Accuracy and Safety of Image Guided Percutaneous Injection of Gelified Ethanol (Discogel®) in the Intervertebral Disc in Dogs

By

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Accuracy of image guided injection of the intervertebral discs and the distribution and safety of a gelified ethanol product was investigated in two phases. In the first phase, the ability to accurately perform percutaneous injection of gelified ethanol into intervertebral discs of cadaver dogs using three imaging modalities was evaluated (n=14). Injections were performed at random sites along the vertebral column (n=78 discs). Systematic dissection was performed to confirm the location of the injected preparation. The material was successfully delivered to the nucleus pulposus in 55 of 78 (71%) intervertebral discs. Forty-nine of 78 (63%) injections had leakage of the injected material including 10 (13%) with material in the vertebral canal. The success of injections did not differ by site (p=0.9337) or body weight (p=0.3273). The odds of a successful injection without significant leakage were 12 times higher when using computed tomography (CT) compared to ultrasound (p=0.0026), and trended towards significantly higher with CT than fluoroscopy (p=0.0620).

In the second phase, the feasibility of image guided injection developed in phase I was tested in 10 healthy dogs at the lumbosacral disc. Successful
injection of the gelified ethanol was achieved in all 10 dogs, with material evident in the vertebral canal in 4 dogs. Short-term (n=10) and long-term (n=5) follow-up CT and magnetic resonance imaging (MRI) as well as neurological examinations were used to evaluate the effects of the injection. All dogs tolerated the injection well; there were no clinical adverse reactions noted over the study period. The long term follow up CT and MRI studies revealed a static appearance in 3 of 5 dogs with evidence of redistribution of the injected preparation in 2 of 5 dogs.

In conclusion, percutaneous injection of the intervertebral discs is achievable with multiple imaging modalities but is most accurate using CT guidance. Injection of gelified ethanol into the lumbosacral disc of healthy dogs is well tolerated with no adverse effects noted, even with mild leakage of material from the disc. This work provides a solid foundation for the development of this technique as a potential treatment option for dogs with intervertebral disc protrusions.
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Declaration of work performed:

The work presented here is, to the best of my knowledge and belief, original and the result of my own investigations, except as acknowledged.

Formulations and ideas taken from other sources are cited as such. I declare that all the work in this thesis was performed by me with the exception of the procedures indicated below:

Some of the cadaver injections and dissections described in chapter 2 were performed by Dr. Heather Chalmers. Dr. Jeff Caswell also aided in performing cadaveric dissection of the intervertebral discs following percutaneous injections.

Four of the live dog injections described in chapter 3 were performed by Drs. Heather Chalmers or Brigitte Brisson.

Dr. Luis Gaitero performed some of the neurological examinations discussed in chapter 3.

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List of abbreviations:

CT – computed tomography
MRI – magnetic resonance imaging
IVD – intervertebral disc
AF – annulus fibrosus
NP – nucleus pulposus
DLSS – degenerative lumbosacral stenosis
C – cervical
TL – thoracolumbar
L - lumbar
LS - lumbosacral
INTRODUCTION

Intervertebral disc (IVD) herniation is a common and debilitating disease in dogs and humans. Two types of IVD herniation have been described in dogs: extrusion and protrusion (Hansen, 1952). Extrusion of IVD material occurs when the central portion of the IVD, the nucleus pulposus (NP), is propelled through a complete rupture of the outer portion of the IVD, the annulus fibrosus (AF), and into the vertebral canal. IVD extrusion tends to occur in small-breed dogs, particularly chondrodystrophic breeds, and is usually of acute onset (Hansen, 1952; McKee, 2000a; Brisson, 2010; Smolders, 2013; Jeffery, 2013). Protrusion of the IVD occurs more in older, large-breed dogs, and results from partial herniation of the NP into AF tears and annular hypertrophy (Hansen, 1952; Macias, 2002; Brisson, 2010; Smolders, 2013; Jeffery, 2013). Clinical signs associated with extrusion or protrusion of the IVD is attributed to compression of the spinal cord and/or nerve roots. Clinical signs of IVD protrusion tend to be more chronic in duration (Hansen, 1952; Macias, 2002; Brisson, 2010; Smolders, 2013; Jeffery, 2013). Clinical signs in either case can range from spinal hyperesthesia to complete paralysis and loss of sensory function in the limbs (Sharp, 2005; Dewey, 2008). Surgery is a well accepted treatment for acute IVD extrusion with good success rates; however, dogs with IVD protrusion undergoing surgical treatment tend to have a poorer overall recovery rate ranging between 33-88% (Schmid, 1993; McKee, 2000b; Macias, 2002; Moissonnier, 2004; Cherrone, 2004; Kinzel, 2005; Downes, 2009). With the lower success rate, the risks of surgery in cases of IVD protrusion may outweigh the benefits. In fact, clinical postoperative deterioration in neurological status is common after surgical treatments for protrusion of the IVD (McKee, 2000b). Classically, surgical decompression of IVD protrusion requires more spinal cord manipulation than IVD extrusion, which has been considered a main leading factor associated with this higher morbidity (Mckee, 2000b). Poor vascular perfusion of the injured cord, reperfusion injury or low-grade post-surgical vertebral instability can also contribute too less favourable outcomes following surgery for IVD protrusion (Toombs, 2003; Sharp, 2005). Surgery has a more limited success
in dogs with IVD protrusion compared to IVD extrusion; however, traditional medical management also typically fails for these patients as they experience chronic pain and progressive loss of function. For these reasons, less invasive, non-surgical effective treatment options would be beneficial for this condition.

The canine model of IVD disease has been shown to be similar to the disease process seen in humans (Bergknut, 2012). In human medicine multiple minimally invasive procedures have been evaluated for the treatment of clinical signs associated with protrusions of the IVD (Smith, 1964; Smith, 1967; Fraser, 1984; Andreula, 2003; Kelekis, 2005; Raj, 2008; Guarnieri, 2009; Kelekis, 2010). Few reports have been published on minimally invasive injectable therapy and minimally invasive surgical treatment for canine IVD disease (Smith, 1967; Garvin, 1973; Atilola, 1988; Takahasi, 1997; Han, 2007; Carozzo, 2011; Lockwood, 2014). Some of the more studied minimally invasive treatments in humans involve image guided percutaneous injection of a lysing agent into the protruded disc to reduce spinal cord compression and cause the disc to recede from its herniated position. These procedures are termed chemonucleolysis and have the advantage of offering reduction in pain without the risks of invasive surgery. In human patients both fluoroscopy and computed tomography (CT) have been used to facilitate intradiscal delivery of therapeutics (Muto, 2004; Kelekis, 2005; Oder, 2008; La Tessa, 2009; Guarnieri, 2014). Fluoroscopic guided injection of IVDs has been performed in dogs using chemonucleolytic agents (Atilola, 1988; Miyabayashi, 1992; Takahasi, 1997; Han, 2007), but the accuracy of the injection and the use of CT or ultrasound guidance have not been previously described.

Chemonucleolysis has been performed using various substances including chymopapain, collagenase, ozone, and ethanol. In veterinary medicine chemonucleolysis via chymopapain, collagenase and ozone injections have been described (Smith, 1967; Garvin, 1973; Atilola, 1988; Miyabayashi, 1992; Han, 2007). All have shown some promise in the treatment of chronic protrusion of the IVD; however, chymopapain has been associated with hypersensitive and anaphylactic reactions, which has led to its withdrawal.
Nucleolysis using chymopapain was successful in the treatment of IVD herniations in humans, therefore there is a high demand for a new safer product that has the same efficacy. A new product, Discogel®, has been developed that is a combination of ethanol and ethylcellulose (Theron, 2007). The ethylcellulose increases the viscosity of the ethanol creating a gelified product, which allows better control during injection and less dispersion of the injected material from the injection site (Theron, 2007). At the moment, clinical studies including over 400 human patients with cervical or lumbar disc herniations report success rates of up to 92% based on resolution of pain, minimal or no activity limitations and the ability of the patient to return to work (Theron, 2007; Theron, 2010; Stagni, 2012; de Seze, 2013; Bellini, 2013). The gelified ethanol product was also reported to cause no morphostructural changes in muscular and neurological tissue following injection of the soft tissues and vertebral canal in a porcine model (Guarnieri, 2010). The early success of treating disc herniation in humans with this new compound is encouraging for its application to this debilitating and important veterinary disease.
CHAPTER I – GENERAL LITERATURE REVIEW

I. INTERVERTEBRAL DISC DISEASE

Anatomy and Function of the Intervertebral Disc

The vertebral column of the dog is composed of multiple vertebrae, each separated by an intervertebral disc (IVD), with the exception of the first 2 cervical vertebrae (Hansen, 1952; Evans, 1993). Each IVD is made up of four components, the nucleus pulposus (NP), annulus fibrosus (AF), a transitional or perinuclear zone and the cartilaginous end plates (Figure 1.1) (Hansen, 1952; Evans, 1993; Bray, 1998a; Bergknut, Smolders, Grinwis, Hagman, Lagerstedt, Hazewinkel, Tryfonidou, & Meij, 2013).

Figure 1.1 Dissection of a healthy IVD in a dog

NP = Nucleus Pulposus; TZ = Transition zone; AF = Annulus Fibrosus; CEP = Cartilaginous End Plate
These structures work together to absorb and redistribute strains and forces experienced by the animal (Hansen, 1951; Evans, 1993; Bray, 1998a; Brisson, 2010; Bergknut, Smolders, et al., 2013). The IVD is able to maintain stability of the vertebral column most effectively when faced with 4 main physiologic forces; tension and compression, torsion, and shearing (Hansen, 1952; Brown, 1957; Farfan, 1970; White, 1990; Bray, 1998a; Benninger, 2004; Heidiger, 2009). An understanding of the normal anatomy and function of the IVD is important to comprehend IVD disease and the mechanisms and targets of different treatment options.

The embryonic development of the vertebral bodies is the result of site-specific changes induced by the notochord (Sinowatz, 2010). In regions where the notochord is surrounded by the developing vertebral bodies the notochord degenerates, while between the developing vertebral bodies the notochord persists and forms the NP (Evans, 1993; Sinowatz, 2010). During development the AF differentiates from sclerotomes surrounding the notochord (Sinowatz, 2010).

