
PET – Positron emission tomography
RFA - Radiofrequency
ROI - Region of interest
WL – window level
WW – window width
Chapter I: Introduction and literature review.

1.1 General introduction

Cancer is the leading cause of death in veterinary patients, with an increasing prevalence in older patients and some breeds of dogs such as Bernese Mountain Dog, Golden Retriever, Scottish Terrier, Bouvier des Flandres, and Boxer (Fleming, 2011). Pet owners are increasingly motivated to pursue curative or palliative oncologic treatments in order to improve and extend the life of their companion. If possible, surgery is employed, because it often leads to the best local tumor control, and to curative results (Culp, 2016). Chemotherapy and radiation therapy are also widely used, and these techniques are combined with surgery for better outcomes (Culp, 2016). However, some diseases are not amenable to surgery, and the presence of metastases usually makes the prognosis worse and may negatively influence owners’ decision regarding the treatment to implement. Intravenous chemotherapy can be effective for some macroscopic diseases, however it is uncommon to see a durable response (Weisse, 2015). Also, chemotherapy is costly, and requires frequent visits to the hospital for drug administration. Tumor resistance to treatment occurs with chemotherapy and with radiation therapy (Weisse, 2015; Culp, 2016). Because of these therapeutic challenges, there is a need for new and more effective technologies. In recent years, several new therapeutic options have emerged to expand the tools that can be used against cancer in veterinary patients, and further improve prognosis. The use of minimally invasive surgery is rapidly increasing in veterinary medicine (Bleedorn, 2013), including the implementation of minimally invasive techniques in oncologic surgery (Mayhew, 2014). Interventional radiology combines diagnostic imaging techniques and minimally invasive local delivery techniques of medications or devices (Weisse, 2015). These techniques are employed as curative or palliative treatments, or to reduce tumor size to become surgical candidates. These regional techniques are
inspired from what is done in human medicine. Often, local tumoral effects, rather than systemic effects, will lead to death. For example, life threatening lower urinary tract obstruction can occur secondary to a transitional cell carcinoma involving the urinary bladder trigone or urethra. To decrease the incidence of this, interventional radiology techniques have been described. Palliative stenting for example relieves malignant obstructions (Weisse, 2015; Culp, 2016). Intra-arterial chemotherapy is dedicated to specific tumor types that are not surgically resectable, but that are sensitive to certain chemotherapeutic agents, allowing local delivery of high concentrations of chemotherapeutic drugs to the tumor. The specific catheterization of arteries feeding the tumor minimizes systemic effects of the drug (Weisse, 2015; Culp, 2016). Trans-catheter arterial embolization is performed via injection of small particles in the tumoral capillary bed to obstruct blood flow, leading to local ischemia and tumoral necrosis (Weisse, 2015; Culp, 2016). Chemoembolization involves super-selective intra-arterial chemotherapy delivery followed by particle embolization (Weisse, 2015; Culp, 2016). Percutaneous tumor ablation techniques are amongst the most recently developed techniques; their applications in human medicine keep expanding fast, and they are just emerging in veterinary medicine (Culp, 2016).

Thermal ablation is an image-guided minimally invasive technology, which has been used in humans for the past two decades, for palliative or curative treatment of malignancies (Bhardwaj, 2010 a; Webb, 2011; Hinshaw, 2014). It reduces overall costs, morbidity and mortality compared to standard surgical procedures (Nishikawa, 2011; Tombesi, 2013). This technique is as effective but less invasive compared to surgical resection of small tumors (Ong, 2009; Nishikawa, 2011; Tombesi, 2013). Typically, thermal ablation relies on the percutaneous insertion of an electrode or antenna into the target tumor under imaging guidance (most often ultrasound or CT, more rarely MRI guidance), but it can also be performed with endoscopy (laparoscopy mostly, or thoracoscopy) or by open surgical access (Simon, 2005; Murakami, 2011; Simo 2011; Webb, 2011; Veltri, 2012; Sun, 2015; Zaidi, 2016). The goal of the application of thermal energy is the
induction of focal selective tissue necrosis at the site of the target tumor. Above a certain temperature threshold, irreversible cellular damage by coagulation necrosis is induced (Ahmed, 2011; Hinshaw, 2014). Two main categories of thermal ablation are described in the literature: those based on heat modalities and those based on tissue freezing techniques. The first category includes radiofrequency, microwave, high-intensity focused ultrasound, and laser ablation. The only freezing technique is cryoablation (Simon, 2005; Ahmed, 2011; Webb, 2011; Hinshaw, 2014). Cryoablation will not be further considered in the manuscript because the technique is cost prohibitive, has restricted uses, and potential systemic complications that are not seen with heat based ablation modalities (Bagia, 1998).

Because of the promising results shown in human medicine with thermal ablation, this novel technique merits evaluation in animals. In human medicine, hospitals generally have one (or maximum two) thermal ablation techniques available, mainly because the generator units are costly, and different training is required for each technique. The focus of this research is on the microwave ablation technique. This work investigates the possibility to resterilize and reuse microwave ablation antennas safely, in order to decrease the cost of use of this technique in veterinary patients. Given the paucity of data regarding microwave ablation in veterinary medicine, preliminary data must be obtained before putting this technique into clinical practice. This work will evaluate a technique to perform microwave ablation in long bones of normal dogs, and its potential application to diseased bones with neoplasia.
1.2 Thermal ablation

1.2.1 Mechanisms and physics of local tissue heating in thermal ablation

General principles of tissue heating in thermal ablation rely on two phases. First, the thermal ablation device actively induces heat in the tissue focally around the applicator (antenna, electrode, laser fiber). The type of energy delivered and type of interaction with the tissue varies depending on the system. With microwave technology, electromagnetic waves are generated and applied via the antenna to a target tissue. Heat is then generated by two mechanisms. The main one is dielectric hysteresis, or polarization, produced by microwaves that affects dipole molecules, such as water (Clark, 2013). These molecules have an unequally distributed electrical charge, and when submitted to an external electromagnetic field, they continuously attempt to reorient and realign with the variations in charge produced within this oscillating electromagnetic field by rotating and oscillating extremely rapidly (Veltri, 2012). This agitation of water molecules produces friction and heat in the surrounding tissue, leading to coagulation necrosis. A minor mechanism is ionic polarization, causing collision of ions, which converts kinetic energy into heat (Simon, 2005; Ahmed, 2011; Liang, 2013). In tissues with high water content (most solid organs and tumors), the absorption of electromagnetic energy and heat production is higher than in tissues with low water content (fat) (Puentes, 2012; Clark, 2013).

Second, the heat generated diffuses through the tumor via thermal tissue conduction. This additional high tissue heating is different from the direct heating effects generated around the applicator. This effect is dependent on the type of device used and on the intrinsic characteristics of the tissue targeted. For instance, primary hepatic tumors such as hepatocellular carcinoma have a better conduction than a cirrhotic hepatic parenchyma (Ahmed, 2011). It has also been shown that the temperature at which cell death occurs depends on the type of tissue. Therefore, the
thermal dose (total amount of heat administered for a given time) needed to reach cellular death varies between tissues (Ahmed, 2011). This is why the accepted standard threshold temperature of 50°C above which coagulation necrosis occurs must generally be exceeded. Not only is thermal dosimetry dependent on the type and amount of energy applied, but also on distance, and there is a need to take into account the rate of heat transfer within a tissue when planning a target ablation (Mertyna, 2009).

Thermal ablation is a good choice of treatment of tumors because the dielectric properties of cancer cells are 10 to 30% higher than healthy tissues, and the water content is also different, which allows the cancer cells to be heated more easily than normal tissues (Puentes, 2012).

### 1.2.2 Overview of different techniques of thermal ablation

Radiofrequency ablation is the most used and described thermal ablation technique in humans. It is based on the application of a high frequency alternating current through an electrode introduced in the target tissue zone. The current travels from the electrode to a grounding pad applied directly on the skin. This technique generates ionic agitation and focal heating. Additional thermal diffusion may lead to further expansion of the ablation zone (Schramm, 2007). The temperature can reach up to 100°C with radiofrequency ablation (Ahmed, 2011). Two drawbacks to radiofrequency have been recently highlighted: higher temperatures cannot be reached because there is an increase in the impedance of the tissue during the ablation, which further limits the application of additional current, and ablation zone size and shape can vary depending on the surrounding environment of the target lesion (Liu, 2006; Ahmed, 2008).

High intensity focused ultrasound uses high power, highly focused ultrasound beams targeted to a specific point in the body to induce tissue vibration
and heat. Usually when the focusing is adequate, temperatures above 50°C are
reached in the target tissue. However, this technique is currently not routinely
employed in humans, and additional research is needed to further investigate its
potential clinical applications (Webb, 2011).

For laser ablation, an optic laser fiber is introduced in the target tissue, and
conduction of photon energy leads to the production of heat, above 50°C.

Microwave ablation employs an antenna inserted in the target tissue, which
produces electromagnetic waves. This generates tissue heating by dipole molecular
rotation and ionic polarization (detailed above). This phenomenon quickly increases
kinetic energy and tissue temperature (Hinshaw, 2014). Higher temperatures (up to
140-150°C) are reached more quickly compared to radiofrequency ablation. The
ablation zone is also usually larger, more regular (less affected by the proximity of
large vessels for example, as explained below) and tissue necrosis can be reached in
tissues with higher impedances to electric current, such as lung, bone or dessicated
tissue (Webb, 2011). Solid organ tumors in dogs are often found late in the course of
disease, and larger ablation zones make MWA favorable over other thermal ablation
methods. The lack of grounding pads needed for MWA, and the ability to ablate
tumors in high impedance tissues such as bone and lung gives MWA the greatest
potential for successful use in veterinary medicine.

1.2.3 Advantages of microwave ablation

Microwave ablation techniques present several advantages compared to the
other frequently used thermal ablation radiofrequency technique. Higher intra-
tumoral temperatures are reached, and the increase in temperature is quicker,
reducing ablation times (Clark, 2013; Hinshaw, 2014). Microwaves are not limited
by an increase in impedance of the tissues, which at a certain temperature prevent
electrical waves from propagating. Therefore, temperatures higher than 100°C (up
to 140-150°C) can be achieved and microwaves can theoretically be used in tissues with high impedance and poor conductivity, such as lung or bone (Clark, 2013; Hinshaw, 2014). Microwave energy penetrates deeper, leading to a larger tumoral ablation volume (Awad, 2007; Tombesi, 2013). Complete histological necrosis of lesions up to 5cm in diameter have been achieved (Zanus, 2011). It has been shown that microwave heating leads to uniform coagulative necrosis, with a uniform absence of viable tumor in the ablation zone and a clear demarcation zone between ablated and non-ablated tissue (Swift, 2003; Simon, 2005; Bhardwaj, 2009; Garrean, 2009; Simo, 2011). In a clinical setting, all of these properties lead to less applicators needed per ablation site, and the ablation margins are more easily obtained in comparison with radiofrequency (Hinshaw, 2014). It is also more efficient on cystic structures (Simon, 2005).

Microwave energy is less affected by the heat-sink effect, which particularly decreases the efficiency of radiofrequency ablation (Simon, 2005; Awad, 2007; Garrean, 2009; Ahmed, 2011; Clark, 2013). The heat sink effect makes reference to the fact that blood flow draws heat from the ablation zone, and that blood perfusion itself cools the tissues. It has been shown that microwave ablation is efficient in close proximity to large vessels (>3mm in diameter) (Clark, 2013). An explanation is that active heating is more developed in microwave energy, and is less affected by the cooling mediated by the blood vessels via conduction. The induction of proinflammatory cytokines with microwave ablation is also minimal compared to that with radiofrequency ablation (Kang, 2015). Simultaneous application of multiple antennas can be used to increase the size of the ablation zone and decrease the exposure time. Because of the inherent properties of the electromagnetic waves, no grounding pad is necessary, which reduces the risk of burns and is an advantage in animals since no additional hair clipping is required (Simon, 2005). Microwave ablation can be used in patients with pacemakers and metallic implants, contrary to radiofrequency for instance, which is contraindicated in patients with surgical hardware because of the risk of conducting current through it (Nazario, 2011; Liang, 2013). Furthermore, the development of cooled-shaft MWA antennas has allowed higher output and longer duration of ablation, thus further decreasing the risk of
skin burns (Zhang, 2015). For these reasons, MWA is growing in use for human thermal ablation of cancers in various organs, and why the authors selected MWA for their research, over another thermal ablation technique.

1.2.4 Disadvantages of microwave ablation

Because of the relative novelty of microwave ablation in human medicine, in spite of the numerous publications, clinical data and experience are still considered small. Also, systems from different manufacturers are different (varying antenna design, frequency, or cooling systems for example), and therefore it may be challenging to make a comparison between different systems' performance. As well, a learning curve is associated with microwave ablation use. In particular, safety must be paid attention to, because of the larger ablation zones obtained than with other modalities such as radiofrequency, that may potentially damage the surrounding structures. These limitations are amplified in veterinary medicine, due to the paucity of data currently available regarding microwave ablation.

An additional drawback that applies more specifically to veterinary medicine is that microwave ablation technology is expensive. The microwave generator purchased by the investigators (Sulis VpMTA generator system, Microsulis Medical Ltd, Hampshire, UK) prior to the research (in 2012) was about CAD $55000. Moreover, the cost of a microwave antenna is also quite expensive: one antenna for the above-mentioned unit is currently more than CAD $2000. Costs associated with the procedure seem prohibitive for veterinary medicine, and constitute a first obstacle to overcome. The ability to reuse these antennas in multiple patients would help reduce the cost per procedure. An overview of reprocessing techniques is exposed hereafter, in order to explain why the authors thought it would be possible to reprocess the antennas, and how they chose the reprocessing technique for the antennas.
1.2.5 Microwave ablation equipment

A microwave ablation system is composed of three elements: a generator, a low-loss coaxial flexible cable, and the antenna. The cable connects the antenna to the generator and transmits the electromagnetic waves produced in the generator by a magnetron (generates microwaves using the interaction of a stream of electrons with a magnetic field) or solid-state amplifier to the antenna, to the target tissue (Clark, 2013). Currently, most of the antennas are based on the needle-like model, thin and coaxial interstitial; interstitial meaning that it enables percutaneous use. Larger ablation zones are also obtained with this model (Pisa, 2001; Cavagnaro, 2011; Clark, 2013). Depending on their physical properties, there are different types of antennas that are associated with a different size and shape of the electromagnetic field, and therefore different shapes of ablation zones (Liang, 2013; Hinshaw 2014). The most commonly used antenna diameter is 14-16G (2.1-1.65 mm). Coaxial cables have excellent propagation characteristics, but are larger in diameter, more cumbersome, and more prone to heating than the simple wires used in radiofrequency ablation (Ward 2013). However, additional techniques such as cooled-shaft antennas (by circulation of chilled saline or compressed carbon dioxide) prevent over-heating of the antenna, allowing the use of higher output power and the creation of larger ablation zones (Liu, 2007; Ahmed, 2011; Clark, 2013). In humans, current microwave antennas are single usage instruments. The antenna used in this project can be used for up to 8 ablations in the same procedure for one patient, according to the recommendations given by a company representative.