In the mature animal, the NP is located eccentrically within the IVD and has a gelatinous consistency and appearance (Hansen, 1952; Evans, 1993). The NP is composed of proteoglycans that contain multiple glycosaminoglycan subunits and are mixed with a relatively low amount of type II collagen fibers (Hansen, 1952; Ghosh, 1977; Cole, 1985; Cole, 1986). Within the NP, the glycosaminoglycans create a strong osmotic gradient and thus attract and bind water (Hansen, 1952; Ghosh, 1977; Cole, 1985; Cole, 1986). This high water-binding capacity of the NP is closely related to the elasticity and therefore the ability of the IVD to respond to compressive forces (Humzah, 1988). The normal NP has a relatively low amount of collagen, mainly type II collagen, similar to articular cartilage, with no traces of type I collagen (Bray, 1998a; Nerlich, 1998; Mckee, 2000a). The NP is bound circumferentially by the AF and cranially and caudally by the cartilaginous end plates (Hansen, 1952).

The mature AF is composed mainly of fibrous tissue arranged in incomplete
rings of parallel fibers termed lamellae that are interlocked with each other by interlamellar fibers (Hansen, 1952; Evans, 1993). The lamellae are more numerous ventrally and thus the AF is thicker ventrally than dorsally; this creates the offset position of the NP mentioned above (Hansen, 1952; Evans, 1993). Closer to the NP, the fibers of the AF lose their structure and are progressively more disorganized (Hansen, 1952; Evans, 1993; Bergknut, Smolders, et al., 2013). This zone is frequently referred to as the transition or perinuclear zone (Evans, 1993; Bergknut, Smolders, et al., 2013).

Interestingly, it has been found that in chondrodystrophic dogs the transitional zone is wider than other breeds resulting in a less distinct transition (Hansen, 1952; Braund, 1975). The dorsal and ventral two thirds of the AF connect to the epiphysis of the adjacent vertebral bodies while the inner third connects directly to the cartilaginous end plate (Hansen, 1952). The peripheral lamellae of the AF also have connections to the dorsal and ventral longitudinal ligaments of the vertebral column (Bray, 1998a). As different forces compress the NP, the AF must react to constrain the NP via tightly packed arrangement of the lamellar fibers (Bray, 1998a). The AF also plays an important role in providing flexible connections between adjacent vertebral bodies (Michalek, 2012). Although the normal AF contains some type II collagen fibers at the innermost layers, the majority is type I collagen (Evans, 1993; Nerlich, 1998; Raj, 2008). This is similar to other types of fibrous connective tissue including most ligaments (McIlwraith, 1996). The high proportion of type I collagen allows the AF to respond well to bending or stretching movements, however because of the lack of type II collagen the AF responds poorly to compressive forces (Bray, 1998a).

The cartilaginous end plate is a thin layer of hyaline cartilage that separates the cranial and caudal vertebral bodies from the IVD and represents the anatomic limit of the disc (Hansen, 1952). Both the NP and the AF come into contact with the cartilaginous end plate (Hansen, 1952). The major functions of the cartilaginous end plate include confinement of the AF and NP within the appropriate anatomic boundaries and facilitation of diffusion of nutrients between the vertebral bodies and the IVD (Hansen, 1952; Crock, 1984).
The structures that support the IVD play an important role in the normal function of the spine (Hansen, 1952). The vertebral column of the dog is composed of multiple vertebral bodies; most typically 7 cervical, 13 thoracic, 7 lumbar, 3 fused sacral and a variable number caudal vertebrae (Hansen, 1952; Evans, 1993). Each vertebra is separated from the adjacent ones by the IVDs, with the exception of first 2 cervical vertebrae (Hansen, 1952; Evans, 1993). The dorsal and ventral longitudinal ligaments run the entire course of the spine to the level of the sacrum supporting and stabilizing the vertebral bodies along its dorsal and ventral aspects and confining the IVD (Hansen, 1952; Evans, 1993). In the thoracic region, the dorsal longitudinal ligament is augmented by the intercapital ligaments, which runs transversely between the right and left ribs from the 2nd to 11th thoracic vertebrae (Hansen, 1952; Evans, 1993). The intercapital ligament between the 11th ribs is smaller in size (Evans, 1993). Additionally, another ligament, the ligament of the head of the rib, is found extending from the head of the rib, to the IVD and the adjacent vertebral bodies (Hansen, 1952; Evans, 1993). Along the dorsal aspect of the vertebral canal, the ligamenta flava (yellow ligament) joins the arches of adjacent vertebrae providing additional stability (Evans, 1993). The continuous supraspinous ligament covers the dorsal aspects of the vertebral spinous processes while the individual interspinous ligaments bridge the base of adjacent spinous process (Evans, 1993). Dorsally, the caudal articular process of the more cranial vertebra and the cranial articular process of the more caudal vertebra form bilateral synovial articulations (Evans, 1993). Additional support and stabilization of the vertebrae is provided by the paraspinal musculature (Hansen, 1952).

**Terminology**

The terminology of IVD pathology in veterinary medicine has historically been confusing with overlap and inconsistent use of terminology. This has recently been addressed and adaptation of terminology used in human medicine and neuroimaging has been recommended (Levine, 2015). The terminology used throughout this thesis is based on these recent recommendations.
Intervertebral Disc Degeneration

Both cellular and biochemical changes occur within the IVD during normal maturation and aging, leading to alteration of the biomechanical properties of the IVD that can cause dysfunction (Hansen, 1952; Cole, 1986; Bergknut, Smolders, et al., 2013). Specifically, a decrease in proteoglycan and increase in collagen content cause the NP to lose its hydrostatic properties and take on a more rigid form (Hansen, 1951; Hendry, 1958; Cole, 1986; Pearce, 1987; Bergknut, Smolders, et al., 2013). These changes to the IVD matrix impair the already limited ability of the IVDs to receive nutrients leading to further degeneration (Horner, 2001; Bergknut, Smolders, et al., 2013). The degenerative changes result in inappropriate discal response to compression forces via redistribution of forces to the end plate and AF (Adams, 1996; Meij, 2010). Eventually, this process may lead to thickening and microfractures of the endplates and tears in the AF (Boos, 2002; Bergknut, Smolders, et al., 2013; Bergknut, Meij, et al., Hagman, de Nies, Rutges, Smolders, Creemers, Lagerstedt, Hazewinkel, & Grinwis, 2013). This thickening of the vertebral end plates leads to further impairment of diffusion of nutrients and fluid creating a cyclical degenerative pattern (Nachemson, 1970; Horner, 2001; Benneker, 2005; Bergknut, Smolders, et al., 2013). In dogs, two specific models of IVD degeneration have been described; chondroid metaplasia and fibrous metaplasia (Hansen, 1951; Hansen, 1952).

In chondrodystrophic dogs chondroid metaplasia is the more common form of degeneration and is characterized by a transformation of the NP to chondroid tissue (Hansen, 1951; Hansen, 1952). This process occurs at a young age and typically all IVDs follow a similar course of degeneration (Hansen, 1951; Hansen, 1952; Braund, 1975). Chondroid metaplasia is associated with marked dehydration of the NP, which is related to a decrease in the proteoglycan content of the NP (Hansen, 1951; Hansen, 1952; Cole, 1986). Additionally, within the NP there is an increase in type I collagen, which is normally not present (Hansen, 1951; Bray, 1998a; Bergknut, Smolders, et al., 2013). These alterations result in a change of the normally gelatinous NP to a firm tissue, which in many instances becomes partially mineralized (Hansen,
As the degeneration of the NP progresses, the distinction between the NP and AF becomes less recognizable (Hansen, 1952). In chondrodystrophic dogs, alteration of the AF occurs subsequent to degeneration of the NP (Smolders, 2013).

Fibrous metaplasia was first described as a continuous, progressing maturation process and not specifically as a model of degeneration, although concurrent degeneration was often seen (Hansen, 1951; Hansen, 1952). Fibrous metaplasia shares many of the same characteristics as chondroid metaplasia, however it occurs at a later age (Hansen, 1951; Hansen, 1952). In the majority of non-chondrodystrophic dogs, the gelatinous nature of the NP is retained over most of their life; however, in advanced age a more dehydrated, fibrous NP develops (Hansen, 1951; Hansen, 1952). During fibrous metaplasia of the IVD, the NP becomes organized into lobules and with continuing degeneration the interlobular septae thicken, creating fibrillar bundles encompassing the NP cells (Hansen, 1951; Hansen, 1952; Bray, 1998a; Smolders, 2013). In cases of fibrous metaplasia, degenerative changes in the AF are observed concurrently, or in some cases, earlier, than those in the NP, indicating that annular degeneration may not always be accompanied by NP degeneration (Hansen, 1951; Hansen, 1952; Bray, 1998b; Smolders, 2013). As the AF becomes damaged, small tears can occur and the NP may herniate in between these tears (Hansen, 1952). Fibrous metaplasia occurs anywhere along the vertebral column and can affect more than one IVD but does not typically affect all IVD equally (Hansen, 1951; Hansen, 1952). Calcification of the NP is very rare in fibrous metaplasia (Hansen, 1952).

Although differentiation between either fibrous or chondroid metaplasia has been done since the initial description of these categories, there is increasing support that this distinction is not always clear and the processes are more similar than often described (Hansen, 1952; Smolders, 2013; Kranenburg, 2013). These studies have found chondroid metaplasia of the NP in some non-chondrodystrophic dogs that is similar to that seen in chondrodystrophic dogs (Hansen, 1951; Hansen, 1952; Bray, 1998b; Smolders, 2013;
Kranenburg, 2013). The early onset of degeneration in chondrodystrophic dogs suggests a genetic or conformational component, while the longer duration of changes seen in non-chondrodystrophic dogs is more suggestive of a ‘wear and tear’ etiology (Hansen, 1951; Hansen, 1952; Stigen, 1993).