Currently, several microwave ablation devices are available on the market (Hoffmann, 2013). Generally, one microwave antenna is used at a time, but some generator systems allow the use of up to three antennas, enabling large and spherical ablation zones (Hoffmann 2013). Two types of frequencies are approved: either 915 or 2450 MHz (Clark, 2013). Investigators have theorized that
microwaves of 915 MHz can penetrate more deeply and achieve higher peak temperatures than microwaves of 2450 MHz; the lower the frequency, the greater the penetration and therefore the larger the ablation zone (Sun, 2012). As a consequence, insertion of fewer antennas is needed with 915MHz microwave antennas to achieve the same ablation zone size (Hoffmann, 2013). More recently, other authors have noted that these previous observations were made without controlling for the power delivered to the tissue, and therefore may not be valid (Hinshaw, 2014). The same authors also declare that lower frequency microwaves do not penetrate deeper in the near field. However, they state that 915MHz antennas usually result in longer ablation zones. Therefore, uncertainties remain regarding direct comparisons between system performances.

Depending on the type of antenna and type of targeted tissue, slightly different settings of power and time of application are recommended. Output power and time of application can be individually set on the generator, independent of the tissue being ablated. An output from 60 to 180W and an application time of 5-10min are generally used. For example, to ablate liver lesions, the settings of the generator are usually set at an energy application of 50-80W for 5-10min sessions. The size of the ablation zone is dependent on the energy deposited, local tissue interactions and heat loss. In one study, lesion diameter was the only prognostic factor for obtaining complete ablation with microwave devices, with increased treatment failure for larger lesions (Veltri 2012). Overall, the final ablation zone size currently reached is 3 to 5cm in diameter using a single percutaneous electrode (Strickland 2002). Multiple overlapping thermal lesions can be employed to achieve the ablation of a larger tumor. The design of the microwave antenna is very important in determining the size and shape of the ablation lesion. Several types of antennas have been designed to control energy delivery, and several experimental studies in ex vivo organs have been performed to assess the size and shape of the thermal lesions induced by different types of microwave antennas (Awad, 2007; Cavagnaro 2001; Sun, 2012; Qi, 2012; Hoffmann 2013). Original models, especially the older first generation (without antenna cooling and therefore limited to low power and short
durations), tended to produce ablation zones with an elongated appearance, with the largest diameter along the axis of the applicator. More recent generations result in a more rounded ablation zone (Hinshaw, 2014). A more rounded shape of the ablation zone avoids undesired burns to adjacent structures such as the body wall, that is more likely to happen with an elongated ablation zone.

The system used in this study is the Sulis VpMTA generator (Microsulis Medical Ltd, Hampshire, UK), with the Accu2i pMTA applicator. The Sulis VpMTA unit combines the electrical power source and a 2.45 GHz microwave generator. The Accu2i pMTA applicator is a water-cooled 15G (1.8 mm) microwave antenna that is configured for percutaneous insertion, in addition to open and laparoscopic procedures. It creates a near spherical coagulation zone of up to 5.6 cm in 6 min at maximum power. The Acculis system employs two different temperature monitoring systems, one is built for internal feedback and the other comprises two temperature probes for tissue monitoring adjacent to the ablation zone. A study demonstrated low complication rates and a low local recurrence rate of only 2% with the Acculis microwave ablation, with a single treatment of 31 patients with 89 unresectable liver tumors (Bhardwaj, 2010 b).

1.2.5.2 Thermal monitoring system

As evoked above, microwave antennas can be coupled to a thermal monitoring system, to monitor in real time the tissue temperatures during the ablation. These temperatures are displayed in real time, during the ablation, on the generator unit. It is important to verify that temperatures reached within and at the margins of the ablation zone are adequate, and also that adjacent tissue temperatures remain below the range inducing irreversible damage. For that purpose, thermal monitoring needles (thermistors) are inserted in the tissue through a non-conducting needle trocar during the ablation (Liang 2013). One to three thermal monitoring needles are generally inserted at 5 to 10mm from the tumor margins.
Death from coagulation necrosis of the neoplastic tissue depends on the temperature applied within the lesion, and time of application. Around 40–45°C, irreversible cell damage occurs only after prolonged exposure (from 30 to 60 minutes). At higher temperatures, the time required to achieve irreversible damage decreases exponentially (Chu, 2014). Total tumor necrosis in the liver is obtained at 54°C maintained for at least 3 minutes (non-instantaneous thermal lesion). If 60 °C or above is reached instantaneously, rapid protein denaturation occurs, which is immediately cytotoxic and leads to coagulative necrosis (instantaneous thermal lesion) (Liang, 2013). The lesion boundary is usually characterized as an isothermal contour of 54°C +/- 0.5°C (Nan, 2010). Above that temperature, the changes linked to protein dehydration induced in the tissue are irreversible, even if the tissue is allowed to return to the initial temperature (Lopresto, 2012). For tumors considered high-risk, such as those less than 5mm away from vital tissues such as gallbladder, bile duct, blood vessels or gastrointestinal tract, the temperature of the tumor margins should not reach above 54°C. If a temperature of that value is detected, the emission of microwaves is stopped, and not resumed before the temperature decreases below 45°C (Liang, 2013).

1.3 Instrument reprocessing in veterinary and human medicine

As the cost of single use antennas may make microwave ablation cost prohibitive in veterinary medicine, it is necessary to determine whether the antennas can be reprocessed and reused safely. Cleaning and sterilization is essential to kill bacteria and other microorganisms, and eliminate bacterial residuals such as lipopolysaccharide complex (on the outer wall of gram negative bacteria). This avoids infections and secondary reactions such as fever, hypotension, changes in white cell count, or intravascular coagulation (Tessarolo, 2006; Boiano, 2015). Many reviews are available on disinfection and sterilization (Rutala, 2013; Barnden,
Steam sterilization is highly effective and environmentally friendly. Steam sterilization under pressure using an autoclave is the most common and cost-effective method of achieving instrument sterilization. However, the development of heat and/or moisture sensitive devices has prompted the expansion of other sterilization techniques, to prevent damage of these medical instruments. The first low temperature chemical method developed was based on ethylene oxide (Phillips, 1949). However, the sterilization method based on ethylene oxide requires strict health, safety and environmental regulations, and the processing times are long (Rutala, 2012; Boiano, 2015). The International Agency for Research on Cancer (IARC) has determined that ethylene oxide is carcinogenic to humans (WHO/IARC, 2012). More recent sterilization techniques that are safer and more efficient include hydrogen peroxide gas plasma, vaporized hydrogen peroxide, immersion and vapor phase peracetic acid (Rutala, 2012; Boiano, 2015), and more marginal techniques such as low-temperature steam formaldehyde (Kanemitsu, 2005). Usually, high throughput facilities have sterilization systems based on either steam, ethylene oxide, or hydrogen peroxide gas plasma (Boiano, 2015).

Instrument reprocessing involves steps prior to sterilization, such as cleaning and disinfection. Improper reprocessing can lead to contamination at any stage of the process (Smith, 2011; Funk, 2014). Inadequate cleaning can leave biomaterial on instruments that will not be removed by further steps of the reprocessing. If instruments are not soaked long enough in disinfectant, resistant particles such as *Staphylococcus aureus*, mycobacteria, bacterial spores, fungi, and some viruses (human immunodeficiency virus for example) may not be killed. Residual protein may promote the adhesion of bacteria via specific adhesion receptors, or inhibit the sterilization process (Amaha, 1954; Piroth, 2008). Moreover, not all cleaning agents inactivate prions (McDonnell, 2013). Residual moisture may also impair sterilization efficiency (Vessoni Penna, 1999). Therefore, reprocessing steps prior to sterilization must be followed rigorously.
The established Spaulding classification for patient-care items and equipment disinfection and sterilization distinguishes critical, semi-critical, and non-critical items according to the degree of risk for infection involved in use of the items (Rutala, 2012). Critical items are those contacting sterile tissues, such as surgical instruments. Semi-critical devices contact only mucous membranes (endoscopes eg.), and non-critical items contact only intact skin (stethoscopes eg.). Microwave ablation antennas should be in the critical class, because they enter in contact with sterile body tissues or fluids, and they would confer a high risk for infection if they are contaminated with microorganisms. Although microwaves may lead to organism death in the ablation zone, contamination may occur along the insertion path of the antenna in the body up to the target. Items in the critical category should be purchased as sterile or be sterilized with steam if possible, but heat-sensitive objects require a treatment with a low temperature technique such as hydrogen peroxide gas plasma or ethylene oxide (as well, peracetic acid) (Rutala, 2012).

In human medicine, reuse of medical or surgical devices after sterilization has been described, although subjected to controversy and legislative regulations (Cohoon, 2001). It can result in significant costs savings in emerging economies (Funk, 2014), as well as a reduction in the amount of waste that originates from single-use devices (Evangelista, 2015). A study showed that hydrogen peroxide plasma gas sterilization was efficient and safe for repeated use of electrophysiology catheters, for up to 5 uses. Visual inspection of the catheters by microscopy was suggested before each use though, because one catheter out of ten displayed fraying of the insulation at the insulation-electrode interface (Bathina, 1998). In that study, estimated cost savings per catheter amounted to $2000. There are however some concerns about reuse of single-use devices for minimally invasive surgery, because the material they are made of (rubber or plastic for example), is less robust than the material of reusable devices. This may lead to more difficult decontamination and sterilization, and also potentially to mechanical failure (Chan, 2000; Hamamci, 2002;
Roth, 2002; Yoon, 2012). Repetitive use of reusable devices, such as endoscopes, may increase the risk of infection, and would suggest a limited number of times that instruments can safely be reused (Lee, 2015). Reprocessing can also lead to mechanical damage of the devices. A study showed that reprocessing of arthroscopic shaver blades was associated with mechanical damage and contamination by chemicals (collagen, hydroxyapatite, and salts) of the blades (Kobayashi, 2009). In spite of this, there is evidence that reuse of devices (most often reusable devices) after sterilization is frequent and often efficient (Jung, 2003; Seavey, 2013; Evangelista, 2015). Moreover, in face of the technological evolution that brings more complicated medical and surgical devices on the market, published recommended practices on how to reprocess instruments is constantly being updated (Seavey, 2013).

In veterinary medicine, reuse of single use devices is very common. The main purpose of this is to lower the cost of otherwise very expensive devices by splitting the cost over several procedures. A recent article studied the efficacy of decontamination and sterilization of a single use laparoscopic surgery port (Coisman, 2013). Decontamination of the ports was performed with rinsing with tap water, soaking in an enzymatic cleaner, and brushing. Sterilization was performed with ethylene oxide at 50°C and >30% relative humidity. No positive bacteriologic culture was noted on any of the sterilized ports, therefore the procedure was deemed efficient. Another recent study has investigated the impact of three different sterilization techniques on the bioadhesive properties of nylon and polyethylene lines used for stabilization of canine stifle joints (Gatineau, 2012). Results showed that hydrogen peroxide gas plasma compared favorably with other techniques of sterilization such as ethylene oxide and steam.
1.3.1 Hydrogen peroxide plasma sterilization efficiency

Sterilization with hydrogen peroxide gas plasma is a recently developed low temperature sterilization technique. This technique of sterilization has proven to be a suitable alternative to ethylene oxide sterilization. It involves a synergistic reaction between hydrogen peroxide and low temperature gas plasma. Gas plasmas are generated in a closed chamber under vacuum after excitation of the gas molecules with energy (radiofrequency or microwave energy) to produce charged particles, many of which are free radicals. The proposed mechanism of action is that free radicals are able to interact with essential cell components and disrupt the metabolism of microorganisms. This is further enhanced by using hydrogen peroxide gas, which already possesses germicidal properties (Rutala, 2012). Hydrogen peroxide gas plasma is safer than ethylene oxide sterilization in the way that contact of the operator with liquid or plasma hydrogen peroxide is limited by the fact that the hydrogen peroxide is contained in a cassette-like device, that is opened only once the sterilizer door is closed (Boiano, 2015). This prevents inhalation or contact with the eyes or the skin, which are the primary ways of occupational exposure to hydrogen peroxide (Boiana, 2015). Additionally, the byproducts include water vapor and oxygen, which have no toxicity potential, and do not require methods of aeration for elimination.

In human medicine, several studies have demonstrated the efficiency of sterilization with hydrogen peroxide plasma. Hydrogen peroxide plasma gas sterilization is effective against both lipid and non-lipid viruses: a study showed that H1V type 1, human hepatitis A virus, respiratory syncytial virus, vaccinia, herpes simplex virus type 1, and poliovirus type 2 were inactivated with this type of sterilization (Roberts, 1998). It is also effective against Cryptosporidium parvum oocysts (Vassal, 1998). This method is also successful in sterilizing bronchoscopes contaminated with Mycobacterium tuberculosis (Bär, 2001). Prions can also be sterilized with hydrogen peroxide plasma (Rogez-Kreuz, 2009). A study has
investigated the efficiency of hydrogen peroxide plasma sterilization for electrophysiology catheters (Tessarolo, 2006). Prior to sterilization, the catheters were decontaminated by immersion in a chlorine solution, then rinsed under tap water, and immersed again in an enzymatic detergent. Manual brushing and wiping was then performed just prior to sterilization with hydrogen peroxide plasma. It was found that the sterilization technique led to a reduction in endotoxin contamination and safe reprocessing of single-use non-lumen electrophysiology and ablation cardiac catheters. In this study, a few catheters had endotoxin fragments after the cleaning and decontamination step, but none had such pyrogenic fragments after the sterilization with hydrogen peroxide plasma, therefore, the sterilization step was crucial in achieving appropriate decontamination of the devices. This has an advantage over other conventional sterilization techniques such as steam or low temperature sterilization techniques such as ethylene oxide, because the latter do not destroy the lipopolysaccharide and do not impair the pyrogenic potential of endotoxic fragments.

There are some limitations to sterilization with hydrogen peroxide plasma. One study compared three techniques of low temperature sterilization (ethylene oxide gas, hydrogen peroxide gas plasma, and low temperature steam formaldehyde sterilization) on items contaminated with *Bacillus stearothermophilus* spores, and found that plasma sterilization may be unsuccessful under certain conditions, especially for instruments with complex shapes and narrow lumens (Kanemitsu, 2005).

A recent veterinary study investigated the impact of three different sterilization techniques on the bioadhesive properties of nylon and polyethylene lines used for stabilization of canine stifle joints (Gatineau, 2012). The three sterilization methods included hydrogen peroxide gas plasma, ethylene oxide and steam. Bacterial adherence was tested with *Escherichia coli* and *Staphylococcus epidermidis*. Results showed that in most cases, bacterial adherence was similar or lower when hydrogen peroxide gas plasma was used, compared with ethylene oxide
and steam. Moreover, bacterial adherence (determined by bacterial cultures and number of CFUs) was higher after steam sterilization for almost all samples, which led the authors to state that steam sterilization appeared less suitable for polyethylene and nylon lines.

Because of the evidence regarding the efficiency of hydrogen peroxide plasma as a low temperature sterilization technique, in association with the fact that microwave ablation antennas are classified as critical items according to the Spaulding classification, and are heat sensitive (especially because of the silicone coating, and chip with electrical circuit present on the pumping chamber), in the first part of the project, the authors chose to perform the sterilization of the antennas with hydrogen peroxide plasma. This decision was reached in collaboration with the sterilization department at the authors’ institution. If antennas can be successfully reprocessed and reused, this may allow a cost-effective use of microwave ablation antennas.