Degenerative IVD changes in humans share several similar characteristics to both chondroid and fibrous metaplasia in dogs. As such, the dog has been used as a model of IVD degeneration in many studies (Singh, 2005; Erwin, 2006; Sether, 1990; Levine, 2011; Bergknut, 2012). Similar to dogs, in humans affected with IVD degeneration the disc has an altered gross appearance and becomes less organized histologically (Raj, 2008). A study comparing IVD degeneration in humans to that in both chondrodystrophic and non-chondrodystrophic dogs found that the histological appearance, histological grading, collagen and proteoglycan content were similar between all three groups (Bergknut, 2012). This study concluded that the canine is a suitable model for human IVD degeneration (Bergknut, 2012). Due to the difference in the timeline of IVD degenerative changes, it has been suggested that depending on the type of study it may be more appropriate to use one type, chondrodystrophic or non-chondrodystrophic dogs, over the other (Bergknut, 2012). Due to the early onset of degeneration affecting all IVDs, chondrodystrophic dogs may be better suited for studies assessing treatments aimed at impeding degeneration or long-term studies assessing the process of degeneration (Bergknut, 2012). Conversely, the disease process of IVD degeneration in non-chondrodystrophic dogs is more similar to humans and therefore non-chondrodystrophic dogs may be more suited for investigating treatments for clinical IVD degeneration (Bergknut, 2012). This includes similarities between lumbosacral disease in dogs and humans (Bergknut, 2012).

Intervertebral Disc Herniation

Degenerative changes of the IVD have been observed in clinically normal dogs and may be considered an age related change (Hansen, 1951; Hansen, 1952). However, these degenerative changes can also lead to significant
pathology (Hansen, 1951; Hansen, 1952). The pathology of clinically significant IVD disease is most commonly related to an alteration of the location of the IVD material related to either a partial rupture of the AF (Hansen type II) or complete rupture of the AF (Hansen type I) (Hansen, 1951; Hansen, 1952). In more recent literature these two processes have been referred to as IVD extrusion (Type I) or IVD protrusion (Type II) (Bray, 1998b; Mckee, 2000a; Brisson, 2010; Smolders, 2013; Kranenburg, 2013). Intervertebral disc extrusion is typically associated with chondroid metaplasia in chondrodystrophic dogs while IVD protrusion is typically associated with fibrous metaplasia in non-chondrodystrophic dogs. However, there is substantial overlap between these categories (Hansen, 1951; Hansen, 1952; Mckee, 2000a; Bergknut, 2012; Kranenburg, 2013).

In IVD extrusion, an abnormal distribution of forces in the IVD causes damage to the AF, including loosening and fragmentation of the normal lamellae. Eventually, this allows the NP to be propelled from the center of the IVD through a complete rupture of the AF and into the vertebral canal (Hansen, 1951; Hansen, 1952). Histologically, these extrusions have been classified as acute, with the presence of disc material and hemorrhage, sub-acute that has the addition of inflammation and chronic, where fibrous adhesions are seen between the material and the dura (Hansen, 1952). Intervertebral disc extrusion tends to cause more severe and acute clinical signs compared to protrusion, however both may include ataxia, back pain, and variable neurological deficits depending on the site and extent of spinal cord compression (Hansen, 1952; Mckee, 2000a; Brisson, 2010; Jeffery, 2013).

As mentioned above, fibrous metaplasia has been described as a normal maturation process in which the changes seen occur over a slower period of time (Hansen, 1952). This maturation process is characterized by a variable fibroid appearance to the NP, which often occurs adjacent to the transitional zone (Hansen, 1952). Over time, this can lead to compensatory hypertrophy and cyclic damage to the AF, creating a combination of gross thickening and small tears in the AF (Hansen, 1952; Jeffery, 2013). Partial herniation of the NP into these tears occurs in a manner that, combined with the annular
thickening, can result in protrusion of the IVD into the vertebral canal (Hansen, 1952). Intervertebral disc protrusion may cause neurological dysfunction via spinal cord compression; however pain is the most common clinical sign (Macias, 2002; Cherrone, 2004).

Both types of disc herniation may lead to physical compression of the spinal cord or nerves. This leads to the variable pain and neurological deficits that are clinically recognized (Jeffery, 2013). However, other factors may contribute to pain in these patients (Fadda, 2013). It has been demonstrated that clinical signs are not only related to the physically herniated disc material, but also to the concurrent biochemical alterations triggered by the inflammatory reaction (Olmarker, 1993; Martin, 2002; Mulleman, 2006). These molecular changes include increases in macrophages, increase in interleukin 1 beta and a release of prostaglandin (Martin, 2002). Inflammatory changes within the vertebral canal have been shown in dogs following IVD extrusion (Hansen, 1952; Hasegawa, 2000; Shimizu, 2010; Fadda, 2013; Kranenburg, 2013). In an experimental study where autologous AF and NP material was placed into the vertebral canal of dogs, an age related inflammatory reaction was seen with NP material in older dogs (Hasegawa, 2000). In addition to this, a lack of inflammatory reaction was evident in younger dogs with NP material placed in the vertebral canal and in all age groups with AF material placed in the vertebral canal (Hasegawa, 2000). Although there has not been a direct correlation with the presence of inflammatory changes and spinal pain in dogs, it is reasonable to postulate that the presence of inflammation may play a pivotal role in IVD disease, similar to that seen in human patients (Kranenburg, 2013).

**Diagnosis of Intervertebral Disc Disease**

Diagnostic imaging is essential to the accurate diagnosis of IVD disease and is especially critical prior to interventional treatment (Robertson, 2011; Jeffery, 2013). Appropriate spinal imaging provides essential information about localization and severity of lesions and guides therapy (Duval, 1996; Ito, 2005; Levine, 2009; Brisson, 2010; Robertson, 2011; Pease, 2011; Jeffery, 2013).
Survey radiographs, myelography, computed tomography (CT), and magnetic resonance imaging (MRI) have all been assessed for diagnosing IVD disease in dogs (Kirberger, 1992; Lamb, 2002; Levine, 2009; Brisson, 2010; Robertson, 2011; Bos, 2012; Jeffery, 2013). The selection from among these different imaging tests is dependent on patient factors, such as signalment and clinical signs, logistical factors such as cost and availability, as well as clinician factors that include both evidenced based and experiential criteria (Robertson, 2011).

Despite the ultimate necessity of advanced imaging for accurately diagnosing IVD disease, non-contrast radiography has long served as the first line modality for evaluating the spine, helping to rule out fractures or aggressive bone lesions such as neoplasia or discospondylitis (Jeffery, 2013). Survey radiographs of dogs affected with IVD herniation may show signs of narrowed and/or wedge shaped IVD space, narrowed joint space at the synovial articulations, narrowed intervertebral foramen, and possibly mineralized herniated disc material (Kirberger, 1992; Lamb, 2002; Brisson, 2010; Murakami, 2014). Although the presence of radiographic changes was found to be strongly associated with site of disc herniation, there was a high degree of false-positive and false-negative findings (Murakami, 2014). Non-contrast radiography is inaccurate for IVD herniation and lacks information on the severity of spinal cord compression; therefore, radiographs alone are considered inadequate for diagnosis prior to intervention (Kirberger, 1992; Lamb, 2002; Hecht, 2009; Brisson, 2010; Robertson, 2011; Murakami, 2014).

Myelography, a technique resulting in opacification of the subarachnoid space by intrathecal injection of positive contrast, has been utilized for many years for the diagnosis of spinal cord compressive lesions (Brisson, 2010; Robertson, 2011; Bos, 2012; Jeffery, 2013). When compared to surgical findings, the agreement of lesion localization and lateralization using myelography has been reported to range from 50-100%, with most publications reporting sensitivity around 80% (Mckee, 2000a; Hecht, 2009; Israel, 2009; Brisson, 2010; Robertson, 2011; Bos, 2012). Although the accuracy of myelography is moderate to high for the diagnosis of IVD disease, small lesions may be missed or the diagnosis may be impeded by technical
difficulties including poor opacification of the subarachnoid space caused by extensive cord swelling (Lamb, 1994; Hecht, 2009; Israel, 2009; Robertson, 2011). Appropriate lesion lateralization can be difficult with myelography (Bos, 2007; Hecht, 2009). This is problematic for thoracolumbar surgical approaches, which require a lateral approach (Bos, 2007; Hecht, 2009). Although rare, adverse reactions have been associated with myelography including seizures, asystole, intracranial subarachnoid hemorrhage, and exacerbation of myelopathy (Widmer, 1992; Lewis, 1992; Carroll, 1997; Baron, 2002; Packer, 2007; da Costa, 2011; Robertson, 2011; Israel, 2009). Because of the complications and the increased availability of non-invasive cross-sectional imaging such as CT and MRI the use of myelography has declined in recent years (Robertson, 2011; Jeffery, 2013).

Computed tomography has also been used to diagnose spinal cord compressive lesions either alone or in conjunction with myelography (Mckee, 2000a; Hecht, 2009; Israel, 2009; Newcomb, 2012; Robertson, 2011). For IVD herniation, the sensitivity of CT alone has been reported to be similar to myelography with reported sensitivities between 80-90%, but is suggested to have a higher sensitivity in diagnosing chronic lesions compared to myelography (Hecht, 2009; Israel, 2009; Robertson, 2011; Cooper, 2014). It is likely that for chondrodystrophic dogs, in which mineralization of the herniated portion of the IVD is more common, CT without contrast will lead to an accurate diagnosis in the majority of cases (Newcomb, 2012; Robertson, 2011). Computed tomography in conjunction with myelography has been reported to have a higher sensitivity for localization and defining lesion characteristics than either CT or myelography alone, however it continues to have the risk of adverse reactions described with myelography and is more time consuming and costly (Dennison, 2010; Robertson, 2011).