1.4 Microwave ablation techniques

The antenna is usually inserted into the body through natural orifices and veins (intracavitary antennas), or percutaneously (interstitial antennas) (Cavagnaro, 2011). Image guidance can be performed under various modalities. Most of the time, percutaneous microwave ablation is performed under ultrasound guidance (Ahmed, 2011; Webb, 2011; Qi, 2012; Liang, 2013). Ultrasound allows real time visualization of the location of the lesions, monitoring the microwave antenna insertion into the target tissue, and following the progression of the necrosis, visible for some minutes as an area of transient hyperechogenicity, caused by bubbles produced during the ablation, exceeding the diameter of the tumoral lesion (Garrean, 2009; Veltri, 2012; Liang 2013). Contrast-enhanced ultrasonography can be used to better highlight unablated lesions, which enhance, contrary to ablated
lesions. Ultrasound can be also used for follow-up. This modality is very practical and likely the least expensive. However, the lesions cannot always be accurately located, especially deep ones, and/or the insertion of the antenna into the target cannot accurately be monitored. Computed tomography is another widely used modality to locate the lesion and guide the insertion of the antenna, and is the more generally used imaging modality in humans (Simon, 2005; Nazario, 2011). Magnetic resonance imaging is not routinely used but has been reported for guidance of percutaneous thermal microwave ablation of a locally recurrent prostate carcinoma in 5 patients (Chen, 2000). More recently, positron emission tomography (PET)/CT has been proposed as an advantageous modality because it combines anatomic and metabolic information (McLoney, 2014). This makes it a more accurate pre-ablation staging technique for patients with hepatic and pulmonary malignancies, and may also alter treatment decisions. Moreover, used during the procedure, it can allow better targeting of lesions that are difficult to see with other imaging techniques. Finally, PET/CT has proven to be more sensitive to detect residual or recurrent hepatic or pulmonary neoplastic lesions in the follow-up period (Mc Loney, 2014). Microwave ablation can also be performed under direct visualization, by laparoscopic or open surgical access (Ahmad, 2005; Simon, 2005; Strickland, 2005; Padma, 2009; Simo, 2011).

Taking the example of percutaneous ultrasound-guided microwave ablation of any abdominal organ, patients are placed in supine position, standard surgical preparation and draping are performed, and local anesthesia administered at the point of entrance of the skin (one or several depending on the number of antennae used) to the peritoneum, along the antenna tract (Simon, 2005). Some patients require light to heavy sedation for the procedure. The skin is pricked and the antenna inserted into the target tissue. If several antennas are used, they are inserted parallel to each other and placed from 1 to 2.5cm apart. Multiple thermal lesions can be created by successively withdrawing the antenna needle tip from the previous lesion, redirecting it and then re-applying an electromagnetic field. Multiple overlapping thermal lesions can also be performed for the ablation of a
large target. The treatment is prolonged until the desired temperature (monitored with thermistor needles) is reached. If overheating is detected, the treatment is stopped. In the liver, for curative treatments, ablation margins of apparently healthy tissue of 5 to 10mm are recommended, except in cirrhotic livers or if the lesion is adjacent to critical organs, where an ablation margin <5mm or a conformational ablation are performed (Liang, 2013). After the procedure, the site is covered with a sterile dressing and pressure applied to the puncture site. The immediate post-operative care is limited because patients usually stay in hospital for 4 to 6 hours of bed rest, observed for 1-3 additional days and then discharged if no complication is observed (Liang, 2013). For example, at Toronto General Hospital, patients are released from hospital the same day as the procedure, following a brief observation period.

The first follow up is recommended at 1 week post-procedure, and no later than 4 weeks. Then, subsequent follow-ups are recommended every 3 to 4 months. They involve repeated imaging examinations, such as contrast-enhanced CT, MRI or ultrasound. Sometimes, adjunctive blood tumor marker level tests are also required (Veltri, 2012). Complete response is considered achieved if no evidence of enhancement in the mass is visible on contrast-examination at 1 month post-procedure (Liang, 2013). During follow up the lesions normally progressively diminish in size and remain non-contrast enhancing. Diminution in size of the lesion can be explained by removal of dead cells from the ablation site, or by formation of fibrous tissue containing myofibroblasts around the dead cells, that induces contraction of the lesion.

1.4.1 Clinical applications of MWA in humans

There are currently only few reports of the uses of thermal ablation or more specifically microwave ablation in veterinary patients. However, thermal ablation has proven its usefulness for the ablation of malignant processes in several tissues
in humans, such as liver, kidney, lung, bone, breast, prostate, uterus, thyroid and other (Simon, 2005; Dupuy, 2009; Ahmed, 2012; Nazario, 2011; Veltri, 2012; Clark, 2013). Reviews of uses of different techniques of thermal ablation in different organs are available (Hinshaw, 2014). Results similar to classical surgical or laparoscopic techniques regarding local control, survival and complications rate have been demonstrated (Bhardwaj, 2010 b; Webb, 2011; Tombesi, 2013; Xu, 2015). Decreased morbidity with ablation compared with open surgery or laparoscopy has even been demonstrated by some authors (Nishikawa, 2011; Tombesi, 2013; Hinshaw, 2014). The techniques of thermal ablation have also gained popularity in the treatment of non-surgical candidates where a tumor may be in a poor location for resection, or as an adjuvant therapy to surgery for the ablation of small-sized metastases (Bhardwaj, 2010 b; Linecker, 2016; van Amerongen, 2016). Other advantages include synergy with other cancer treatments for unresectable neoplasia and repeatability of the procedure if there is incomplete ablation or further metastatic growth (Simon, 2005; Kang, 2015; Tang, 2016). Microwave ablation is more specifically used in certain organs listed below.

Liver - Microwave ablation is the second most frequent thermal ablation technique in the liver, just after radiofrequency. However, its use is gaining in popularity over radiofrequency for hepatic ablations in some institutions, because current microwave systems have advantages over radiofrequency (explained above), which leads to similar or superior clinical results (Hinshaw, 2014). It is used for the ablation of primary hepatic tumors such as hepatocellular cell carcinoma in patients with cirrhotic livers (Simon, 2005; Ong, 2009; Bhardwaj, 2010 a; Bhardwaj, 2010 b; Lloyd, 2011; Webb, 2011; Tombesi, 2013). In a recent study, high-powered (80-100W) MWA has been demonstrated safe and effective in the treatment of hepatocellular carcinomas up to 8 cm; although local recurrence was possible, two-year survival rates were high (Zhang, 2015). As overlapping ablations and several sessions of MWA are possible, tumor size does not appear to impact complete ablation rates or local recurrence rates for focal hepatic malignancies (Alexander, 2015). Yet, some authors state that regarding large hepatocellular carcinomas (over
3 cm), in order to avoid high rates of local recurrence, transarterial embolization followed by ablation is recommended (Lu, 2013; Peng, 2013). Conversely, small hepatocellular carcinomas (up to 2-3 cm in diameter) can be treated with microwave (or radiofrequency) ablation alone, if the location is suitable to the procedure. Percutaneous MWA is performed safely in hepatic tumors close to critical organs such as the heart and diaphragm in human medicine (Asvadi, 2016; Carberry, 2016). It is also considered for the ablation of hepatic metastases (Ong, 2009). The most frequently treated hepatic metastases are from colorectal cancer (Simon, 2005; Jones, 2011), because with this cancer the liver is very often the only site of metastasis. Since the metastases are limited to the liver, thermal ablation improves the prognosis for the patients treated for the primary tumor. The difficulty increases with the target size, since the ablation must comprise 1cm margins of the metastasis (Webb, 2011). Microwave ablation is considered a curative treatment if the patient has 1 hepatic nodule with a diameter <5cm or a maximum of 3 nodules <3cm, when no portal vein cancerous thrombus or extra-hepatic spread are present (Liang, 2013). Other current indications for hepatic microwave ablation include benign lesions, such as giant hemangiomas, hepatic adenomas and cysts (Cui, 2003; Rhim, 2004; Zagoria, 2004; Rocourt, 2006; Hinshaw, 2007). In these cases, the goal of the ablation is to control the signs due to the mass effect, or to prevent hemorrhage or malignant transformation, such as for adenomas.

Kidney – The majority of the renal lesions treated by ablation are renal cell carcinomas. As in the liver, a minority of benign lesions may also be amenable to ablation, such as for oncocytes and angiomyolipomas (Castle, 2012; Gregory, 2013). Emerging data on microwave ablation for renal cell carcinoma is promising, and is favored in some institutions (Hinshaw, 2014). Microwave ablation is especially interesting for small exophytic lesions, because the ablations are quick, and few applications are needed. However, microwave ablation is not considered the technique of choice when the ablation is performed centrally in the kidney, close to vulnerable structures such as the renal pelvis or ureter, for which some institutions prefer cryotherapy (Janzen, 2005; Warlick, 2006; Rosenberg, 2011). Of
note, hydrodissection is very important in renal ablations, because of the close proximity of the ureters, intestines and pancreas, and is used in up to 50% of cases by some (Patel, 2012).

**Lung** - In the lungs, microwave has some advantages over the radiofrequency technique, because the lung is aerated and therefore has intrinsic high impedance, which limits the amount of electrical energy deposited. Microwave ablation has been shown to produce larger and more circular ablation zones in the lungs (Dupuy, 2009). Usually for primary lung tumors, microwave ablation is palliative, providing symptomatic relief to patients for whom conventional modalities have failed (Simon, 2005). In patients with a finite number of metastases in the lungs and primary tumors with favorable biologic characteristics (eg soft tissue sarcoma, renal cell carcinoma and colorectal carcinoma), ablation of these metastases substantially improves the prognosis (Simon, 2005). CT-guided percutaneous MWA therapy has been described for pulmonary neoplasia without surgical treatment, with good effectiveness rate and high two-year survival rates (Sun, 2015). It should be noted that a long approach to the tumor is desirable for microwave ablation (as opposed to the shortest path possible for lung biopsy), in order to avoid ablating the chest wall (resulting in pain and burns), or that ablated lung retraction occurs from the chest wall, potentially resulting in persistent airleaks and bronchopleural fistulas (Hinshaw, 2014).

**Musculoskeletal** - Thermal ablation (including radiofrequency, microwave and cryoablation) is a successful minimally-invasive technique used for ablation of painful bone metastases refractory to standard medical management (Nazario, 2001; Simon, 2005; Murphy, 2013), and curative treatment of benign bone tumors in humans (Pusceddu, 2013; Filippiadis, 2014). It is usually performed with CT guidance. Currently, therapeutic options for osteoid osteoma include almost all thermal ablation techniques (RFA, microwave ablation, laser, MR guided high-intensity focused ultrasound) (Basile, 2014; Filippiadis, 2014), although RFA is proposed as the gold standard (Webb, 2011; Clark, 2013). The main goal of ablation
of osteoid osteoma is to remove pain. On a note, there is no counterpart of osteoid osteoma in animals. Percutaneous thermal ablation can be used as a curative therapy in metastatic disease with less than 3 lesions. However, in most cases, ablation is used as palliative therapy for bone metastases, aiming at pain reduction, local control of disease and tumor decompression (Pusceddu, 2013). Pathophysiologic explanations for pain reduction after ablation include necrosis of the interface between tumor and the pain-sensitive periosteum, tumor decompression, decrease in nerve-stimulating cytokines released by the tumor, and inhibition of osteoclastic activity (Filippiadis, 2014). When the bone is at risk of pathologic fracture or in weight-bearing areas, additional stabilization is required and ablation is usually combined with cementoplasty (polymethylmethacrylate (PMMA)) with or without further metallic augmentation. Local tumor control can also be enhanced with embolization (Murphy, 2013; Pusceddu, 2013; Filippiadis, 2014).

Amongst the previously detailed advantages of microwave over radiofrequency ablation (paragraph 1.2.3), microwaves propagate better than radiofrequency through tissues with low conductivity and poor thermal conduction, which is a huge asset in bone (Brace, 2009). Microwaves are insensitive to bone’s high impedance and penetrate deeper, are less affected by tissue heating or desiccation, and more effective for heating bone tumors than radiofrequency energy. Although cryoablation has been associated with more rapid and more durable pain relief by some, microwave ablation can have some advantages in the treatment of soft tissue tumors. These include the rapidity of propagation, ability to create a large ablation zone, and to ablate almost every tumor type (Hinshaw, 2014). It may be particularly important in the treatment of fibrous tumors such as sarcomas, which may be resistant to cryoablation, and tumors closely adjacent to bone or large vessels (leading to a vascular heat sink effect – see paragraph 1.2.3) (Hinshaw, 2014). Few reports of microwave ablation for bone tumors have been published in the scientific literature. Some recent studies describe successful curative treatment of osteoid osteoma with microwave ablation (Basile, 2014).
Other studies report the use of microwave ablation as palliative therapy for painful bone metastases when these are refractory to conventional approaches such as radiation or chemotherapy (Kastler, 2013; Pusceddu, 2013). With percutaneous microwave ablation of spinal and paraspinal bone metastases, pain relief greater than 50% was achieved immediately in 9/10 patients and lasted for an average of 4.85 months (range 0.5-15 months) (Kastler, 2013). In another study with percutaneous microwave ablation of axial skeleton metastases, a reduction of pain and improvement of quality of life was observed in all patients, with a mean pain score reduction by 92% during the 3-month follow-up period (Pusceddu, 2013).

In the current literature there is a lack of a standard protocol of microwave ablation in bone to produce variably sized ablations. In the study where percutaneous microwave ablations of spinal and paraspinal bone metastases were performed, an Acculis MTA-2 generator (4.5 GHz) with a 17G antenna was used. Mean ablation time was 4.75 minutes (1-13 minutes) with an average of 4.2 cycles per ablation lasting from 30 seconds to 3 minutes with an ablation power ranging from 30 to 160 W. Obtained lesion size ranged from 12mm to 70mm (Kastler, 2013). In the study where percutaneous microwave ablations of axial skeleton metastases were performed, a 2.45 GHz microwave generator (AMICA-GEN; HS Hospital Service, Aprilia, Italy) was used, with energy delivered via a 14 or 11 G water-cooled interstitial antenna (HSAMICA; HS Hospital Service). The mean procedure time was 7 minutes +/- 5 minutes, and the mean net delivered power was 50 W +/- 20W. Lesions of less than 3.5cm in maximal dimension were treated with one antenna, lesions more than 3.5cm were treated with one or two antennas, but no further precision regarding ablation zone sizes obtained was provided (Pusceddu, 2013). The study demonstrating the more consistent results used a 16G antenna (HS, Aprilia, Italy), connected to a generator (HS, Aprilia, Italy) delivering a power of 20 W for 2 minutes. The ablation area was measured at 1 month by MRI follow-up and had a mean ablation area of 21 x 12 x 14 mm$^3$ (Veltri, 2012).
Published literature in humans also indicates methods to use microwave antennas for ablation in bone. If the cortex is intact, a bone marrow biopsy needle can be used to pierce the cortex and serve as a coaxial introducer for the antenna to reach the lesion (Pusceddu, 2013). Once the antenna is in the tumor, the introducer is retracted before energy delivery is initiated so it does not interfere with microwave emission by the antenna tip. The antennas used in this situation are usually 20cm long to allow sufficient retraction of the cannula from the ablation zone. Once the ablation is completed, if osteoplasty is warranted, the antenna is withdrawn and the introducer left in situ to be subsequently used for the cementoplasty procedure. If the cortical bone is lysed or interrupted by the tumor, the antenna is directly inserted in the lesion without the need for a coaxial introducer (Pusceddu, 2013). One study specifies that 2cm of the active tip of the microwave antenna has to be kept inside the bone to avoid damage to the surrounding vital structures (Basile, 2014). Other studies use techniques such as cooling with chilled saline, gas dissection or placing a sterile glove filled with cooled saline on the skin to protect sensitive structures adjacent to the ablation zone (Fillipiadis, 2014).

**Adrenal glands** - Microwave ablation has also been performed in patients with adrenal cortical carcinoma and adrenal metastases. Given the cystic nature of adrenal metastases, microwave ablation is considered the best option (Simon, 2005).

**Prostate** - In the prostate, palliative percutaneous microwave therapy under MR imaging guidance has been reported with good clinical response at 6 months in patients with locally recurrent prostatic carcinoma (Chen, 2000).

**Body wall** - A preliminary study has shown that ultrasound guided microwave ablation was safe and effective at a median follow-up of 13 months in 11 patients for the ablation of metastatic abdominal wall nodules (Qi, 2012).
Pancreas – MWA of locally advanced pancreatic cancer has been reported in a limited number of cases, however the experience is currently limited. In fifteen patients, partial necrosis of the pancreatic neoplasia was achieved adequately, without major morbidity or mortality (Linecker, 2016). However, given the paucity of the data, MWA, alone or in combination with chemotherapy, is still considered under investigation for locally advanced pancreatic cancer (Linecker, 2016).

Even though MWA is a promising technique, there are still some uncertainties regarding its therapeutic impact on non-resectable neoplasms of certain organs, such as locally advanced pancreatic cancer (Linecker, 2016). This is mainly due to its recent advent and subsequent low number of cases treated. However, more and more studies are performed regarding multimodal combinations of MWA with other therapeutic approaches, to enhance its efficiency (Linecker, 2016; Kang 2015; Thornton, 2016; Urban, 2016).

Ablation sizes are variable between tissues. Some charts are available for different ablation zone sizes expected according to different settings and in different organs. The manufacturer of the microwave ablation system used by the authors has charts available for the liver, muscle and kidney (Appendix I). However, such data is not available in other organs, such as bone. Moreover, ablation sizes obtained in canine patients may be different than in humans, because the histopathology and morphology of the tissues may be different. For example, Haversian system and canal diameter are smaller in dogs compared to humans. Moreover, humans being bipeds, their femurs contain more Haversian bone, which is not the case in the dog (Hillier, 2007). Although bone marrow composition is similar in humans and dogs (hematopoietic tissue, blood vessels and adipocytes) (Fry, 2007), proportions of these elements may be variable depending on the age and species of the subjects. This is why in the second phase of this study, the authors focused on investigating the behaviour of microwaves in canine bones.
1.4.2 Complications of microwave ablation

Following microwave ablation, side effects can occur, but rarely result in morbidity (Loyd, 2011). Examples of morbidity reported include pain, asymptomatic pleural effusions, low-grade fever and general malaise that are usually self-resolving. The potential major complications such as collateral damage by heating non-targeted structures is reduced compared to radiofrequency but remain infrequent (Garrean, 2009; Clark, 2013). For thermal ablation in the liver, major complications can include bile duct stenosis, uncontrollable bleeding, liver abscess, colon perforation, skin burn secondary to the high thermal efficiency of microwaves, and tumor seeding (Veltri, 2012; Liang, 2013). To avoid damage to vital structures in the vicinity of a tumor, such as the gallbladder or major bile duct when ablating a hepatic tumor, monitoring of temperatures adjacent to the ablation site with a thermal monitoring system attached to the microwave unit is performed (Li, 2015). Portal vein thrombosis following microwave ablation in the liver has also been documented, and apparently, creating ablation zones near vessels with slow blood flow, such as in cirrhotic patients, can potentially increase the risk of vascular damage and thrombosis. In comparison, creating an ablation zone near high velocity vessels results in a rapid thermal transfer and does not lead to localized temperatures elevations (Chiang, 2012).

1.4.3 Thermal ablation in veterinary medicine

Studies have been published in human medicine journals regarding thermal ablation in animals, as models. A group described transurethral ultrasound thermal therapy of implanted transmissible veneral tumors in an in vivo canine prostate model (Hazle, 2002). However, there is a paucity of literature reporting the use of thermal ablation in veterinary medicine.

Percutaneous ultrasound guided radiofrequency heat ablation has been
reported in the treatment of hyperparathyroidism in dogs (Pollard, 2001). In that study, the parathyroid nodules targeted measured from 0.3 to 1.1 cm in length. The procedure was performed under general anesthesia. The technique used was a little different from what is reported in humans; the radiofrequency antenna was not inserted directly in these small nodules. Rather, a 20 gauge over-the-needle catheter was advanced in the nodule under ultrasound guidance, then an insulated wire connection was established between the radiofrequency unit and the catheter stylet. They specified that the radiofrequency unit had to be equipped with a ballast resistor because the high impedance of the needle would otherwise have prevented adequate energy application. The catheter sleeve acted as an insulator to protect the surrounding tissues. Then, they started each ablation at 10W for 10-20 seconds. The efficiency of the ablation was monitored by seeing echogenic bubbles at the site of insertion of the antenna. If no echogenic bubbles were seen, the wattage was increased by increments of 5W (up to a maximum of 20W) every 10 seconds (up to a maximum total time of 90 seconds), until they became apparent. The needle sometimes had to be redirected to ablate the entire nodule, because the amount of tissue exposed to heat ablation had a radius of about 2-3mm only. Out of 11 dogs, the treatment was effective in 8 (after one treatment in 6, and two treatments in 2), and unsuccessful in 3. Complications described included hypocalcemia in 5 of the 8 successfully treated dogs, and transient voice change in one dog. Another study performed a few years later compared this method of parathyroid ablation with parathyroidectomy and percutaneous ultrasound guided ethanol ablation (Rasor, 2007). Radiofrequency ablation compared positively to parathyroidectomy (90% resulted in control of hypercalcemia for a median of 581 days versus 94% of parathyroidectomies for a median of 561 days), and had better results than ethanol ablation (72% of which resulted in control of hypercalcemia for a median of 540 days).

The second use in which radiofrequency ablation has been described in veterinary medicine is the treatment of hyperthyroidism in cats (Mallery, 2003). In that study, heat ablation was applied to the abnormal thyroid lobe found with the
combination of scintigraphy and ultrasound. If both lobes were abnormal, ablation was performed on the largest lobe. Out of the 9 hyperthyroid cats enrolled, all became euthyroid for a mean of 4 months (0 to 18). However, hyperthyroidism recurred in all. Complications described included transient Horner’s syndrome in 2 cats and non-clinical laryngeal paralysis in one.

A recent article compared different imaging techniques to assess radiofrequency ablation lesion size in livers of healthy dogs (Moon, 2017). Conventional ultrasonography, strain elastography, and contrast-enhanced ultrasonography were used immediately after, 30 to 60 minutes after, and 3 days after RFA, and contrast-enhanced (three-phase angiogram) CT was performed 30 minutes and 3 days after RFA. The authors found that CT was the most reliable technique to determine ablation lesion size, however they also state that all the modalities studied were appropriate to monitor the ablation size in real time.

A few reports about the use of microwave ablation in veterinary medicine have been recently published. One of them describes the use of laparoscopic guided MWA of hepatic metastases in three dogs (Case, 2015). Ablation of the metastases was performed after resection of the primary malignancy. The hepatic metastases were between 5 and 30mm in size. No complication was observed. Another recent study describes thoracoscopy assisted MWA of pulmonary metastasis in a dog (Boston, 2016). The patient had a previous history of appendicular osteosarcoma, treated with standard therapy, and had a solitary 2.1 cm by 2.3 cm pulmonary presumptive metastatic lesion. Video-assisted microwave ablation of the pulmonary nodule was performed after insertion of a microwave antenna in the lesion, using two ablation cycles of 45 W for 10 minutes and 65 W for 3 minutes. Autologous whole blood was applied at the entry site of the probe. No complication was noted peri-operatively. Two months after the procedure, the patient was doing very well, but no repeat imaging was available.

Aside from these few cases described in the veterinary literature, potential
uses of microwave ablation in veterinary patients include intra-operative ablation of small (1-5 cm) metastatic tumors in the liver and/or spleen during surgical resection; intra-operative ablation of small pulmonary nodules (1-5 cm) during lung lobectomy to reduce the number of lung lobes removed; percutaneous ablation of pulmonary masses or nodules; percutaneous ablation of primary bone tumors such as osteosarcoma or metastatic bone tumors; percutaneous ablation of liver, spleen, and kidney tumors; and stereotactic MRI-guided ablation of intra-axial brain tumors.

1.5 Rationale, objectives and hypotheses of the research

1.5.1 Microwave ablation antenna reuse and reprocessing treatment

Based on the human model, percutaneous thermal ablation would represent a minimally invasive cancer therapy for animals. This novel technique could be highly relevant to clinical health by increasing survival and decreasing pain in oncologic patients. Yet, thermal cancer ablation techniques are scarcely currently reported in the veterinary literature with the cost of the equipment being a limiting factor. For example, a microwave antenna is purchased for more than CAD $2000, and is licensed for single use in human medicine. Reprocessing and reusing microwave antennas would be a solution to decrease associated costs, and it would allow access for our veterinary patients to this potentially promising therapy. Given that antennas can be used several times during one procedure in one patient, if they can safely be resterilized, they could potentially be reused several times in different patients. Reprocessing of other single-use instruments is currently performed with success in both veterinary and human hospitals (Coisman, 2013).

The first objective of this research project is to assess the lifespan of microwave antennas following repeated use in the livers of bovine cadavers, with
their reprocessing and sterilization. Bovine livers made a good study system because they had been used as a model by previous authors (Lopresto, 2012; Hoffmann, 2013), because of their large size and because of their availability. Microwave antennas (Accu2i pMTA applicator, with a 1.8mm shaft, internally cooled, 2.45GHz operating frequency, generator powers from 60W to 140W and ablation time 0 to 6 minutes per use) used with the Sulis VpMTA generator system (Microsulis Medical Ltd, Hampshire, UK) will be tested. Sterilization will be performed with hydrogen peroxide gas plasma. The objective and hypotheses made by the authors are below.

**Objective 1:** To determine whether the structural and functional integrity of microwave antennas will be maintained with repeated use in bovine liver cadavers and reprocessing.

**Hypothesis 1:** Microwave antennas will remain structurally and functionally sound for up to six total cycles (use and reprocessing) with variable numbers of ablations performed for each use (1, 2 or 3) in bovine liver specimens.

The outcomes evaluated for each ablation will be the size and the shape of the ablation zones, the temperature just outside of the ablation zone, and the microscopic structural assessment of the antenna following thermal ablation. This project will constitute a crucial first step towards assessing the safety and efficacy of reusable antennas prior to clinical trials and implementation in clinical patients.

### 1.5.2 Application of microwave ablation in canine osteosarcoma of long bones

In the current human literature there is a lack of a standard protocol of microwave ablation in bone to produce variably sized ablations. Microwaves may behave differently in canine bone marrow compared to human’s. A chart for sizes of
bone microwave ablation zones in dogs, prior to its clinical use in our patients, is required. These charts have been produced in other tissues (Appendix I is a table of different ablation sizes obtained at different settings in liver, muscle and kidney, produced by the manufacturer of the antenna used in the study). Developing a technique to perform bone microwave ablations in dogs, and assessing the different ablation zone sizes expected in normal and diseased bone tissue is a crucial first step prior to the use of microwave ablation in clinical veterinary patients with primary or metastatic bone tumors. This data will be necessary for subsequent research and before the use of microwave ablation can be recommended in clinical trials. This novel, minimally invasive technique with percutaneous or intra-operative use is anticipated to decrease pain in oncologic patients with bony metastases, as described in humans, and could be an additional asset in limb sparing techniques.

The second objective of this research is to describe a technique to perform microwave ablation in long bones of dogs. Also, the authors aim at developing a protocol for microwave ablation zone sizes in normal canine bone at different settings, and correlating this to ablation zone sizes performed in cadaver bones affected with neoplasia.

**Objectives 2:**

1. To describe a technique to perform microwave ablation in long bones of normal dogs.
2. To establish different microwave ablation zone sizes in normal canine bone at different settings.
3. To correlate microwave ablation zone sizes in normal bones to ablation zones performed in cadaver bones affected with neoplasia (osteosarcoma or metastatic neoplasia).

**Hypotheses 2:**

1. Microwave ablation will be feasible in long bones of normal dogs.
2. Ablation zone sizes will be established at different settings for microwave ablation in long bones of dogs.

3. The data obtained during ablations in normal canine bones will be applicable to ablation of canine long bones with primary or metastatic neoplasia.
Chapter 2: Effects of repeated use and resterilization on structural and functional integrity of microwave ablation antennas.

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This was published as an article in the AJVR (American Journal of Veterinary Research), April 2017, issue 78, volume 4.

2.1 Abstract

OBJECTIVE
To determine effects of repeated use and resterilization on structural and functional integrity of microwave ablation (MWA) antennas.

SAMPLE
17 cooled-shaft MWA antennas (3 groups of 5 antennas/group and 2 control antennas).

PROCEDURES
1, 2, and 3 ablations in livers of bovine cadavers were performed at the maximum recommended settings. Antennas were cleaned and sterilized in hydrogen peroxide plasma, and the process was repeated (reprocessing cycle; n = 6). Control antennas were only sterilized (6 times). Aerobic and anaerobic bacterial cultures were performed, and antennas were assessed for damage with microscopy.

RESULTS
6 cycles were completed. Thirteen of 15 MWA antennas remained functional for up to 4 cycles, 10 were functional after 5 cycles, and only 7 were functional after 6 cycles. Progressive tearing of the silicone coating of the antennas was observed, with a
negative effect of the number of cycles on silicone tearing. Size of the ablation zone decreased mildly over time after cycles 5 and 6; however, this was not considered clinically relevant. No significant changes in shape of the ablation zones were detected. All cultures yielded negative results, except for an isolated case, which was considered a contaminant.

**CONCLUSIONS AND CLINICAL RELEVANCE**

Structural and functional integrity of the microwave antennas remained acceptable during repeated use and reprocessing for up to 4 cycles. However, there was a decrease in functional integrity at cycles 5 and 6. We suggest that these microwave antennas be subjected to ≤ 3 reprocessing cycles. Antennas should be carefully examined before reuse.

**2.2 Introduction**

Thermal ablation is a procedure used for the treatment of cancer in humans. However, there are few literature reports specific to veterinary patients (Pollard, 2001; Hazle, 2002; Mallery, 2003; Rasor, 2007; Case, 2015; Boston, 2016), except for a few studies (Awad, 2007; Mertyna, 2009; Lopresto, 2012; Sun, 2012; Hoffmann, 2013) that involved animals with experimentally induced conditions. Thermal ablation applications in humans include the treatment of primary and metastatic neoplasia of the liver (Simon, 2005; Ahmed, 2011; Veltri, 2012; Clark, 2013) and various other cancers (eg, lungs (Simon, 2005; Dupuy, 2009; Ahmed, 2011; Clark, 2013), bones (Simon, 2005; Ahmed, 2011; Nazario, 2011; Clark, 2013), breasts (Clark, 2013), thyroid gland (Clark, 2013), and kidneys (Simon, 2005; Ahmed, 2011; Clark, 2013)). Thermal ablation induces irreversible cellular injury by creating a focal high-temperature environment (> 54°C for at least 3 minutes or 60°C reached instantaneously) (Goldberg, 1996; Nan, 2010; Ahmed, 2011). Several thermal ablation methods exist, including radiofrequency, MWA, laser, cryoablation, and high-intensity focused ultrasound (Simon, 2005; Ahmed, 2011; Webb, 2011). Various methods of thermal
Ablation can reduce overall costs, duration of hospitalization, postoperative pain, morbidity, and fatalities, compared with outcomes for standard and laparoscopic surgical procedures (Ahmed, 2010; Nishikawa, 2011; Webb, 2011; Zanus, 2011; Tombesi, 2013; Xu, 2015). Thermal ablation has gained popularity for use in the treatment of nonsurgical candidates in which a tumor may be in a location that precludes resection or as an adjuvant treatment to surgery for the ablation of small-sized metastases (Bhardwaj, 2010b; Linecker, 2016; von Amerongen, 2016). Other advantages include synergy with other cancer treatments for unresectable neoplasms and repeatability of the procedure when there is incomplete ablation or further metastatic growth (Simon, 2005; Kang, 2015; Tang, 2016).