Discography has been used to evaluate IVD degeneration, including AF tears and IVD protrusion (Erlacher, 1952; Barthez, 1994; Ramirez, 1998; Derby, 2005; Raj, 2008). In human patients, discography has been utilized to help direct minimally invasive treatment, especially in patients with multiple sites of IVD degeneration (Smith, 1967; Fraser, 1982; Theron, 2010). Discography
has been described in dogs, with the majority of the studies being in normal subjects (Barthez, 1994; Garrick, 1964; Wrigley, 1984; Kahanovitz, 1986). Although discography has been reported to have a high sensitivity for IVD pathology, it is invasive and can lead to complications such as discospondylitis, protracted back pain syndrome (in humans), and accelerated degeneration of the normal IVD (Kluner, 2006; Carragee, 2009). The last of these has been highly debated for decades, with some canine studies showing no changes in the IVD following discography while other studies in humans have suggested that discography accelerates degenerative changes (Kahanovitz, 1986; Carragee, 2009). A prospective, matched-cohort study comparing the MRI changes over a 10 year period between individuals that underwent discography (n=52) using a 22 or 25 gauge needle and individuals that only had MRI examinations (n=50) found that there was a significantly greater rate of disc degeneration in those who received the discography procedure, with 35% of the discography group having progression of degenerative changes and only 14% of the control group having degenerative changes (Carragee, 2009). However, this finding may have been affected by selection bias; patients that received discography were likely at a higher risk of degeneration than controls as it was recommended by a physician to undergo this diagnostic test (Carragee, 2009). The researchers tried to control for this by comparing untreated higher lumbar IVDs in those individuals who received injections and control individuals and found no significant differences (Carragee, 2009). In animal models it has also been reported that accelerated IVD degeneration can be induced by puncture of the IVD (Sobajima, 2005; Masuda, 2005; Rousseau, 2007). Needle puncture of the IVD with 16-21 gauge needles has been found to cause degeneration in rabbits (Sobajima, 2005; Masuda, 2005). In human patients discography may be useful in differentiating incidental degenerative discs from those that are responsible for clinical pain by stimulating a pain response during injection (Derby, 2005; Iatridis, 2012). In veterinary patients discography is performed under general anesthesia or heavy sedation and therefore the ability of discography to differentiate incidental degenerative IVDs from ones causing clinical pain is likely limited. Discography is therefore uncommon in veterinary medicine and has essentially been replaced by advanced cross-sectional imaging.
Magnetic resonance imaging is unmatched in its ability to provide spinal cord and IVD detail and therefore has been considered the first choice for spinal imaging by many (da Costa, 2010; Robertson, 2011; Bos, 2012; Jeffery, 2013). Magnetic resonance imaging was found to be more accurate compared to myelography and non-contrast CT for determining surgical lesions in dogs with IVD extrusions (Bos, 2012; Cooper, 2014). Magnetic resonance imaging also may provide prognostic information (Ito, 2005). In dogs with paraplegia, hyperintense regions within the spinal cord seen on T2-weighted images that were the length of L2 were associated with a poor prognosis for functional recovery (Ito, 2005). This was especially true if the dogs had concurrent loss of deep pain perception or if the hyperintense region was greater than 3 times the length of L2 (Ito, 2005).

Multiple studies have compared the diagnostic accuracy of myelogram or CT to surgical findings for IVD disease (Schulz, 1998; da Costa, 2006; Hecht, 2009; Israel, 2009; Dennison, 2010; Newcomb, 2012; Bos, 2012; Cooper, 2014). The accuracy for correct diagnosis of site and lateralization using myelogram or CT are reported from 53-83% and 66-89%, respectively (Schulz, 1998; Hecht, 2009; Israel, 2009; Dennison, 2010; Newcomb, 2012; Bos, 2012; Cooper, 2014). The majority of these studies confirmed localization and lateralization of the lesions based on surgical findings, which has been questioned as an appropriate gold standard (Schulz, 1998; Hecht, 2009; Israel, 2009; Cooper, 2014). In many cases of acute extrusion it has been shown that material is found bilaterally, while surgery is only routinely performed on one side, therefore there may be discordance with surgical findings and imaging studies (Schulz, 1998; Hecht, 2009; Israel, 2009; Cooper, 2014). As imaging results are interpreted by the surgeon prior to treatment, the surgeon may be biased when recording results or making surgical approaches (Cooper, 2014). These studies should also be interpreted in light of the recognized selection bias; the majority of cases were chondrodystrophic dogs with acute onset of clinical signs. This may have lead to increased accuracy of CT as these dogs are more likely to have mineralization of the IVD material (Schulz, 1998; Hecht, 2009; Israel, 2009;
Cooper, 2014). When both chondrodystrophic and non-chondrodystrophic dogs with various causes of acute myelopathy were assessed, CT was found to have an overall accuracy of 66% compared to the surgery or necropsy confirmed diagnosis (Dennison, 2010). There is support that in chondrodystrophic dogs, non-contrast CT will diagnose IVD extrusions in the majority of cases and is a good initial test; however if no lesion is seen augmentation with myelography or an MRI is recommended (Dennison, 2010; Robertson, 2011). Performing CT following myelography has been reported to improve the agreement between imaging findings and surgical findings, resulting in up to 97% accuracy when the modalities are combined (Dennison, 2010). If MRI is unavailable, many consider the combination of CT-myelography to be the next best choice (Robertson, 2011; Newcomb, 2012). Studies comparing the accuracy of myelography and/or CT to MRI show support for MRI being more accurate than myelography and at least equal or more accurate than CT for the diagnoses of IVD herniations (Suwankong, 2006; da Costa, 2006; Bos, 2012; Cooper, 2014). These studies have similar limitations to those mentioned above, mainly including the use of surgery as a gold standard (Suwankong, 2006; Bos, 2012; Cooper, 2014). Compared to surgical findings, MRI has an accuracy of 98.5-100% for correct localization and lateralization of lesion (Bos, 2012; Cooper, 2014).

There have been few published reports for evaluation of disc degeneration prior to the onset of clinical signs in dogs (Seiler, 2003; Bergknut, Auriemma, et al., 2011; Kranenburg, 2013). Magnetic resonance imaging not only provides information on compression of the spinal cord, which is critical for surgical intervention, it also provides information on the state of degenerative changes to the IVD, which correlate to the T2 relaxation properties of the IVD (Pfirrmann, 2001; Lotz, 2012; Pearce, 1991). Currently, MRI is accepted as the most accurate and least invasive diagnostic test for IVD degeneration (Sether, 1990; Pfirrmann, 2001; Seiler, 2003; Bergknut, Grinwis, et al., 2011; Lotz, 2012; Kranenburg, 2013). Early detection of IVD degeneration and early protrusion is desirable as this could allow for early intervention and more conservative, minimally invasive therapies. This may include preventative therapies in high-risk patients such as German Shepherd Dogs with
lumbosacral stenosis or Dachshunds with a history of IVD herniations. Additionally screening tests may be beneficial for procurement of working dogs (Linn, 2003).

On T2 weighted images, any difference in water content between tissue types is exploited; in a properly hydrated normal IVD, there is clear distinction between the normal NP and AF. The NP has homogenous high signal intensity while the AF has low signal intensity (da Costa, 2010; Sether, 1990; Besalti, 2005; Bergknut, Auriemma, Wijsman, Voorhout, Hagman, Lagerstedt, Hazewinkel, & Meij, 2011; Bergknut, Grinwis, Pickee, Auriemma, Lagerstedt, Hagman, Hazewinkel, & Meij, 2011; Pfirrmann, 2001; Sobajima, 2005). Imaging based grading systems for degenerative changes have been developed in human and veterinary medicine and are largely based on detecting alterations in disc size and shape and reduction in water content expected in degenerative IVDs (Sether, 1990; Besalti, 2005; Bergknut, Auriemma, et al., 2011; Bergknut, Grinwis, et al., 2011; Pfirrmann, 2001; Sobajima, 2005; Bergknut, 2012; Sether, 1990; Seiler, 2003). The most common imaging based grading system used in human medicine is the Pfirrmann system, which has also been validated for use in both chondrodystrophic and non-chondrodystrophic breeds with excellent intra-observer and inter-observer agreement and good agreement with histological changes (Bergknut, Auriemma, et al., 2011; Bergknut, Grinwis, et al., 2011). The Pfirrmann grading system ranges from 1 to 5 and includes assessment of the IVD based on 4 categories; structure of the disc, distinction between NP and AF, signal intensity and height of IVD (Table 1.1) (Pfirrmann, 2001). Although the agreement between MRI findings, gross morphological findings, and histopathological findings has been found to be good, it is poorer with more severe changes (grades 4 and 5) (Bergknut, Grinwis, et al., 2011; Bergknut, Meij, et al., 2013; Kranenburg, 2013). It has been reported that intra-discal therapies are not applicable with more severe grading (grade 5 and most of grade 4) therefore grade 3 degenerative IVDs may be the most relevant target for intra-discal treatment options in humans (Lotz, 2012). The challenge with grade 3 IVD degeneration is that there is poor correlation with clinical signs and therefore treatment may not be indicated (Lotz, 2012).
Table 1.1. Classification of intervertebral disc degeneration on magnetic resonance imaging. The classification system as reported by Pfirrmann et al. 2001.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Structure</th>
<th>Distinction of Nucleus and Annulus</th>
<th>Signal Intensity</th>
<th>Height of Intervertebral Discs</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Homogenous, bright white</td>
<td>Clear</td>
<td>Hyperintense, isointense to cerebrospinal fluid</td>
<td>Normal</td>
</tr>
<tr>
<td>II</td>
<td>Inhomogeneous with or without horizontal bands</td>
<td>Clear</td>
<td>Hyperintense, isointense to cerebrospinal fluid</td>
<td>Normal</td>
</tr>
<tr>
<td>III</td>
<td>Inhomogeneous, gray</td>
<td>Unclear</td>
<td>Intermediate</td>
<td>Normal to slightly decreased</td>
</tr>
<tr>
<td>IV</td>
<td>Inhomogeneous, gray to black</td>
<td>Lost</td>
<td>Intermediate to hypointense</td>
<td>Normal to moderately decreased</td>
</tr>
<tr>
<td>V</td>
<td>Inhomogeneous, black</td>
<td>Lost</td>
<td>Hypointense</td>
<td>Collapsed disc space</td>
</tr>
</tbody>
</table>