Microwave ablation is the most recent development in tumor ablation techniques. Ablations can be performed percutaneously by use of image guidance (Simon, 2005; Webb, 2011; Veltri, 2012; Sun, 2015) (most often ultrasound- or CT-guided procedures) or endoscopy (Simon, 2005; Murakami, 2011; Simo, 2011; Webb, 2011; Boston, 2016; Zaidi, 2016) (primarily laparoscopy but also thoracoscopy) or with open surgical access (Simon, 2005; Webb, 2011). Electromagnetic waves are emitted by an antenna that is inserted into the tumor. Electromagnetic waves agitate the water molecules within the target volume, which produces friction and heat and results in irreversible coagulative necrosis (Veltri, 2012; Clark, 2013). Most of the clinical and research studies of humans have been performed with radiofrequency ablation and liver tumors. The main advantages of MWA over radiofrequency ablation include higher intratumoral temperatures (Simon, 2005; Clark, 2013; Tombesi, 2013), larger tumor ablation volumes (Simon, 2005; Awad, 2007; Zanus, 2011; Clark, 2013; Tombesi, 2013) (up to 5.5 cm in diameter per ablation and larger tumors with overlapping ablations), more rapid ablation procedures (Simon, 2005; Awad, 2007; Clark, 2013; Tombesi, 2013) (4 to 8 min/ablation), optimal heating of cystic masses (Simon, 2005), a reduction in pain during procedures (Simon, 2005), and homogeneous heating of tumors (Simon, 2005; Clark, 2013; Tombesi, 2013). In contrast to radiofrequency ablation, MWA does not require grounding pads, which makes MWA ideal for veterinary patients that usually have a thick coat, and avoids
complications from grounding pad burns (Simon, 2005). Microwaves can propagate through many tissue types, including lung and bone tissues that have high impedance, with a reduced heat-sink effect adjacent to blood vessels, compared with results for radiofrequency ablation (Simon, 2005; Awad, 2007; Garrean, 2009; Ahmed, 2011; Nazario, 2011; Clark, 2013). Induction of proinflammatory cytokines is also minimal with MWA, compared with that for radiofrequency ablation (Kang, 2015). Furthermore, the development of cooled-shaft MWA antennas has allowed higher output and longer duration of ablation, which thus decreases the risk of skin burns (Zhang, 2015). For these reasons, application of MWA is growing for use in thermal ablation in humans with cancers in various organs. In a recent study (Zhang, 2015), high-powered (80 to 100 W) MWA was found to be safe and effective for the treatment of hepatocellular carcinomas up to 8 cm in diameter in humans. Although local recurrence was possible, 2-year survival rates were high (Zhang, 2015). Because overlapping ablations and several sessions of MWA are possible, tumor size does not appear to impact complete ablation rates or local recurrence rates for focal hepatic malignancies (Alexander, 2015). Percutaneous MWA can be performed safely in hepatic tumors close to critical organs such as the heart and diaphragm of humans (Asvadi, 2016; Carberry, 2016). In addition, MWA has been used in less conventional organs. For example, CT-guided percutaneous MWA treatment has been described for pulmonary neoplasia without surgical treatment, with a good effectiveness rate and high 2-year survival rates (Sun, 2015). Even though the use of MWA is promising, because of its relatively recent advent, there are some uncertainties regarding its therapeutic impact on nonresectable neoplasms of certain organs (Linecker, 2016). However, additional studies have been performed to evaluate the use of multimodal combinations of MWA with other treatment approaches (Kang, 2015; Linecker, 2016; Thorton, 2016; Urban, 2016).

Microwave ablation holds great promise for veterinary patients as an adjuvant minimally invasive cancer treatment. The use of laparoscopic-guided MWA of hepatic metastases in dogs (Case, 2015) and thoracoscopy-assisted MWA of pulmonary metastasis in a dog (Boston, 2016) have been reported. However, an antenna is costly
(expected price range is approx $1,800 to $2,500 Canadian dollars, depending on the manufacturer) and is labelled for multiple thermal ablations in a single human patient (8 ablations per patient with the antennas used in this study). The ability to reuse antennas for multiple patients would dramatically reduce costs, which would make this a more feasible method of treatment for use in veterinary patients. It also would reduce the costs if 1 antenna could be reused for several steps of a research project or for multiple research projects.

Therefore, the objective of the study reported here was to determine whether structural and functional integrity of MWA antennas would be maintained after repeated use and resterilization. Our hypothesis was that MWA antennas would remain structurally and functionally sound after multiple uses in liver specimens of bovine cadavers with instrument processing between subsequent ablations.

2.3 Materials and Methods

2.3.1 Sample

Seventeen MWA shaft-cooled antennas and a 2.45-GHz generator system were used. The antennas had a 14-cm-long shaft that was 1.8 mm in diameter with a ceramic trocar cutting tip; each shaft was covered with a thin coating of silicon. Available generator power ranged from 60 to 140 W, and ablation times ranged from 10 seconds to 6 minutes for each application. During an ablation, the output power was displayed in real time on the generator, and ablation was aborted when there was a decrease in output power (which would entail an inappropriate temperature in the ablation zone). Appendix II and appendix III display the antenna and the generator unit, respectively.

Bovine livers were obtained from the Meat Science Laboratory of the Department of Animal and Poultry Science at the University of Guelph. Livers were
frozen at –20°C until used in the study. Before the experiments were performed, 1 bovine liver was submitted for aerobic and anaerobic bacterial culture to determine the amount and type of bacteria.

2.3.2 Procedures

The 17 antennas were divided into 3 groups (5 antennas/group; groups 1, 2, and 3), and a group of 2 control antennas (group 4). Ablations (1, 2, and 3) were performed in livers of bovine cadavers with antennas from group 1, 2, and 3, respectively. Ablations were performed at the maximum power (140 W) and application time (6 minutes) recommended by the manufacturer. For each cycle, the order of use of the antennas was randomized by selecting a number from a bag that corresponded to each antenna to be tested.

Twenty-four hours before ablations were performed, frozen bovine livers were thawed and immersed in a temperature-controlled water bath (25°C). Ablations were performed in the temperature-controlled water bath to ensure a consistent temperature of the livers was maintained between the end of the thawing period and the ablation period.

Temperature during ablation was measured in the liver by use of thermistors and recorded every 30 seconds; the thermistors were attached to the microwave unit that displayed the temperatures. Temperatures were measured at locations 2 and 3 cm from the antenna. These distances were chosen to monitor the temperatures within and outside of the ablation zone because the short-axis of the ablation zone in liver expected on the basis of the manufacturer chart at the chosen settings was 4.5 cm in diameter (edge of ablation was 2.25 cm from the antenna). The antenna and 2 thermistors were inserted in a carved foam block attached to a moveable arm to ensure reproducible placement in the livers (Figure 2.1). In a clinical setting, the use of thermistors would not be mandatory; however, thermistors were used as an
additional tool to monitor the behavior of the microwaves in the tissues around the antenna.

The antenna was withdrawn after each ablation, and a metal skewer was inserted in the antenna track into the ablation lesion. This guided dissection of the ablated portion of the liver along the long axis through the center of the ablation zone. Photographs of the ablation zones, each of which included a ruler, were obtained with a digital camera.

A ROI was manually traced around each ablation zone by use of software, and size and shape of the cross-sectional area of the ablation zones were recorded (Figure 2.2). The line of demarcation between the grossly ablated tissue and non-ablated zone was determined on the basis of color differences of the liver. When the outline was judged to be inexact (subjectively too much grossly ablated liver was not included in the ROI or non-ablated liver was included inside the ROI), the tracing was repeated. The same investigator (C.A.F.) traced all ROIs. The investigator was not aware of which antenna was used to create the ablated zone.

After the ablations for each group were completed, the antennas were soaked in an enzymatic detergent and gently cleaned with a soft-bristled toothbrush to remove blood and other organic material.

No ablations were performed with the control antennas. Control antennas were not soaked in the enzymatic detergent nor subjected to cleaning procedures.

All antennas, including the control antennas, were sterilized with hydrogen peroxide plasma. The water coolant end of the antenna tubing was capped because sterilization with pressurized hydrogen peroxide plasma will not be successful if any water is present. However, the Luer-lock connection of the water coolant end of the antenna allowed water to leak out during depressurization. Therefore, manual modifications were performed. The Luer-lock connection was cut off with a small saw,
and the end was sealed air tight for sterilization with silicone tubing hermetically closed with plastic zip ties (Figure 2.3). This was a permanent modification that did not alter the use of the antennas but allowed proper sterilization. To prevent water leaking from the water pump at the point, the magnetic plunger was strapped down with elastic bands.

After sterilization following the first set of ablations was completed, all antennas were submitted to the Animal Health Laboratory at the University of Guelph for aerobic and anaerobic bacterial culture. Samples for aerobic culture were plated on Columbia blood agar and MacConkey agar plates and incubated in 5% CO₂ and atmospheric conditions, respectively. Plates were incubated at 35°C and examined for the presence of bacterial growth after incubation for 24 and 48 hours. Samples for anaerobic culture were plated on brucella and phenylethyl alcohol agar plates and incubated at 37°C in an anaerobic chamber with anaerobic conditions (10% hydrogen, 5% CO₂, and 85% nitrogen). For all subsequent ablation cycles, 1 antenna from each of the 4 groups was randomly (using random sampling by selecting numbers from a bag that corresponded to each antenna) selected for aerobic and anaerobic bacterial culture.

Antenna tips were carefully inspected for damage and photographed before initial use and after each ablation. Inspections were performed by use of stereo light microscopy in the Laboratory Services Division of the Agriculture and Food Laboratory at the University of Guelph. Loss of structural integrity was determined by subjectively identifying damage to the ceramic tip and silicone-coated shaft. Each antenna was examined systematically along its entire length to assess tearing of the silicone coating or staining of the ceramic tip (yellow instead of white) and to identify any brown spots or particles deposited on the antenna.

After the evaluation was completed, antennas were reused. A reprocessing cycle was comprised of ablation, cleaning, and sterilization. Antennas were used in up to 6 reprocessing cycles.
2.3.3 Statistical analysis

Size of ablation zones was evaluated by use of an ANOVA, with antenna as a categorical factor and time as a continuous explanatory variable, by use of a mixed linear model. The model initially included a quadratic value for time as well as interactions between antennas and time and between antennas and the quadratic value for time; terms that were not significant were removed from the model. To assess ANOVA assumptions, residual analyses were conducted. Residuals were tested for normality by use of 4 tests (Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling). In addition, residuals were plotted against the predicted values and explanatory variables used in the model. Residual analyses were performed because they may have revealed outliers, unequal variances, the need for data transformations, or other issues that would need to be addressed.

A binary logistic model was used to estimate by use of a generalized linear mixed model the probability of oval-shaped ablation zones as a function of specific antennas and time. Ablation zones were recorded as oval or non-oval. Silicone tearing was recorded as absent or present (at the tip, at the junction between the tip and shaft, on the shaft, or a combination of these). Because there was insufficient data for silicone tearing, incidence of brown spots, and staining of the antennas, it was only possible to examine 1 factor at a time (ie, antennas or cycles). Thus, instead of a logistic regression analysis, Pearson chi-2 tests were used. Monte-Carlo P values were computed to investigate whether these responses differed among antennas or among cycles.

Temperatures were plotted against time. Because the plot was extremely variable among antennas, regressions with cubic terms were fitted to each antenna and each ablation separately. Then, the components (intercept and linear, quadratic, and cubic components) of the equation for each antenna or ablation were analyzed separately by evaluating the distribution of data. The null hypothesis that each
component equaled zero was tested.

Kaplan-Meier time-to-event plots were created and used to examine time to antenna failure. For all tests, significance was set at values of $P < 0.05$.

### 2.4 Results

Six reprocessing cycles were completed with each of the 15 antennas of groups 1, 2, and 3. Six sterilization cycles were completed with both of the control antennas.

Regarding size of the ablation zones, the ANOVA assumptions appeared to be reasonably met and the data did not require transformation. Overall mean of the cross sectional areas of the ablation zones, adjusted to the mean number of cycles, was 20.77 cm$^2$ (95% confidence interval, 19.76 to 21.77 cm$^2$). Up to cycle 4, size of the ablation zones did not change significantly ($P = 0.064$). For cycle 5 and 6, there was a significant ($P < 0.001$) decrease in size of the ablation zones over time (−0.28 cm$^2$/cycle [95% confidence interval, −0.43 to −0.14 cm$^2$]). The number of reprocessing cycles did not significantly ($P = 0.176$) affect shape of the ablation zone. The expected shape of the cross-sectional area was ovoid; however, in most cases, the margins of the ablation were irregular and the shape was amorphous because heat spread along the path of hepatic blood vessels that were filled with water from the water bath (Figure 2.4).

Temperatures recorded were quite variable and not normally distributed; hence, it was not possible to fit a unified statistical model. Medians of coefficient for the quadratic and cubic components for temperature differed significantly ($P < 0.001$) from 0 by use of 1-sample signed-ranked tests, and the linear component also differed significantly ($P = 0.018$). The resulting equation relating temperature to time was as follows: $\text{temperature} = 25.5302 + (0.22286 \times \text{time}) + (0.997003 \times \text{time}^2) + (-0.081585 \times \text{time}^3)$. Temperatures recorded at 2 cm from the antennas sometimes remained low
or only increased slightly during ablation (instead of the expected high increase). Conversely, temperatures recorded at 3 cm from the antennas sometimes had a spike of high temperature (instead of the expected low values). Some recorded temperatures had a steady increase during the 6 minutes of ablations, whereas others plateaued (maximum, 70°C) after a few minutes. All combinations of high or low temperatures and increasing or plateauing could be recorded at the thermistors at 2 and 3 cm from the antennas.

Progressive tearing of the silicone coating of the antennas was observed over the cycles (Figure 2.5). There was a significant ($P < 0.001$) negative influence of the number of cycles on silicone tearing. Silicone tearing was seen in 2 of 15 antennas after cycle 1, 11 of 15 antennas after cycle 2, and all 15 antennas after cycle 3.

Mild yellow staining and brown spots on the tip of the antennas were evident after cycle 1 (Figure 5). Number of cycles did not significantly ($P = 0.44$) influence the occurrence of yellow staining; all antennas were stained after cycle 2. There was a significant ($P < 0.001$) effect of the number of cycles on the occurrence of brown spots. Beginning with cycle 2, all antennas had spots.

No bacterial growth was detected after sterilization for all antennas of all groups after 6 reprocessing cycles, except for 1 antenna. A few *Staphylococcus haemolyticus* (1 to 10 colonies) were isolated from that antenna (which was in group 3) after cycle 5. Bacterial culture of the bovine liver prior to the ablation experiments yielded *Bacillus cereus* and *Escherichia coli* ($>10^5$ cfu/ml colonies) but no anaerobes.