Although the Pfirrmann system has been validated in dogs using human based gross pathological grading schemes, the fact that human cartilaginous end plates are wider than those in dogs, this may make these grading schemes inappropriate (Bergknut, Auriemma, et al., 2011; Bergknut, Meij, et al., 2013; Kranenburg, 2013). A novel veterinary histological grading system has been developed based on a commonly used grading system in human patients (Boos, 2002; Bergknut, Meij, et al., 2013). This histological grading system is based on the morphology of the AF, chondrocyte metaplasia of the AF, tears and cleft formations, chondrocyte proliferation of the NP, presence of notochordal cells in the NP, matrix staining of the NP with Alcian blue/Picosirius red staining, endplate morphology, new bone formation and subchondral bone sclerosis (Bergknut, Meij, et al., 2013). It has been validated in chondrodystrophic and non-chondrodystrophic dogs using a reliable gross pathological scoring system (Thompson scheme) and the glycosaminoglycan content of the NP as gold standards (Thompson, 1990; Bergknut, Meij, et al., 2013). Significant correlation was found between this modified histological grading scheme and the Pfirrmann MRI grading scheme.
in dogs supporting the use for evaluating IVD degeneration using these schemes, however no significant correlation with the severity of clinical signs was found (Kranenburg, 2013). More advanced techniques have been used to classify and evaluate disc degeneration (Lotz, 2012). These include T1 time constant, T2 mapping, diffusion imaging, MR spectroscopy and positron emission tomography (Lotz, 2012). Since T2 relaxation times of IVD correlate well with the proteoglycan content and possibly with the overall hydration level, T2 mapping may be useful to evaluate degenerative changes (Lotz, 2012; Pearce, 1991; Marinelli, 2009). In fact, the T2 changes have even been suggested to be different between discs that cause pain and those that do not, with the T2 relaxation being lower in painful discs and therefore T2 mapping may be useful in predicting clinical discs as well as monitoring post-treatment (Lotz, 2012; Boos, 1997).

As discussed, various imaging modalities are successful in diagnosing IVD disease and there have been many studies assessing which imaging modality is best for making the diagnosis (Hecht, 2009; Israel, 2009; Robertson, 2011; Newcomb, 2012; Bos, 2012; Dennison, 2010; Reynolds, 2013; Cooper, 2014). The choice of which imaging modality is usually dictated by availability, cost, specific patient factors such as breed, age, and duration of clinical signs. Also, the clinical suspicion of whether extrusion or protrusion of the IVD is likely may affect imaging modality choice. It is generally accepted that if available and patient factors allow for it (such as no metal or pacemaker present), MRI is the first choice of imaging tests for diagnosing IVD disease and ruling out other diseases such as fibrocartilagenous emboli, neoplasia or hematomas which may cause similar clinical signs (Robertson, 2011). However, CT and/or myelography are also generally accepted diagnostic tests (Robertson, 2011; Bos, 2012; Jeffery, 2013).

II. DEGENERATIVE LUMBOSACRAL STENOSIS

Degenerative lumbosacral stenosis (DLSS) is an important syndrome in dogs, which is generally considered separately from other types of IVD disease. Degenerative lumbosacral stenosis is an acquired condition in which clinical
signs are attributed to compression of the cauda equina (Ramirez, 1998; De Risio, 2000; Worth, 2009; Meij, 2010; Jeffrey, 2014). Degenerative lumbosacral stenosis arises from narrowing of the vertebral canal and/or intervertebral foramina secondary to local anatomic changes. These may include alterations of the AF, interarcuate ligament, sacral lamina and synovial joint capsule (Oliver JE, 1978; Tarvin G, 1980; Chambers, 1989; Watt, 1991; Jones, 1996; Mayhew PD, 2002).

Anatomy

The caudal extent of the spinal cord narrows gradually forming the conus medullaris, which in dogs terminates at the level of 6th to 7th lumbar bodies (Fletcher, 1966; Evans, 1993; Meij, 2010). The cauda equina originates from the conus medullaris and extends from the 6th lumbar to approximately the 5th caudal vertebrae and is composed of multiple lumbar, sacral and caudal spinal nerves all enveloped by meningeal sheaths (Evans, 1993; Meij, 2010). The nerve roots of the cauda equina traverse dorsally or slightly dorsolaterally over the IVD between the 7th lumbar and the 1st sacral vertebrae, a site commonly called the lumbosacral (LS) junction. The 7th lumbar and 1st sacral vertebral bodies are stabilized in a similar manner to other vertebral bodies by the IVD itself, two synovial articulations, ventral and dorsal longitudinal ligaments, interarcuate and interspinous ligaments, and the surrounding muscle and fascia (Meij, 2010; Evans, 1993; Demoulin, 2007).

Ultimately the clinical signs seen in DLSS are attributed to a compression of the nerve roots of the cauda equina (Chambers, 1989; Ramirez, 1998; Worth, 2009; Meij, 2010; Jeffrey, 2014). The peripheral nerves originating from the cauda equina include the femoral, sciatic, pelvic, sacral, and pudendal nerves, and the nerves of the tail (Meij, 2010). These nerves have a variety of functions including control of pelvic limb movement, urinary bladder function, anal sphincter control, and tail tone (Meij, 2010). Alteration of the normal pathway of the cauda equina can have a variety of impacts on neurological function depending on which nerve roots are involved. Cauda equina compression occurs when there is narrowing of the vertebral canal and/or
intervertebral foramina (Ramirez, 1998; Jeffrey, 2014). The causes of narrowing can be multifactorial and may involve IVD degeneration, LS instability or misalignment, soft tissue proliferation, osteochondrosis, vascular compromise or congenital vertebral anomalies including bony stenosis of the vertebral canal (Lang, 1992; Ramirez, 1998; Hanna, 2001; Jones, 2008; Meij, 2010). The pathophysiology of DLSS is incompletely understood, however it is commonly stated that the inciting cause is degeneration of the LS IVD (Worth, 2009; Meij, 2010). This may be attributed to normal age-related degeneration or may be associated with unfavorable biomechanics of the LS junction creating disc degeneration (Worth, 2009; Meij, 2010). As DLSS is seen more commonly in large breed dogs, with German Shepherd Dogs and working dogs being over-represented, it is likely there are both congenital and developmental factors, which contribute to degeneration (Lang, 1992; Schmid, 1993; Morgan, 1993; Danielsson, 1999; Hanna, 2001; Fluckiger, 2006; Worth, 2009; Meij, 2010; Smolders, 2012). Working dogs are over-represented in studies published on DLSS; it is unclear if this is due to the impact of increased activity of these dogs, greater tendency for clinical signs to be recognized, a larger proportion of these dogs being Shepherd breeds, or a combination of these factors (Linn, 2003; Worth, 2009). The IVD degeneration associated with DLSS is similar to that seen with fibrous metaplasia (Worth, 2009; Meij, 2010). This includes loss of the AF integrity and migration of an abnormal NP through tears in the AF eventually causing protrusion of the IVD (Worth, 2009; Meij, 2010). Epidural fibrosis may also occur as a component of DLSS and was reported in 8 of 9 dogs that underwent surgical decompression for DLSS (Jones, 1996). Although compression of the nerve roots has been commonly stated as the causative factor for clinical signs, it has also been found that the cauda equina region is relatively resistant to compression, as compressions of up to 40% have been reported in non-clinical dogs (Danielsson, 1999; Axlund, 2003). Therefore, it is hypothesized by some that discogenic pain may cause clinical signs, similar to that seen in humans (Danielsson, 1999). However, since discogenic pain alone would not be expected to cause neurological deficits observed in dogs with DLSS, a multifactorial process is considered likely. This may include a dynamic component to the lesion.
Motion of the LS junction likely plays a role in the onset and/or propagation of the degenerative changes seen in DLSS and may contribute to clinical signs (Meij, 2010; Jeffrey, 2014). Many studies have supported a decreased range of motion in dogs with DLSS compared with those of normal dogs (Schmid, 1993; Mattoon, 1993; Gradner, 2007). It is uncertain if this alteration in motion is a consequence or cause of DLSS as there is a lack of association between radiographic changes and altered mobility (Gradner, 2007). Abnormalities and asymmetry (known as tropism) of the anatomical features of the synovial articulations have also been evaluated as a cause of altered motion at the LS junction resulting in DLSS (Seiler, 2003; Rossi, 2004; Benninger, 2006; Suwankong, 2008). It has been shown that German Shepherd Dogs have a smaller synovial articulation joint angle with the joint being more sagittally orientated in the caudal lumbar spine and more joint tropism than other breeds of dogs (Seiler, 2003; Rossi, 2004; Suwankong, 2008). The relationship between these synovial articulation alterations and IVD degeneration in German Shepherd Dogs is not clear as some studies have shown a significant association and other studies found no significant correlation (Seiler, 2003; Rossi, 2004; Suwankong, 2008). Hence, at present no relationship between these synovial articulation alterations and clinical signs has been established (Rossi, 2004; Suwankong, 2008). The presence of transitional LS vertebrae, an abnormal vertebra between the last lumbar and first sacral vertebra, may lead to altered biomechanics of the LS junction (Morgan, 1968; Morgan, 1993; Fluckiger, 2006). Multiple studies have shown a strong association with the presence of LS transitional vertebrae and DLSS (Morgan, 1993; Fluckiger, 2006). Although dynamic factors at the LS junction are likely to play a pivotal role in the pathophysiology of DLSS, these are poorly understood and at present there is a lack of correlation between clinical disease and anatomical findings.

**Diagnosis**

The diagnosis of cauda equina compression and any association with DLSS may be supported by physical and neurological examination findings in many
cases. However patients may present with more vague clinical signs such as reluctance to rise or climb stairs without neurological deficits and only minimal pain, and the diagnosis may be more challenging (Suwankong, 2008; Meij, 2010; Jeffrey, 2014). Many different imaging modalities have been utilized to assess DLSS and with the increased availability of CT and MRI some headway has been made into the imaging of the LS region, however there remains much uncertainty (Ramirez, 1998).

Discography and epidurography have been used individually or together to diagnose DLSS with correct diagnosis made in 89% (n=16) of cases using surgical or necropsy findings as a gold standard (Barthez, 1994). Identification of compressive lesions may be ameliorated if radiographs are performed in both flexion and extension (Barthez, 1994; Ramirez, 1998). The sensitivity of these tests for diagnosing DLSS vary and are likely increased by both patient selection and interpretation biases leading to increased false positive diagnosis (Barthez, 1994).

Cross-sectional imaging (CT/MRI) is currently used frequently for the diagnosis of DLSS (Ramirez, 1998; Suwankong, 2006; Worth, 2009; Meij, 2010). Using either CT or MRI, evidence of increased soft tissue at the LS space, with loss of epidural fat, and/or narrowing of the intervertebral foramina may be observed in dogs with DLSS (Adams, 1995; Jones, 1996; Jones, 2000; Suwankong, 2006; Suwankong, 2008). In dogs with clinical signs consistent with DLSS and these associated imaging findings, cauda equina compression should be suspected (Jones, 1996). The agreement between MRI and CT has been shown to be very high when assessing for findings related to DLSS (Suwankong, 2006). Advantages of MRI include improved soft tissue contrast resolution, detection of early disc degeneration, improved evaluation of the dural sac and epidural fat, and visualization of displacement of the nerve roots while still allowing adequate assessment of the foramen (Sether, 1990; Ramirez, 1998; Worth, 2009; Meij, 2010).

It has been found that young healthy German Shepherd Dogs evaluated with MRI may have a higher rate of LS degeneration and mal-alignment between
the caudal endplate of L7 and the cranial endplate of S1 compared to other large breed dogs (Amort, 2012). In this study, the categorization of IVD degeneration was based on a previous validated system of 4 grades (Seiler, 2003). Although there was a significant difference between grades of degeneration of the IVD between German Shepherd Dogs and other breeds, both groups had individuals categorized in all 4 grades. This data was analyzed as continuous data with the results being expressed as a rational number. The grading scheme may be more appropriately classified as categorical data with an ordinal scale; the differences between grades are not necessarily the same at each interval but they are in order of severity. If the data were handled differently, it is uncertain if significant difference would have been maintained.

Despite the increased information acquired, available cross-sectional imaging modalities continue to have low specificity for the diagnosis of DLSS because of overlap in the imaging findings of those individuals who have clinical signs and those without (Jones, 1996; Mayhew, 2002; Axlund, 2003; Worth, 2009; Jeffrey, 2014). Clinically normal dogs can have mild (<25% of the vertebral canal) or even moderate (25-50% of the vertebral canal) bulging of the IVD, which may be accentuated with dogs in extended positions (Axlund, 2003; Jones, 2008; Suwankong, 2008). In 27 dogs, a lack of correlation between MRI findings suggestive of nerve root compression and clinical signs has been found (Mayhew, 2002). Because of the lack of correlation between the MRI findings of compression and clinical signs, it can be hypothesized that compression of the nerve roots may only be one factor leading to clinical DLSS in dogs (Mayhew, 2002). The majority of cases in this study were only mildly affected and therefore in more severe cases imaging findings may be better correlated with clinical diagnosis (Mayhew, 2002). Many other studies have shown there is a very good correlation between the CT and MRI imaging diagnosis but a lack of agreement between imaging diagnosis and surgical findings (Jones, 2000; De Risio, 2001; Mayhew, 2002; Suwankong, 2006). An observational study of 35 dogs with DLSS which underwent pre-operative CT and MRI followed by decompressive surgery found that although there was near perfect agreement between the CT and MRI findings, these were at best
only moderately associated with the surgical findings (Suwankong, 2006). These authors hypothesized that differences in position between imaging and surgery and difficulties in assessing disk protrusion, epidural fat distribution and spinal nerve swelling at surgery contributed to the discrepancy (Suwankong, 2006). Imaging studies have also been shown to lack correlation with post-operative outcome in dogs and humans (Herno, Saari, Suomalainen, & Airaksinen, 1999; Herno, Partanen, Talaslahti, Kaukanen, Turunen, Suomalainen, & Airaksinen, 1999; Jones, 2000). In a prospective study of 12 dogs there was no correlation between the severity of disc degeneration or degree of IVD protrusion noted on preoperative imaging and the post-operative patient outcomes which supports that other factors are involved in DLSS in dogs (Jones, 2000).

The lack of correlation between imaging findings and surgical findings, as well as the variable post-operative outcomes in these patients, make it difficult to assign clinical significance in association with degree of LS IVD protrusion observed during MRI/CT. This is important because a false positive diagnosis may result in surgical treatment that does not result in resolution of clinical signs. It is therefore important to interpret cross sectional imaging findings in conjunction with history, clinical and neurological examination (Jones, 1996).

Dynamic imaging studies have been used for assessment of DLSS (Jones, 2008; Suwankong, 2006). It has been shown that the foraminal area and LS angle are smaller when dogs are placed in extension compared to flexion, however the clinical significance of this is uncertain (Jones, 2008). The changes in foraminal area and LS angle were not significantly different between dogs with clinical signs compared to those without (Jones, 2008). However, there was a significant linear relationship between changes in foraminal area and LS angles in dogs with clinical signs (Jones, 2008). The authors hypothesized that these parameters may be useful as early predictors of DLSS (Jones, 2008). However, at present the clinical utility of this observation is limited, as there are no cut-off values for LS angle or percentage change available (Jones, 2008).
Along with imaging modalities, electrodiagnostics has been evaluated for assessment of canine DLSS (Delamarter, 1990; Sisson, 1992). Spontaneous electromyographical changes including fibrillation potentials, bizarre high frequency discharges, and positive sharp waves have been associated with compressive lesions but do not confirm a cause of compression (Sisson, 1992; Meij, 2010). Electromyographic changes were found to be more accurate in diagnosis of significant compressive lesions compared to discography and epidurography in 13 dogs (Sisson, 1992). This finding suggests that imaging and electrodiagnostics may be used in a complimentary manner to improve the diagnostic interpretation of lesions seen on imaging modalities.

III. TREATMENT OPTIONS

Treatment of Intervertebral Disc Herniation

There continues to be much discussion in the literature about the most appropriate treatment for IVD herniation in dogs. Many different non-surgical and surgical treatments have been reviewed (McKee M., 2000; Meij, 2010; Brisson, 2010; Jeffery ND, 2013). Treatment for certain forms of IVD herniation is more straightforward, while for others a clear consensus on the treatment of choice is not established and in some cases treatment success is considered low.

Treatment of Intervertebral Disc Extrusion

Conservative management of extrusion of the IVD has been shown to be successful in some cases (Davies, 1983; Macias, 2002; Mann, 2007; Levine, 2007). It has been reported that 50% of cases will respond favorably to conservative management (Levine, 2007). This expected improvement of clinical signs contributes to the challenge in evaluating therapies for IVD disease; many dogs will show improvement no matter what treatment is chosen. One of the major challenges in interpreting the current literature is the lack of standardized conservative management options utilized. Many
different forms of conservative management have been described and typically consist of cage rest, and non-steroidal anti-inflammatory or glucocorticoid administration although other treatment options such as acupuncture and physiotherapy have at times, also been included (Mann, 2007; Levine, 2007; Janssens, 2009; Joaquim, 2010). Additionally, the majority of these studies are retrospective and not randomized and therefore case selection may be a factor in the reported success rates.

The surgical treatment options for canine IVD disease have been the focus of many review publications (Mckee, 2000b; Macias, 2002; Brisson, 2010). Surgery is considered the most appropriate treatment for acute IVD extrusion, especially when neurological deficits are present (Mckee, 2000b; Macias, 2002; Moissonnier, 2004; Cherrone, 2004). Patients with back pain alone can also benefit from surgery (Sukhiani, 1996). The most commonly performed surgical procedures include: ventral slot decompression for cervical IVD extrusion, hemilaminectomy or mini-hemilaminectomy (pediculectomy) for IVD extrusion in the thoracolumbar region, and dorsal laminectomy or lateral foraminotomy for IVD extrusion at the LS junction (Cudia, 1997; Mckee, 2000b; Macias, 2002; Cherrone, 2004; Moissonnier, 2004; Godde, 2007; Brisson, 2010). Reported surgical success rates for treatment of IVD extrusion range from 82%-99% in dogs that retained deep pain sensation preoperatively and are significantly lower at 25%-52% in those dogs with no deep pain sensation at the time of presentation (Cudia, 1997; Macias, 2002; Ferreira, 2002; Cherrone, 2004; Forterre, 2008; Brisson, 2010; Scmeid, 2011; Aikawa, 2012). The range of success rates may be attributed to variations in surgical approach, differences between study designs, and the variations that exist between cases.

There are few studies that directly compare conservative and surgical outcomes in dogs. An early study reported little difference in outcome following conservative or surgical management in dogs with less severe neurologic deficits (dogs that showed pain, ataxia or were paraplegic but retained bladder function and conscious pain perception) (Davies, 1983). However, 30% of dogs treated conservatively had recurrence and 13% had
residual ataxia (Davies, 1983). None of the dogs treated surgically in this study had reported recurrence or residual ataxia (Davies, 1983). The rates of recurrence in this study are similar to other more recent studies, which report recurrence following conservative management approaching 50% (Mann, 2007; Levine, 2007). Recurrence rates for surgically treated cases of IVD extrusion are reported to be lower, with one randomized controlled clinical trial reporting recurrence rates of 18% for dogs receiving surgical fenestration only at the lesion location and recurrence rates of 8% for those receiving multiple site fenestrations (Brisson, 2011).

When interpreting the current literature and trying to evaluate whether surgical or conservative treatment method will result in the most optimal outcome in patients with IVD extrusion there are some inherent difficulties. The majority of the current studies are retrospective in design, use inconsistent terminology, lack control groups, and lack quantitative or long term follow-up (Jeffery, 2011; Van Wie, 2013; Levine, 2015). Other challenges arise from the fact that there are very few randomized clinical trials, therefore in many studies the client would make the ultimate decision regarding treatment course. It can also be speculated that dogs that present at large referral hospitals, where the majority of studies are performed, do so because their clinical signs are more severe and/or their owners are seeking advanced therapeutic options. This introduces a selection bias; it is likely that dogs with mild clinical signs attributed to IVD herniation in the general practice setting are treated conservatively more frequently and if they respond well to conservative management they are unlikely to be referred. It is also likely that dogs with mild clinical signs are managed conservatively without advanced imaging, while those with more severe clinical signs are more likely to have diagnostic imaging. This may be a further challenge when comparing conservative to surgical management of IVD herniation, as the quantity of disc herniated is not defined if no imaging is performed. An ethical challenge arises when designing studies to evaluate the success of conservative management because surgical management is widely regarded to alleviate pain and result in normal ambulation more rapidly than conservative management (Davies JV, 1983; Davis, 2002; Brisson, 2010). These factors, combined with the
usual limitations of veterinary research such as funding and case numbers, are likely responsible for the lack of randomized clinical trials in this field.