A total of 13 of 15 antennas remained functional up to cycle 4. Failure of 1 antenna was detected prior to use at cycle 3; the antenna could not pump cooled saline solution into the tubing. One antenna could not be sterilized at cycle 4. Sterilization was stopped because of water leakage, likely from the pumping chamber of the antenna, which could not be hermetically sealed. After 6 cycles, only 7 antennas
remained functional. Three antennas failed at cycle 5, and 3 additional antennas failed at cycle 6 (Figure 2.6). Reasons for failure included various alerts given by the microwave generator system that stopped the ablations (a defective applicator error message, and a coolant error message) in each of 2 antennas prior to use at cycle 5 and 6, a coolant error message during ablation for 1 antenna at cycle 6, and a bent antenna tip after sterilization of 1 antenna prior to use at cycle 5.

2.5 Discussion

Results of the present study indicated that reprocessing of a particular single-use microwave antenna can be safely and effectively performed. This is consistent with findings from a similar study (Coisman, 2013) in which investigators found that a single-use laparoscopic surgery port could be safely reprocessed.

Overall, the structural and functional integrity of the antennas remained acceptable during repeated use at maximal recommended settings and reprocessing up to 4 cycles. However, there was a decrease in functional integrity at cycles 5 and 6, with 6 antennas becoming nonfunctional at these cycles (3 at cycle 5 and 3 at cycle 6).

After cycle 4, there was a mild decrease in size of the ablation zone. However, it is unlikely that a decrease of $0.28 \text{ cm}^2$ would be clinically important. Indeed, the ROIs were manually traced; therefore, it was possible that this introduced bias in the accuracy of the measurements and could have accounted for the variability of the size of the ablations zone. Nonetheless, this decrease was small and represented approximately 1.35% of the mean cross-sectional area of the ablation zones obtained in the present study ($20.77 \text{ cm}^2$). Therefore, it is unlikely that this change over time would compromise clean margins of tumor ablations. Moreover, it is possible to overlap ablations (Simon, 2005; Ahmed, 2011; Zanus, 2011), which could provide an option to achieve a successful ablation if a single ablation zone was too small.
Alternatively, if the ablation was performed at lower settings (and not the maximal settings, as in the study reported here), the wattage or duration (or both) for the ablation could be increased to provide a larger ablation zone. Up to cycle 4, significant ($P = 0.064$) changes in the size of the ablation zone were not detected; however, only a small sample size was used.

The established Spaulding classification for patient-care items and equipment disinfection and sterilization distinguishes critical, semicritical, and noncritical categories according to the degree of risk for infection involved in use of the items (Rutala, 2008). Microwave ablation antennas should be placed in the critical category because they would confer a high risk for infection if contaminated with microorganisms. Items in the critical category should be purchased as sterile or be sterilized with steam, if possible, but heat-sensitive items can be treated with hydrogen peroxide gas plasma.

Structural alterations of the antennas were only evident at the tip, as observed microscopically, and included yellow staining, brown spots, and silicone tearing. Yellow staining of the tip was suspected to be attributable to oxidation of hepatic pigments. The brown spots were likely tiny pieces of charred tissue from the ablated area. This had no overall impact on sterility as all cultures but 1 yielded negative results. Moreover, the single positive culture result of *S haemolyticus* was most likely attributed to an environmental contaminant. Indeed, *S haemolyticus* is a part of the normal skin flora in humans and other animals (Ruzauskas, 2014; Livshiz-Riven, 2015). Fewer than 10 colonies were isolated, and no *S haemolyticus* was isolated from the bovine liver cultured prior to the experiments. Therefore, it was unlikely that this growth originated as a result of contamination of a liver during the ablations but was more likely originating from a human during either collection of the swab of the antenna, or plating the swab.

Silicone coating of the antenna is required for removal of the antenna from the ablated target. During preliminary experiments, some antennas with a missing or worn
silicone tip broke while inside a liver. However, the mild silicone tearing noted in the study reported here did not affect the ability to extract the antennas from the tissue of any ablation zone. Thus, the amount of silicone tearing observed in this study did not prevent appropriate functionality of the antennas. It is uncertain whether damage to the silicone coating occurred during removal of the antennas or during reprocessing. Silicone can induce certain inflammatory and chemotactic cytokines in macrophages (Vijaya Bhaskar, 2015). However, in ablated and dead tissue, the potential of an inflammatory reaction is fairly low. Moreover, silicone tearing was detected after cycle 1, which indicated that tearing may occur in the tissue during use in a single patient, especially if multiple ablations are performed. To our knowledge, there are no reports that silicone tearing of MWA antennas has resulted in complications. This appears to support the hypothesis that mild silicone tearing during MWA has no clinical relevance.

Temperatures were recorded around the antenna by the thermistors with the intent of monitoring the temperature inside, and more importantly just outside, the ablation zone. In a clinical setting, use of thermistors adjacent to an antenna is not mandatory. The generator displays in real time the output power, and the ablation is terminated if the output power decreases. This ensures that the appropriate temperature inside the ablation zone is achieved. Variability of the recorded temperatures was attributed to the heating of water from the water bath that filled the hepatic blood vessels. This would be minimized by circulating blood in live patients (Garrean, 2009; Ahmed, 2011) and could also be explained by slight differences in thermistor position. Monitoring of temperatures adjacent to the ablation site with a thermal monitoring system attached to the microwave unit is critical in a clinical setting to avoid damage to vital structures in the vicinity of a tumor (eg, gallbladder or major bile duct) when ablating a hepatic tumor in humans (Li, 2015). In dogs, the common bile duct is extrahepatic; however, this could be a concern depending on the size and location of the hepatic lesion that is being ablated.

In the present study, there was a sharp decrease in the number of functional antennas at cycles 5 and 6. One antenna could not be sterilized after cycle 4; the
sterilization procedure was stopped because of a loss of the hermetic seal. Except for 1 antenna during cycle 6, all failures were apparent or indicated by an error signal from the control unit prior to use. In a clinical setting, it is necessary to have a spare new antenna available in case of such a failure prior to the procedure. The only failure that occurred during ablation was at cycle 6. Ablations can be overlapped, so it is possible that a new antenna could be inserted to complete a procedure. We do not recommend use of antennas after they have been subjected to 6 cycles of use and reprocessing.

One of the major goals of the present study was to reduce the cost of MWA procedures to allow greater access to this method. Three reprocessing cycles would decrease the cost for antennas by one-fourth, although there would be costs for cleaning and sterilization.

The study had some limitations. First, the results and our recommendations are limited to the type of microwave antenna that was used in this study. Currently, there are several MWA systems commercially available, and antennas from various manufacturers have different designs. Because of differences in materials and cooling systems, it may not be possible to apply the sterilization process used in the present study to other types of microwave antennas, or they may react differently to the reprocessing cycles. Second, we had to cut off the Luer lock to ensure an air-tight seal for sterilization. This was necessary for appropriate sterilization of this type of antenna. We do not believe that there is any risk associated with this modification because it only affects the part of the antenna that pumps water and would not penetrate a patient. In a clinical setting, this may represent a disadvantage because of the additional manipulation needed before sterilization; however, it can be quickly and easily performed. Finally, the ablation zones were measured on manually traced ROIs of a cross-sectional area. The potential introduction of a measurement error by use of manually traced ROIs has been mentioned previously. Moreover, because of the design of the study, only a 2-dimensional area (cross-section) of the ablation zone was measured, rather than a 3-dimensional volume. According to manufacturer data, the ablation lesion should be perfectly ellipsoidal; therefore, the cross-sectional area of a
lesion should be representative of its volume. However, given that the ablation zones were sometimes not perfectly ovoid, it is possible that results for volume of an ablation zone would have differed.

On the basis of results for the present study, we suggest a maximum of 3 reprocessing cycles for the type of MWA antennas that were evaluated (ie, 4 uses) to minimize the risk of antenna failure. A larger number of reprocessing cycles is possible, but this would most likely have to be for each individual antenna (eg, if the control unit did not display a failure signal prior to subsequent use). We recommend careful examination of antennas prior to reuse. Although the antenna is designed to be a single patient-use product, the ability to reuse this instrument in multiple patients will dramatically reduce costs for clinical and research purposes. Furthermore, it must be reiterated that these results may not be applicable to other types of MWA antennas or to MWA antennas from other manufacturers.
Footnotes

a. Accu2i pMTA applicator, Microsulis Medical Ltd, Hampshire, UK.
b. Sulis VpMTA generator, Microsulis Medical Ltd, Hampshire, UK.
c. Microsulis Medical Ltd, Hampshire, UK.
d. Accu5i MTA temperature probe kit, 20 cm, Microsulis Medical Ltd, Hampshire, UK.
e. Sony Cyber-shot DSC-W50, 6.00 megapixels, 3.00 X zoom, Sony Electronics Inc, San Diego, CA.
g. Asepti-Zyme, Ecolab Inc, Saint Paul, Minn.
h. Fisherbrand amber natural rubber latex tubing, 3/16 X 1/16 wall, Fisher Scientific, Markham, ON, Canada.
i. Concept 400, Baker Ruskinn Global, Bridgend, South Wales, UK.
j. Nikon SMZ1500 stereoscope with a DS-Fi2 camera, Nikon Canada, Mississauga, ON, Canada.
m. PROC GLIMMIX, version 9.2, SAS Institute Inc, Cary, NC.
2.6 Figures

Figure 2.1—Photographs of an antenna and 2 thermistors inserted in a carved foam block to ensure reproducible placement for each ablation. The liver was placed in a temperature-controlled water bath (25°C).
Figure 2.2—Photograph of an ROI manually drawn around the margins of an ablation zone and used to measure cross-sectional area in a bovine liver. The central portion of the tract along the insertion of the antenna has a charred halo (white arrows). The scale on the left side is in centimeters.
Figure 2.3—Photograph of the tube of an antenna from which the Luer lock was removed with a small saw and sealed air tight for sterilization with hydrogen peroxide plasma to avoid water leakage (A). The air-tight seal was accomplished by use of rubber tubing and standard commercial zip ties. Photograph of a magnetic plunger, which is strapped down with elastic bands to ensure it did not come out of the water pump (B).
**Figure 2.4**—Photograph of an ablation performed near major blood vessels, which resulted in spreading of the ablation zone along the path of a vessel in the liver (white arrows) and a nonoval ablation zone. The scale on the left side is in centimeters.
Figure 2.5—Photographs of MWA antennas examined by use of stereo light microscopy. A—Tip of an unused antenna. B—Tip of an antenna after cycle 3 (a reprocessing cycle comprised ablation, cleaning, and sterilization). Notice the silicone tearing, yellow staining of the tip, and yellow spots on the tip. C—Tip of an antenna after cycle 1. Notice the mild yellow staining of the tip and a few yellow spots on the tip. Black bar scales = 2 mm.
Figure 2.6—Kaplan-Meier time-to-event curve illustrating the number of antennas that failed during 6 cycles of use (a reprocessing cycle comprised ablation, cleaning, and sterilization). Notice the sharp decrease at cycles 5 and 6, whereby 3 antennas failed at each of those cycles.
Chapter 3: Development of a microwave ablation protocol of normal canine long bones and bones with osteosarcoma.

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This study has been submitted to the American Journal of Veterinary Research (AJVR), and will be presented in abstract form (podium) at the conference of the Veterinary Interventional Radiology & Interventional Endoscopy Society in Cabo San Lucas, Mexico, June 22nd-24th, 2017.

3.1 Abstract

OBJECTIVE
Describe a technique to perform microwave ablation (MWA) in long bones of normal dogs, and develop a protocol for MWA zone sizes at different settings. Correlate MWA zones in normal bones to bones with neoplasia.

SAMPLE
11 femurs and 11 tibias from normal dogs, and 3 limbs with osteosarcoma (OSA).

PROCEDURES
Ablations at 4 different settings (80W-30 sec, 80W-50 sec, 120W-30 sec, 120W-50 sec) were randomized in the proximal and distal metaphyses of 11 normal femurs and tibias. A hole was drilled transversally, a MWA antenna inserted into the medullary cavity through a trocar, and the ablation performed. In the bones with OSA, ablations were performed at 120W-50 sec.
RESULTS
In the normal bones, a 3-way interaction was found between the type of bone, the location of ablation, and the combination of settings (p=0.052). Therefore, it was not possible to establish a rigorous chart of ablation zone sizes according to different combinations of settings. In the bones with OSA, some ablations were difficult to delineate due to pathological fractures. Overall the ablation sizes were slightly larger than in normal bones.

CONCLUSIONS AND CLINICAL RELEVANCE
A microwave bone ablation technique is described in normal canine long bones. For the 4 settings tested, it was not possible to build a rigorous chart of ablation zone sizes. This highlights the importance to monitor the ablation with diagnostic imaging. The MWA technique was applied to bones with OSA. Further work is needed to assess ablation zone size with different settings in OSA bones.

3.2 Introduction

Thermal ablation is used for the treatment of painful bone metastases (Nazario, 2011; Pusceddu, 2013; Filippiadis, 2014) and curative treatment of benign bone tumors in humans (Basile, 2014). It is usually performed with computed tomography (CT) guidance. Guidelines in human medicine are that percutaneous thermal ablation can be used as a curative therapy in metastatic disease with less than 3 lesions (oligometastatic), malignant lesions that are slow-growing and less than 3 cm in diameter each, or benign lesions such as osteoid osteoma, osteoblastoma less than 3 cm in diameter, or chondroblastoma (Gangi, 2010). However, in most cases, ablation is used as palliative therapy for bone metastases, to achieve pain reduction, local control of disease and tumor decompression (Simon, 2005; Pusceddu, 2013). When the bone is at risk of pathologic fracture or in weight-bearing areas, additional stabilization is required and ablation is usually combined with cementoplasty with or without metallic implants (Urban, 2016). Local tumor control can also be enhanced with embolization.
(Pusceddu, 2013; Filippiadis, 2014). In comparison with other thermal ablation techniques, microwaves propagate better through tissues with low conductivity and poor thermal conduction, such as bone (Brace, 2009; Hinshaw 2014). Microwaves resist bone’s high impedance and penetrate deeper, are less affected by tissue heating or desiccation, and are more effective for heating bone tumors (Webb, 2011). Reports of microwave ablation (MWA) for human bone tumors have been published. Some recent studies describe successful curative treatment of osteoid osteoma with MWA (Basile, 2014). Other studies report the use of percutaneous MWA as palliative therapy for painful axial and appendicular bone metastases when these are refractory to conventional approaches such as radiation or chemotherapy. Good pain relief for an average of 3-5 months and improvement of quality of life were achieved (Kastler, 2013; Pusceddu, 2013).

In the current medical literature there is a lack of a standard protocol of MWA to produce variably sized ablations in bone. Different authors have used different types of generators from different companies with different antennas (including a 4.5 GHz generator with a 17G antenna and a 2.45 GHz microwave generator with 14 or 11G water-cooled interstitial antenna) to perform percutaneous MWA of spinal and paraspinal bone metastases (Kastler, 2013; Pusceddu, 2013; Veltri, 2012). Amongst these studies, times of application varied from 30 seconds to 13 minutes, and power from 20 to 160W. Some of the studies used several cycles per ablation. Ablation sizes amongst the studies ranged from 1.2 cm to 7 cm, however no details were given about which settings produced what exact lesion size and at what location this was obtained, or if overlapping ablations were necessary.

Published literature in humans also indicates methods to use microwave antennas for ablation in bone. If the cortex is intact, a bone marrow biopsy needle can be used to pierce the cortex and serve as a coaxial introducer for the antenna to reach the lesion (Pusceddu, 2013). Once the antenna is in the tumor, the introducer is retracted before energy delivery is initiated so it does not interfere with microwave emission by the antenna tip. Once the ablation is completed, if osteoplasty is
warranted, the antenna is withdrawn and the introducer left in situ to be subsequently used for the cementoplasty procedure. If the cortical bone is lysed or interrupted by the tumor, the antenna is directly inserted in the lesion without the need for a coaxial introducer (Pusceddu, 2013).

Based on the human model, percutaneous thermal ablation could be a minimally invasive cancer therapy for animals. It could increase survival and decrease pain in oncologic patients with bony metastases, as described in humans, and could be an asset in limb sparing techniques. As there is variable data in the human literature regarding protocols of MWA in bone to produce variably sized ablations, and as microwaves may behave differently in canine bone marrow, there is a need to develop a chart for sizes of bone microwave ablation zones in dogs, before it is used in a clinical setting. Additionally, if the technique and chart for sizes of bone MWA zones is established in normal canine bones, it will be crucial to make sure that this applies to diseased bone, such as osteosarcoma (OSA) or metastasis. This data will be necessary for subsequent research and before the use of microwave ablation can be recommended in clinical trials.

The objectives of this research were 1) to describe a technique to perform MWA in normal canine long bones; 2) to establish different MWA zone sizes in normal canine bone at different settings; 3) to correlate MWA zone sizes in normal bones to ablation zones performed in bones affected with neoplasia (presumed OSA or metastasis). The hypotheses were that MWA would be feasible in normal long bones; that sizes would be established at different settings for MWA; and that the data obtained in normal canine bones would be applicable to ablation of canine long bones with primary or metastatic neoplasia.
3.3 Materials and methods

Microwave antennas with a 1.8mm shaft\(^a\) (15G) used with a 2.45GHz generator system\(^b\) were employed for all experiments.

3.3.1 Normal canine long bones

Eleven femurs and eleven tibias from six normal medium sized canine cadavers were used. Each frozen limb (at – 20°C) was thawed at room temperature 24 hours prior to the ablations. Ablations were performed in both metaphyses of each bone, for a total of 44 ablations. Four different combinations of settings (80W for 30 sec, 80W for 50 sec, 120W for 30 sec, 120W for 50 sec) were randomized in the proximal and distal metaphyses of each bone. These settings were chosen based on the human literature and a previous pilot study.

3.3.2 Osteosarcoma bones

Three canine cadaver limbs were obtained after euthanasia was performed for suspicion of bone neoplasia based on radiographs and/or physical examination and clinical signs. Affected breeds were large and giant breeds. One tibia, one humerus and one radius were affected. The limbs were frozen. It was chosen to perform the ablations at the maximal of the previously tested settings in normal bone (120W, 50 seconds).

3.3.3 Ablation procedures

To perform the ablations in normal bones, after dissection and retraction of the muscles and fascia overlying the long bones, holes were drilled transversally through the metaphyseal cortices with a 2.7mm diameter drill\(^c\) bit. A 13G trocar needle\(^d\) was placed into the drill hole (figure 3.1A), and the antenna inserted into the bone through
the trocar. Antennas were inserted through the cortex so that the active portion of the antenna (the junction between the metal shaft and the ceramic tip) was centered in the medullary cavity (figure 3.1B). The trocar was retracted from the ablation site along the shaft of the antenna prior to the ablation. During the ablations, the temperature at the surface of the adjacent bone cortex, cranial and caudal to the ablation site, were monitored with thermistors. Once the ablation was completed, the coaxial trocar was moved along the antenna, to be put back in place through the ablation site, and the antenna withdrawn, to simulate the setting for further cementoplasty. Wooden tooth-pics were inserted in the ablation track, to mark the tracts. After the ablations were performed, the bones were sectioned in half with a band saw along the long axis (going through the tooth-pics), exposing the ablation zones, which were photographed (figure 3.2A). The ablation zone sizes were measured and recorded (figure 3.2B). The same observer performed all measurements by tracing regions of interest at the margins of the ablation zones on dedicated software. The diameter of the bone at the location of the ablations was recorded. Ablation zone size and bone diameter were registered in a spreadsheet with other parameters such as side (left or right hind limb), bone (femur or tibia), location (proximal or distal metaphysis), and the combination of settings used (80W for 30 sec, 80W for 50 sec, 120W for 30 sec or 120W for 50 sec).

Differences in the ablation procedure of OSA limbs from the normal limbs were as follows. They were first imaged with CT to further characterize the distribution of the lesion, and plan the location of the ablations. It was not intended to ablate the whole lesion, to be able to see and measure each ablation zone separately. Similarly, the ablations were not supposed to overlap. Spacing of the ablations and the number of ablations were determined based on the size of the bone lesion and expected ablation sizes from the previous experiments in normal bone. The location and appearance of the lesion and of the bone also guided the orientation in which the ablations were performed. The cortices of the bones were drilled at obliqued angles (~30-45 degrees) to ensure the microwave emitting portion of the antenna was completely within the medulla. A 13G trocar was positioned through the hole. A 16G tru-cut biopsy needle was inserted through the trocar, or a 13G bone marrow biopsy needle was inserted
through the cortical drill hole, to obtain biopsies for histology. The MWA antenna was then inserted through the trocar into the bone, and the ablation performed as described above. After the ablations, the bones were sectioned, and the exposed ablation zones were photographed, and measured. When needed, additional small pieces of bone marrow and bone cortex were sampled with a scalpel and submitted for histopathology. Histopathology was interpreted by a board certified pathologist (RF).

3.3.4 Statistical analyses

Statistical analyses were performed by a statistician, using a dedicated software$^k$.

The data was analyzed$^l$ as a general linear mixed model. For ablation sizes in normal bones, there was a random effect of cadaver and fixed effect of side (right or left), location of the ablation (proximal or distal), type of bone (tibia or femur) and the combination of settings (power and time). The design was a 4-factor factorial in a randomized block design. In addition to the main effects, all interactions to the 4-way interaction were included in the initial model. Non-significant terms were removed from the model, starting from complicated terms first but preserving the hierarchy. Concerning the analysis of the temperatures for the ablations in normal bones, there was the additional fixed effect of site (cranial or caudal), and the data was incomplete with respect of having all factors combinations. Therefore, only interactions up to 3-way interactions were included in the model. Otherwise, the design was a 5-factor factorial in a randomized block design. The model was simplified in the same way as was done to study ablations sizes.

The assumptions of the ANOVAs conducted were examined via residual analyses. This included formally testing the residual for normalities, using the 4 tests offered by the software (Shapiro-Wilk, Kolmogorov-Smirnov, Cramér-von Mises, Anderson-Darling). In addition, the residuals were plotted against the predicted values
and the explanatory variables used in the models. Such analyses may have revealed the need for data transformation, unequal variances or outliers that need addressing.

For all tests, significance was set at values of $P < 0.05$.

For the bones with suspected neoplasia, descriptive statistics were performed, as only one tibia with a distal metaphyseal lesion was available (no replication), and as no normal humerus or radius had been ablated (confounding factors).

### 3.4 Results

The ANOVA assumptions were met.

#### 3.4.1 Normal canine long bones

MWA was successfully performed in the normal canine femurs and tibias. Ablation zones ranged between 0.325 cm² (ablation in a distal tibia) and 2.28 cm² (ablation in a proximal tibia). Per combination of settings, the median cross-sections of the ablation zones were 0.9925 cm² for 80W-30sec; 0.9747 cm² for 80W-50sec; 0.7785 cm² for 120W-30sec and 0.9586 cm² for 120W-50sec. Appendix IV lists the combination of median sizes and their confidence interval for each combination (bone, settings and location). A 4-way interaction was found between side (left or right hind limb), bone (femur or tibia), location (proximal or distal metaphysis), and the combination of settings. An outlier was present: one of the ablations was centered too cranial in the bone, and the portion of the ablation cranial to the cortex was non-existent (because no tissue surrounded the dissected bone); therefore the ablation zone was artifactually too small. When this outlier was dropped, a marginally significant 3-way interaction was found between the type of bone, the location of ablation and the combination of settings ($p=0.052$). However, since there were two 2-
ways interactions (bone by location, and bone by the combination of settings) that were quite significant (each p<0.01), and because they were entangled, the 3-way interaction had to be used. Therefore, it was not possible to study the effect of varying the settings on the sizes of the ablations, or to establish a rigorous chart of different ablation zone sizes according to different combination of settings. Appendix V lists in a table the significant differences in the 3-way interaction.

On average, the temperatures recorded at the distal metaphyses were higher than at the proximal metaphyses, but this was only significant for the tibias, and not for the femurs. For the tibias, the temperatures at the distal bone were about 37% higher (about 10°C) than the proximal (p<0.0001).

The temperatures recorded at the distal femoral metaphyses were higher by about 6°C compared to the temperatures at the distal tibia (p=0.0097). For a given ablation site, the temperatures measured caudally were on average 10.1% higher than the cranial temperatures (p=0.04).

Overall, the ablations performed with the combination of settings 120W-50sec led to higher recorded temperatures and the combination of settings 80W-30sec led to lower recorded temperatures (p<0.0001).

3.4.2 Osteosarcoma bones

On CT examination, aggressive bone lesions were found centered at the distal tibial metaphysis (mixed lytic and proliferative, with associated pathological bone fracture), centered in the mid aspect of the radial diaphysis (predominantly proliferative), and at the proximal humeral metaphysis (mixed lytic and proliferative, with associated pathological bone fracture) (figures 3.3A, 3.4A and 3.4C). The tibial and humeral lesions were presumed to be primary bone neoplasia such as OSA. Given its location, the radius lesion was presumed to be a bone metastasis over a primary
bone neoplasia. All lesions were subsequently confirmed on histopathology to be osteosarcoma.

In the tibial lesion, four ablations were performed (orientated ~30 degrees from perpendicular) (figure 3.4B). In the radius lesion, three ablations were performed (similar orientation) (figure 3.3B). In the humerus, three ablations were performed: the antenna was inserted vertically from the proximal epiphysis into the medullary cavity of the proximal metaphysis/diaphysis, and sequentially withdrawn proximally (~2 cm between each ablation) (figure 3.4D).

The humeral ablation margins were difficult to delineate, because the lesions macroscopically blended together, and seemed to track within the medullary cavity tissue. The ablation area that could be delineated in the humerus was 1.90 cm2 (figure 3.4E). In the radius, from proximal to distal, the lesions were 1.81 cm2; 1.96 cm2; 2.17 cm2 (median 1.974 cm2). Ablation areas were overall the best delineated in the radius (figures 3.3C and 3.3D). In the tibia, in a proximal to distal order, the ablation areas were 1.26 cm2; 1.84 cm2; 2.16 cm2; 3.51 cm2 (median 2.047 cm2). Subjectively comparing this to the median ablation zone sizes performed at identical settings (120W-50sec) in the distal metaphyses of the normal tibias (median 0.659 cm2), these were slightly larger. However, further statistical comparisons could not be performed as only one OSA tibia was available (no replication), and because no normal humerus or radius had been ablated, limiting comparisons with the OSA humerus and radius (confounding factors).

The temperatures recorded cranial and caudal to the radius and tibia remained low before and after the ablations (radius: temperatures pre-ablations ranging from 19-21°C, temperatures post-ablations ranging from 21-25°C; tibia: temperatures pre-ablations ranging from 20-22°C, temperatures post-ablations ranging from 21-23°C). For the humerus, the temperatures remained overall low, however the temperature caudal to the proximal ablation site spiked to 45°C at the end of the ablation (all of the temperatures pre-ablation ranged from 20-23°C, the other temperatures post-ablation
were 30 and 35°C).

3.5 Discussion

This study describes a microwave bone ablation technique in canine normal long bones. No major modification of the bone ablation technique used in humans was required for application in canine long bones. A hole was drilled through the intact normal cortices to be able to insert the antenna into the medulla.

It was not possible to build a rigorous chart of ablation zone sizes for the 4 settings tested, because a 3-way interaction was present between the type of bone, location of ablation and combination of settings, and no direct interaction was found between the combination of settings and the ablation zone sizes. This highlights the importance to monitor the ablation with diagnostic imaging techniques, performing for example immediate post-ablation imaging with CT, as well as follow-up imaging. In humans, this ensures that the desired ablation size and margins are obtained. Contrast-enhanced CT after MWA shows the area of thermocoagulation as a non-enhancing zone (Simon, 2006). Typically, the ablation size obtained is bigger than the target size, to get appropriate margins, since the target lesion is not excised, but left ablated in situ (Kawamoto, 2007).

The differences in temperature obtained in the normal bones are partially explained by the structure of the bones. Indeed, the tibia has a more narrow shaft distally than proximally, therefore the thermistors were closer to the ablation site distally, partially explaining why the recorded temperatures were higher than proximally. Similarly, it is expected that the highest combination of settings (120W-50sec) would lead to higher temperatures than the lowest combination of settings (80W-30sec). These results should be interpreted with caution, as during the ablations bubbles of boiling liquid were observed at the cortex hole and leaking on either side of the bone. This could have resulted in the temperatures being higher caudal than cranial.
to the ablation site, or why the temperatures recorded at the distal femoral metaphyses were higher than at the distal tibia, which otherwise were not logically explained by the bone morphology or antenna and thermistor location. This could have skewed the results obtained.

It is important to monitor the temperature in real time, and take precautions not to damage the surrounding structures during a procedure of microwave ablation. In human medicine, various techniques and guidelines exist to avoid burns to the tissues adjacent to the ablation site. One study specifies that a certain length (2cm) of the active tip of the microwave antenna has to be kept inside the ablated bone to avoid damage to the surrounding vital structures (Basile, 2014). Other procedures such as cooling with chilled saline, gas dissection or placing a sterile glove filled with cooled saline on the skin to protect sensitive structures adjacent to the ablation zone are also described (Fillipiadis, 2014).

The MWA technique was successfully applied to bones with OSA. If the cortex is very lytic, it is expected to be possible to directly insert the antenna in the tumor. Yet, this was not possible in the three OSA limbs we tested. The degree of cortical lysis was not sufficient to directly penetrate with the trocar, and the cortex had to be drilled. Therefore the authors anticipate that unless bony lysis is extremely advanced, a drill should always be available for the procedure. A trocar was used to simulate the technique needed if cementoplasty was to be performed after the ablation, as is done in humans when the underlying bone is too fragile and at risk of fractures, for example bones subjected to load, disrupted cortex and tumor tissue extending out of the bone, or extensive osteolysis (Pusceddu, 2013). The trocar is needed to guide the application of cement in the proper area. Initial placement of the trocar and drilling should be done with care so as not to induce a pathological fracture.

All final temperatures in the OSA bone ablations were low (very close to the initial temperatures), probably because the diameter of the pathological bones was larger than the normal bones, due to the presence of the neoplasia, creating a mass
effect. The antenna was therefore seated more deeply in the tissue, and the thermistors further away from the ablation zone. In addition, boiling liquid from the ablation site was only seen at the proximal aspect of the humerus, where the temperature recording was high, and the absence of this liquid at the other ablation sites likely gave more accurate (and low) temperature recordings.