For the surgical management of human patients with IVD herniation, minimally invasive endoscopic surgeries have gained rapid use due to reduction of iatrogenic tissue injury while achieving similar surgical success rates. Minimally invasive surgical procedures have been shown to be associated with smaller incision size, less iatrogenic induced injury, reduced intraoperative blood loss, earlier postoperative ambulation (Muramatsu, 2001; Righesso, 2007). However, these have been associated with prolonged procedure time (Righesso, 2007). Other anecdotal drawbacks may include extra equipment, steep learning curves and additional training. In dogs, two minimally invasive approaches to the thoracolumbar spinal canal have been deemed feasible in cadavers (Lockwood, 2014). In this work, minimally invasive approaches to the spinal canal allowed similar removal of simulated IVD material from the spinal canal of cadavers when compared to conventional hemilaminectomy (Lockwood, 2014). The surgical incision was smaller compared to conventional hemilaminectomy, which may translate into less tissue damage in live animals (Lockwood, 2014). The duration of minimally invasive approaches was found to be significantly longer than conventional hemilaminectomy (Lockwood, 2014). This increased duration along with the additional equipment and training required may be reasons why these surgical approaches have not been widely adapted in veterinary medicine.

Despite the described difficulties in assessing the benefits of surgery in dogs with disc herniation, there is general agreement on certain issues. The success of conservative management is broadly understood to be related to initial severity of neurological signs. Even though conservative management may be successful in some mildly affected cases, the recurrence or relapse rates of these patients are high (Davies, 1983; Mann, 2007). Therefore, for dogs that have IVD extrusion causing severe neurological deficits especially with loss of deep nociception, conservative management is considered inappropriate (Davies, 1983; Brisson, 2010).
Treatment of Intervertebral Disc Protrusion

The most appropriate treatment for IVD protrusion is much more equivocal than that of acute IVD extrusion. Choice of treatment may be more challenging based on the typically prolonged duration of clinical signs, uncertainty in rate of progression of the disease and presence of concurrent diseases (Jeffery, 2013). Surgical and conservative management for the treatment of IVD protrusions may have similar likelihood in improving neurological outcome (Macias, 2002). The likelihood of improvement of neurological deficits in dogs with IVD protrusion has been shown to be worse than in dogs with IVD extrusions (Macias, 2002).

Based on multiple retrospective studies, including over 400 dogs, the success rate for surgical treatment of IVD protrusions ranges from 33-88.8% (Schmid, 1993; Macias, 2002; Moissonnier, 2004; Kinzel, 2005; Downes, 2009). Variations in study design including differences in diagnosis of IVD protrusion, surgical techniques, and assessment of patient outcomes, likely contributed to this wide range. Corpectomy, a surgical procedure that removes the IVD and a portion of adjacent vertebral bodies, has been described as a surgical treatment that is possibly associated with higher success rates in chronic IVD extrusions and protrusion (Moissonnier, 2004). It is hypothesized that this is because corpectomy approaches the compression ventrally, without disruption to the spinal cord, which may reduce the risk of neurologic deterioration compared to other procedures (Moissonnier, 2004; Flegel, 2011). Corpectomy has been reported to achieve adequate decompression in 90% (n=46) of dogs based on post-operative CT examination, however 20% (n=10) of dogs required surgical revision (Flegel, 2011). Unfortunately, there was lack of correlation between what was considered adequate decompression and clinical outcome (Flegel, 2011). In dogs with IVD protrusion (n=7) or chronic IVD extrusion (n=8), no dog had worsening of the neurological deficits following corpectomy, however only 43% (n=3) of non-chondrodystrophic dogs were characterized as free from disease at their follow-up period (Moissonnier, 2004).
Partial percutaneous discectomy is another surgical technique that has been described for the treatment of presumptive thoracolumbar IVD protrusions in 331 dogs with high success rates (88.8%) (Kinzel, 2005). This procedure uses fluoroscopy to place a Kirschner wire into the lateral aspect of the IVD and then a trephine is placed through a skin incision over the wire and into the IVD to perform an IVD biopsy (Kinzel, 2005). In this study, an attempt was made to exclude IVD extrusion based on imaging findings, however over 80% of dogs in this study were chondrodystrophic and therefore there may have been overlap between extrusions or chronic extrusion of the IVD and a diagnosis of IVD protrusion (Kinzel, 2005). These studies of thoracolumbar and cervical IVD disease in large, non-chondrodystrophic breeds all had similar limitations including their retrospective nature, limited follow-up that mostly included telephone survey and small number of confirmed protrusions of the IVD. The retrospective nature of these studies means that standardized treatments were not used. Further, neurological scoring based on available medical records has been shown to have poor correlation with the assessment at the time of presentation (Van Wie, 2013). From these retrospective studies it is evident that the treatment for clinically significant protrusion of the IVD is less standardized, has been less thoroughly studied, and compared to the surgical treatment of acute extrusion of the IVD the success rate is lower.

The assessment of surgical outcome in patients with IVD protrusion is challenging because of limitations common to many studies, one of the largest being the accurate differentiation of types of IVD herniation. In almost all studies published to date the differentiation of IVD extrusion from IVD protrusion is based on myelography and confirmed by surgery (Macias, 2002; Moissonnier, 2004; Kinzel, 2005; Flegel, 2011; Downes, 2009; Scmeid, 2011). At present, there is no study looking at the accuracy of myelography or surgery in differentiating the different types of IVD herniation. It is suspected that there is a large degree of overlap in the findings associated with non-dispersed extrusions and protrusions, especially with chronicity. In many other studies, the differentiation is made based on the size and breed of the dog, however it has been established that both chondrodystrophic and non-
chondrodystrophic dogs experience IVD extrusions and protrusions (Hansen, 1952; Cudia, 1997; Macias, 2002; Cherrone, 2004).

It is clear that surgical therapy for IVD protrusion has a more guarded prognosis than IVD extrusion (Mckee, 2000b; Macias, 2002; Brisson, 2010). The reason for the worse prognosis may be associated with the more technically demanding surgery, more manipulation of the spinal cord during surgery, and/or the more chronic nature of the compression (Macias, 2002; Downes, 2009). Furthermore, the clinical signs in these patients are possibly associated with concurrent irreversible spinal cord injury and pathology (Macias, 2002; Downes, 2009). At present, there have been no studies assessing pre-operative imaging findings in dogs with chronic disc protrusion and their potential association with surgical outcomes. Such information would be of great value as it could contribute to improved case selection and more accurate prognostication.

**Treatment of Degenerative Lumbosacral Stenosis**

Various treatments have been evaluated in dogs with DLSS and currently there is no concrete evidence to support one treatment over the others (Jeffrey, 2014). Similar to extrusion or protrusion of the IVD, the majority of studies evaluating treatment options in dogs are retrospective; hence, there is a large amount of variety in case definition, imaging diagnosis, surgical approach, post-operative care and follow-up. It has been suggested that there are a large number of dogs that are placed on a trial of non-steroidal anti-inflammatories or steroids without definitive diagnosis and respond favorably to this medical treatment (Jeffrey, 2014). Degenerative lumbosacral stenosis is similar to human degenerative lumbar disease and based on the human literature conservative management, including pain management, anti-inflammatory treatment, rest, and time are frequently adequate for treatment (Raj, 2008; Guarnieri, 2009).

Retrospective and prospective studies from the last 15 years evaluating results of surgical decompression in over 200 dogs have found success rates
ranging between 67-93%, however complete resolution of signs is only seen in about 50% of these cases (Danielsson, 1999; Jones, 2000; De Risio, 2001; Linn, 2003; Suwankong, 2008; Meij, 2010; Jeffrey, 2014). The most common surgical decompressive technique is dorsal decompressive laminectomy with fenestration or partial discectomy (Danielsson, 1999; De Risio, 2001; Suwankong, 2007; Suwankong, 2008; Jeffrey, 2014). Those factors that make a patient more likely to respond favorably to surgical treatments are incompletely understood. Greater age, chronic progression of clinical signs, more severe clinical signs including urinary and fecal incontinence have all been associated with poorer outcomes following surgical treatment of DLSS (Danielsson, 1999; De Risio, 2001; Linn, 2003; Suwankong, 2008). Although improvement of clinical signs is seen following surgery in approximately 80% of dogs, up to 50% of dogs that show initial improvement may have recurrence of signs (Linn, 2003). In a large retrospective study, severe compressive lesions were significantly associated with a lack of improvement following surgery (Suwankong, 2008).

Biomechanical instability is suspected to be a component of DLSS in dogs (Meij, 2010). The common surgical approaches for decompression of the LS junction are thought to exaggerate this or result in loss of other compensatory stabilization methods (Meij, 2007; Smolders, 2013). Since a dynamic component is suspected to contribute to clinical signs in dogs with DLSS, different surgical methods have been proposed for stabilization of the LS space following decompressive surgery with mixed results (Slocum, 1986; Hankin, 2012; Smolders, 2012; Golini, 2014). Early stabilization techniques included placement of a bridging screw between the vertebral bodies bridging the IVD space or between the articular processes and the ilial wings, while more recently other techniques that provide more rigid stabilization have been evaluated including pedicle screw-rod fixation and transarticular fixation with screws (Denny, 1982; Slocum, 1986; Hankin, 2012; Smolders, 2012; Golini, 2014).

Using a cadaver model, one study found that dorsal laminectomy and partial discectomy at the LS space did not alter forces in flexion and extension (Meij,
2007). Subsequently, it was found that LS stability was decreased following nucleotomy or dorsal laminectomy (Smolders, 2012). The suitability of these cadavers as a model for DLSS is unclear; adjacent musculature was removed which likely reduced LS stability and since both studies utilized cadavers from dogs which were clinically normal they may lack a component of instability already present in some dogs with DLSS (Demoulin, 2007).