The ablation zone sizes obtained in the OSA limbs were larger than in the normal bones. However, statistical comparisons were not possible for the radius and humerus, as similar normal bone ablations were not performed. Statistical comparisons were also not possible for the tibia because the number of OSA ablations were too few. Therefore, only a trend can be highlighted, and further work will be needed to fully correlate the ablation zone sizes obtained in OSA bones to normal bone. Should this tendency persist, larger ablation lesions in OSA bones may be due to different characteristics of the neoplastic tissue compared to normal tissue (lower impedance for example or higher water content).

Some limitations to the study must be acknowledged. First, some ablation lesions were difficult to trace and measure. This was true in normal bones and in the humerus with OSA. This could therefore have led to some imprecision of the measurements of the ablation zone sizes. Ablation margins were especially difficult to trace in the humerus with OSA, and seemed to have spread too far within the medullary cavity beyond the confines of the tumor. Freezing and thawing may have altered the tissue characteristics. Pathological fractures may have contributed to this also. In vivo, limbs with pathological fractures would not be amenable to limb sparing, and the tissues would not have been frozen. Therefore, the authors make the assumption that ablations will be easier to perform in vivo, in appropriate candidates. Second, only four combinations of settings were tested, and it is possible that different settings (with a longer application time for example) would have given larger ablation zones, that would have been significantly different in size from the ones obtained in this study. Also, if performed with a different unit, different settings could have been used, or several applicators introduced at the same time in the lesion (enabling a larger
ablation size). Third, in spite of not being significantly different, some differences were noted in the size of the ablation zones obtained with similar settings. However, it is uncertain that this would be clinically significant, as overlapping ablations can be performed if a larger ablation zone is needed, and as post-ablation imaging is recommended to make sure that complete ablation margins are obtained. Lastly, only three OSA limbs were available for the study, which is a small sample size. Different results regarding the ablation size could be obtained if more OSA limbs had been studied. Moreover, two limbs out of three had pathological fractures, which may have biased the results by altering the shape and size of the ablation zones.

In conclusion, a feasible MWA technique is described for canine long bones. It was not possible to compare ablation zone sizes at different settings. The technique was applied to canine long bones with OSA. Further work is needed to fully evaluate the behaviour of microwaves in neoplastic bone (primary or metastatic) before implementation of MWA clinical trials in oncologic patients. Extension of the technique to bones in living patients before limb amputation or euthanasia will also be necessary to make sure microwaves behave similarly \textit{in vivo} and \textit{ex vivo}. 
Footnotes

a. Accu2i pMTA applicator, Microsulis Medical Ltd, Hampshire, UK.

b. Sulis VpMTA generator, Microsulis Medical Ltd, Hampshire, UK.

c. Drill Model No. LDX120C, 20V MAX* Cordless Lithium Drill/Driver, Black and Decker, Mississauga, ON, Canada.

d. 13G trocar, Spectral Medical Devices, Wilmington, MA, USA.

e. Accu5i MTA Temperature Probe Kit, 20 cm, Microsulis Medical Ltd, Hampshire, UK.

f. Sony Cybershot DSC-W50, 6.00 Megapixels, 3.00 x zoom.


h. CT 16-slice helical scanner GE Bright Speed, General Electric Healthcare, Schenectady, New York, USA

i. 16G tru-cut biopsy needle, E – Z core, Products Group International, Lyons, CO, USA

j. 13G x 3 inch Monoject™ Snarecoil™ Bone Marrow Biopsy Needle, Covidien, Mansfield, MA, USA.

k. SAS 9.2, Cary N.C.

l. PROC MIXED (SAS 9.2, Cary N.C.)
3.6 Figures

Figure 3.1: A - Positioning of a trocar through the hole drilled in the proximal femoral metaphysis. B - Positioning of an antenna for ablation in the hole drilled in the proximal femoral metaphysis. Note: on this photo, the trocar is absent.
Figure 3.2: A - Longitudinal section of a normal femur where ablations have been performed in the proximal and distal metaphyses. B – Same image with ROIs traced around the ablation zone margins.
Figure 3.3: A – CT image of the radius with OSA (sagittal reformat) in a bone algorithm (slice thickness 0.625mm, WW 2180, WL 370) B – Ablation performed in the radius with OSA. Trocars are in place in the previously drilled holes. Two thermistors are in place cranial and caudal to the ablation site. C – Longitudinal section of the radius with OSA where the three ablation zones are very well delineated. D – ROIs have been traced around each ablation zone.
**Figure 3.4:** A – CT image of the tibia with OSA (dorsal reformat) in soft tissue algorithm (slice thickness 0.625 mm, WW 400, WL 40). Pathological fractures are present. B – Ablation performed in the tibia with OSA. Trocars are in place in the previously drilled holes. Two thermistors are in place cranial and caudal to the ablation site. C – CT images of the humerus with OSA (sagittal reformat) in soft tissue algorithm (slice thickness 0.625 mm, WW 400, WL 40). Pathological fractures are present. D – Ablation performed in the humerus with OSA. Trocar is in place in the previously drilled hole. E – Longitudinal section of the humerus with OSA where the 3 ablation zones are indistinct and blend together, tracking along the medullary cavity. There are also pathological fractures proximally.
Chapter 4: General Discussion.

4.1 Conclusions and perspectives of the results in veterinary medicine

Microwave ablation is one of many thermal ablation techniques increasingly used in human medicine. Such treatments are palliative and sometimes curative modalities (Simon, 2005; Dupuy, 2009; Ahmed, 2012; Nazario, 2011; Veltri, 2012; Clark, 2013). It is a minimally invasive cancer treatment option for patients not amenable to surgical resection. It can be used alone or in combination with other treatments such as chemoembolization, radiotherapy or chemotherapy (Simon, 2005; Bhardwaj, 2010 a; Kang, 2015; Linecker, 2016; Tang, 2016; van Amerongen, 2016). Promising results have been published in the literature with short and medium term outcomes with MWA of various neoplasms in people (Alexander, 2015; Sun, 2015; Zhang, 2015). Thermal ablation has a promising potential in veterinary oncology as a minimally invasive treatment for select patients. A few case reports and studies regarding thermal ablation have been published in veterinary medicine (Pollard, 2001; Mallery, 2003; Rasor, 2007; Case, 2015; Boston, 2016). The main process by which MWA leads to cellular death is based on dielectric hysteresis. Advantages of MWA over other thermal ablation techniques such as radiofrequency ablation (the most used in human medicine), include obtaining larger ablation zones in a shorter time, higher intra-tumoral temperatures (Clark, 2013; Hinshaw, 2014), decreased susceptibility to the heat sink effect that leads to decreased ablation lesion size near large vessels that carry away the heat in their vicinity (Simon, 2005; Awad, 2007; Garrean, 2009; Ahmed, 2011), and absence of grounding pads (Nazario, 2011; Liang, 2013). The latter is especially advantageous in our veterinary patients, because no fur clipping is needed; additionally, it avoids the risk of skin burns, and there is no contraindication in specific patients such as those with a pacemaker.

The first obstacle to the use of MWA in veterinary patients is the cost of the technique. A microwave unit costs about $CAD55000. Additionally, a single MWA antenna is more than $CAD2000. Currently, in human medicine, one antenna is for use
in a single patient. It can be used for up to 8 ablations in one patient. In veterinary medicine, it is not uncommon for single use devices to be sterilized and reused safely in several patients (Gatineau, 2012; Coisman, 2013). At the author’s institution, reuse of numerous single use devices is routinely performed without any complication. As these antennas are used in the body cavities, and normal use can lead to damage of the antennas, it is vital to assess the longevity and sterility of the antennas following reprocessing and sterilization before reuse in live patients. The ultimate goal of this research was to determine if it is effective and to decrease the cost of each procedure. The first part of this research partially rejected the hypothesis that MWA antennas would remain structurally and functionally sound for up to six total cycles (use and reprocessing) with variable numbers of ablations performed for each use (1, 2 or 3) in bovine cadaver livers. Indeed, a marked decrease in the number of functional antennas was found at the 5th and 6th cycles. Therefore, to minimize the risk of antenna failure, a maximum of 3 reprocessing cycles (4 uses) is recommended for the type of MWA antenna evaluated. This recommendation is made knowing that the majority of the antennas remained functional up to 4 reprocessing cycles (5 uses), and that some antennas were also functional at subsequent cycles. A larger number of reprocessing cycles is possible beyond the recommendation, but is best determined on a case-by-case basis for each individual antenna. Structurally sound and functional antennas, when attached to the generator, can thus be reused to their maximum but only after careful examination of antenna. Reprocessing damages the thin silicone coating of the antennas over time, mainly from heating, but also from reprocessing. This does not impair the sterility or functionality of the antennas at the minimal amount that occurs. The possibility that fragmented pieces of silicone would induce an inflammatory reaction in the ablated tissue is considered unlikely because the tissue underwent coagulation necrosis, and should not elicit an inflammatory response. More extensive damage to the silicone could lead to the inability to withdraw the antenna from the ablated tissue, and damage to the shaft itself could lead to non-functionality of the antenna including breakdown of connections between the ceramic tip and the shaft, and angulation of the tip. Because of this, examination of the antennas should be performed prior to each reuse, and it should be discarded if any such finding is noted. One new antenna should always be available, to replace a damaged reused antenna. The safe use of one MWA antenna in up to 4 patients will dramatically reduce costs and
help to democratize the use of this novel technique for clinical cases. It will also help decrease the cost of subsequent research involving MWA. These results are only applicable to the type of MWA antenna used in this study, and may not be applicable to MWA antennas from other manufacturers.

One of the advantages of MWA over other thermal ablation techniques such as radiofrequency is the excellent propagation of microwaves in tissue with high impedance and poor conductivity, such as lung or bone (Brace, 2009; Clark, 2013; Hinshaw, 2014). This led to the investigation of potential uses of MWA in bones of veterinary patients. In the human literature, use of MWA is reported as a palliative technique for painful bone metastases that are refractory to conventional treatments such as chemotherapy or radiotherapy, with good short- and medium-term outcomes (Kastler, 2013; Pusceddu, 2013). MWA can also be used as a minimally invasive curative technique for benign bone neoplasms such as human osteoid osteomas (Basile, 2014). However, scant and wide ranging data is available in the literature regarding correlation between power and time settings of the microwave unit and sizes of the ablation zones obtained in bone. Such data exists for other organs such as liver, kidney and muscle, and a chart of ablation sizes in these organs is available from the manufacturer of the microwave unit and antenna used. MWA bone techniques are well described in humans (Pusceddu, 2013; Basile, 2014; Fillipiadis, 2014), however interspecies morphological and histopathological differences could lead to the necessity to refine and adapt the technique for canine patients. Thus, the first step of the second part of the project was to develop a technique to perform MWA in long bones of normal dogs and to establish different ablation zone sizes for different settings. MWA was shown to be feasible in long bones of normal dogs, and the technique was described here. However, ablation zone sizes could not be established at different settings for MWA in long bones of dogs. For the four settings tested, it was not possible to establish a correlation between ablation zone size and the settings used. These ablation zone settings were chosen to allow measurements of ablation zones without overlapping, and were not intended to ablate a large section of bone. This highlights the importance to monitor the ablation with imaging techniques. Monitoring of the ablations by imaging is routinely done in human medicine, ensuring appropriate margins are obtained and that no residual neoplasia is present (Simon, 2005;
Kawamoto, 2007; Nazario, 2011). This also establishes a baseline for follow-up imaging.

The last aspect of the project was to apply the developed technique of microwave bone ablation in normal bones to canine long bones with neoplasia. The third hypothesis was that the data obtained during ablations in normal canine bones would be applicable to ablation of canine long bones with primary or metastatic neoplasia. Promising results were obtained with this pilot study, as microwave ablation was successfully performed in canine bones with OSA. Only descriptive results were obtained regarding the ablation zone sizes in OSA bones. No statistical correlation could be made between the sizes obtained in normal and pathological bones at similar settings. This was because only three OSA limbs were available for this project, and two were on different bones than the normal ones tested. A direct comparison was not possible. Larger ablation zones were obtained in OSA bones compared to the normal bones, however no definitive conclusion can be drawn regarding any potential difference in behavior of microwave ablation in OSA versus normal bone tissue. More data must be collected on OSA bones before drawing such conclusions. Given that CT guidance and monitoring of ablations can and should be performed, it is reasonable that the application of MWA to metastatic or primary bone neoplasms will be accepted. This imaging should ensure that appropriate margins are obtained, even if the procedure needs to be done step by step to treat the entire lesion. MWA could be applied to patients with painful bone metastasis, or as an additional limb sparing technique for small primary bone neoplasms.

4.2 Future research and future potential applications in veterinary medicine

The technique of MWA used for this study and the extensive literature in human medicine (Simon, 2005; Ong, 2009; Bhardwaj, 2010 a; Bhardwaj, 2010 b; Lloyd, 2011; Webb, 2011; Liang, 2013; Tombesi, 2013; Hinshaw, 2014; Alexander, 2015; Zhang, 2015; Asvadi, 2016; Carberry, 2016) make it readily applicable to ablate hepatic metastases, or cytoreducing primary hepatic neoplasms that are non-surgical candidates (to prevent hemorrhage for example). This could be performed
percutaneously with ultrasound or CT-guidance, however, this could also be performed with laparoscopy or an open surgical approach.

Additional research in neoplastic bones would be beneficial before the application of MWA in clinical patients. Preliminary data from this research project is a solid foundation to additional research on this topic.

Additional areas of research and potential applications for MWA in veterinary patients are varied and include ablation of renal, primary or metastatic pulmonary neoplasms as is already performed in human medicine (Simon, 2005; Dupuy, 2009; Hinshaw, 2014; Sun, 2015), and neoplasms in other organs. Potential of this technique is great, and research in veterinary medicine is just starting to explore this. It is expected that MWA use will keep growing and become a more preponderant minimally invasive technique for veterinary patients in the next few years.
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Penna TC, Ferraz CA, Cassola MA. The presterilization microbial load on used medical devices and the effectiveness of hydrogen peroxide gas plasma against Bacillus


Schramm W, Yang D, Wood BJ, Rattay F, Haemmerich D. Contribution of direct


Tessarolo F, Caola I, Nollo G, Antolini R, Guerrera GM, Caciagli P. Efficiency in


APPENDIX

I. MICROWAVE ABLATION SIZES ESTABLISHED AT DIFFERENT SETTINGS IN LIVER, KIDNEY AND MUSCLE, WITH THE SYSTEM USED IN THIS STUDY (Accu2i pMTA APPLICATOR USED WITH Sulis VpMTA GENERATOR, MICROSULIS MEDICAL LTD, HAMPSHIRE, UK).

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<tr>
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<td>3.9 x 4.0cm</td>
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Diameter x length
II. ILLUSTRATION OF THE MICROWAVE ABLATION ANTENNA USED (Accu2i pMTA APPLICATOR, MICROSULIS MEDICAL LTD, HAMPSHIRE, UK).

Chilled saline is pumped and put into motion by the pumping chamber, and circulates in the cable during the ablation to cool down the antenna.
III. MICROWAVE GENERATOR UNIT USED (Sulis VpMTA GENERATOR, MICROSLIS MEDICAL LTD, HAMPSHIRE, UK).
IV. COMBINATION OF MEDIAN SIZES AND CONFIDENCE INTERVALS FOR EACH COMBINATION (BONE, SETTINGS AND LOCATION) IN NORMAL BONES.

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f: femur; t: tibia; d: distal; p: proximal
V. SIGNIFICANT EFFECTS FOR THE 3-WAY INTERACTION IN NORMAL BONES.

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f: femur; t: tibia; d: distal; p: proximal