Assessment of treatment success is challenged by the lack of quantitative outcome measures (Jeffrey, 2014). Many of the researchers utilize follow-up by questionnaire, the majority of which are completed by the owner, while a few studies have utilized more quantitative techniques such as force plate analysis (De Risio, 2001; Linn, 2003; Van Klaveren, 2005; Suwankong, 2008; Smolders, 2012). In a study using force plate analysis in 12 dogs with DLSS, it was found that the pelvic limb propulsive forces were significantly smaller in dogs with DLSS compared to 24 control dogs free of orthopedic disease (Van Klaveren, 2005). At 6 months following decompressive surgery, the force plate analysis measurements improved significantly (Van Klaveren, 2005). These findings were supported by a similar study; in which force plate metrics were seen to improve following decompressive surgery (Suwankong, 2007). While these results are encouraging, this study only included dogs with mild or no neurological abnormalities. Therefore surgical success and improvement of propulsion forces may be more likely in this population.

Minimally Invasive Percutaneous Treatments

Although surgical treatment for large volume IVD extrusions or in patients with severe acute neurological signs is generally preferred, different treatment plans may be suitable for those patients with IVD protrusions and those with mild neurological signs. In contrast to the frequency of surgical decompression performed for dogs, surgical treatment of IVD disease in humans is relatively rare. Surgery has become restricted to the acute more severe cases or patients with chronic back pain that has not responded to other treatments (Guarnieri, 2009). The invasiveness of surgery, the cost and the post-operative complications have all promoted the use of minimally
invasive procedures for percutaneous treatment of IVD herniations (Atilola, 1988). These interventional procedures have been utilized mainly in candidates that have failed short-term conservative management and prior to surgical options with the goal of avoiding surgery while having similar success (Kelekis, 2010). Percutaneous interventional techniques have similar benefits and drawbacks to minimally invasive surgical procedures. The benefits include fewer complications, less iatrogenic induced injury, earlier postoperative ambulation, shorter hospitalization times and less stress (Atilola, 1988; Muramatsu, 2001; Muto, 2004; Righesso, 2007). Anecdotal drawbacks of percutaneous interventional techniques may include steep learning curves and additional training. While surgical decompression will likely remain a mainstay for treatment of acute extrusions, avoidance of surgery would be especially advantageous in dogs with chronic IVD protrusions, due to the lower success rates following surgical intervention (Macias, 2002; Scmeid, 2011). This may be of particular application to DLSS patients, where current surgical decompression techniques have limited success and may even exacerbate dynamic conditions by creating LS instability.

Many different minimally invasive treatments for IVD degeneration and protrusion have been reported and reviewed in the literature (Deen, 2003; Raj, 2008; Guarnieri, 2009). These procedures include chemical, ultrasonographic, laser and thermal methods of nucleolysis, which all share the same goal of dissolution of the degenerative NP. These procedures may be helpful in patients that have non-resolved back pain following conservative management and have contained IVD herniations (Guarnieri, 2009; Kelekis, 2010). Contraindications reported include extruded IVD material due to the increased risk of leakage into the vertebral canal, calcified herniation, or major neurological deficits (Guarnieri, 2009; Kelekis, 2010, Stagni 2012). Some studies have also assessed biologic treatments with the goal of rejuvenating the IVD and preventing further degeneration (Leckie, 2012; Lekie, 2013).

One of the first described minimally invasive treatments was chemonucleolysis with chymopapain (Smith, 1964). Chymopapain is an
enzyme that disrupts the IVD (Smith, 1967). Following the early description of chymopapain, many case series, clinical trials and long term follow up studies were published throughout the next couple decades reporting success rates between 70-90% (Kempen, 1975; David, 1980; Benoist, 1982; Fraser, 1982; Fraser, 1984; Tregonning, 1991; Abdel-Salem, 1992; Wardlaw, 2013). Chymopapain was found to have similar success rates to surgical intervention and better results than conservative management (Fraser, 1982; Fraser, 1984; Tregonning, 1991; Abdel-Salem, 1992; Wardlaw, 2013). Yet, chymopapain was associated with anaphylaxis, discitis and post-operative back spasms and the lack of manufacturing and distribution in the United States has lead to a controversial commercial withdrawal of the drug (Sussman, 1975; Hall, 1983; Smith, 1993; Le Goff, 2002; Theron, 2007; Raj, 2008; Guarnieri, 2009). There have been few studies that examined the effect of chymopapain on dogs (Smith, 1967; Widdowson, 1967; Garvin, 1973; Atilola, 1988; Melrose, 1996). In one of Smith’s earliest studies the use of chymopapain to treat 22 dogs with paralysis is described and 14 of 22 dogs improved with no deleterious effects (Smith, 1967). Another study describing chemonucleolysis in dogs assessed radiographic evidence of narrowing of the IVD space following treatment in normal dogs and reported narrowing of 92% of the cervical disc spaces and 46% of the lumbar disc spaces by day 8 following treatment (Atilola, 1988).

Due to the large success observed during its use, the withdrawal of chymopapain has lead to a desire to find a suitable replacement product. Many other injectable chemonucleolysing agents have been utilized with variable success. These include oxygen-ozone, ethanol, collagenase, chondroitinase ABC, and methylene blue (Brown, 1986; Takahasi, 1997; Riquelme, 2001; Andreula, 2003; Theron, 2007; Peng, 2010). The chemonucleolysing agents that have gathered the most recent support are oxygen-ozone therapy and treatment with ethanol based products, which will be discussed further.

Oxygen-ozone has a proposed mechanism of action that includes anti-inflammatory effects, reduction of nerve root edema secondary to resolution of
venous stasis, improvement of capillary blood perfusion, and oxide reduction of the proteoglycans in the NP resulting in dehydration and shrinkage of the disc (Gallucci, 2007; Han, 2007; Oder, 2008; Guarnieri, 2014). Success rates for human patients treated with oxygen-ozone have been similar to chymopapain, ranging between 65% and 88% (Andreula, 2003; Muto, 2004; Gallucci, 2007; Oder, 2008; La Tessa, 2009). Only few complications have been reported with this procedure and they appear minor (Andreula, 2003). One study assessed the outcome at 18-month follow-up and found success was good in 75% of people including evidence of reduction of herniated volume in 63% of cases (Muto, 2004). There is a logistical disadvantage to this treatment; oxygen-ozone has a short lifespan with decay occurring within 20 seconds, therefore specialized equipment (O₃ generator) is needed on site. Fluoroscopy guided intradiscal injection of oxygen-ozone in dogs has been evaluated (Han, 2007). In this study 5 dogs with chronic neurological deficits were treated with oxygen-ozone and followed up to 20 months (Han, 2007). Four of these dogs regained ambulation within 12 days of treatment (Han, 2007). However this study had variable follow-up times, didn’t evaluate non-chondrodystrophic breeds and had a lack of control dogs.

Ethanol has been used as a therapeutic agent for many interventional procedures due to its necrotizing and lytic abilities (Kawano, 1989). Absolute ethanol has been described as an agent used for chemonucleolysis for the treatment of IVD herniation in humans with good success (Riquelme, 2001). Ethanol acts by causing a molecular split of the remaining proteoglycans of the NP in hopes of either diminishing the nuclear volume resulting indirectly in regression of the protrusion or by lysing the herniated material decreasing compression of the spinal cord (Riquelme, 2001). The major concern with this treatment is the lack of control of the liquid ethanol with the possibility of dissolution into adjacent tissues including the AF, cartilage, dura and nervous tissue (Riquelme, 2001; Theron, 2007). Occasionally patients receiving treatment by percutaneous injections of absolute ethanol complained of transient radicular pain of the leg immediately following the injection (Theron, 2007).
By increasing the viscosity of the ethanol, the distribution can be better restricted to the target area (Theron, 2007). This can be achieved by combining ethanol with ethylcellulose, a gelifying base which is derived from cellulose and has hydrophilic properties and a high potential to become thickened (Sannier, 2004; Theron, 2007). When combined, the ethanol-ethylcellulose preparation has increased viscosity and therefore restricted distribution (Sannier, 2004). The main advantage of the gelified ethanol is that smaller volumes of ethanol can be administered and so that its action is more concentrated at the appropriate location (Sannier, 2004; Theron, 2007).

Theron and colleagues explored the potential use of this gelified ethanol for chemonucleolyzing the IVD (Theron, 2007). Its use has been described in both cervical and lumbar disc herniations in humans and is registered under the trade name Discogel® (Theron, 2007; Theron, 2010). This commercially available preparation also includes a small amount of radiopaque tungsten powder to allow visualization during fluoroscopic or CT guided injection (Theron, 2007). The gelified ethanol initially has a viscous liquid consistency that was easily injected into small gauge spinal needles (18-20 gauge needles) and when it contacted the NP it reportedly reacts with the IVD and changes state to a consistency, which Theron described as a “piece of cotton moistened with alcohol” (Theron, 2007). It is postulated that this change in consistency allows the mixture to provide a soft intradiscal prosthesis and allows for the product (ethanol) to be deposited directly into the NP with increased control and lower doses (Theron, 2007). In a preliminary study of human patients, results were satisfactory with this technique in 89.5% of cervical discs treated and 89.9% of all lumbar discs treated (Theron, 2007; Theron, 2010). Since the study only reported follow up period of 4 years, further monitoring is needed to conclude about long-term complications, if any.

One study has assessed the histopathological changes associated with the administration of radiopaque gelified ethanol in a IVD of a pig (Guarnieri, 2010). In this study, intentional intradiscal, intraforaminal, epidural and intramuscular injections of radiopaque gelified ethanol were performed using
fluoroscopic guidance and then the pig was sacrificed 48 hours following the injection. No morphological changes were noted where radiopaque gelified ethanol was injected, including NP, AF, and root ganglion (Guarnieri, 2010). However this study has some limitations as only one IVD of one pig was injected and the morphostructural changes may have taken more than 48 hours to detect (Guarnieri, 2010).

To date, reported improvement of symptoms following radiopaque gelified ethanol treatment range between 75-91.4% in over 400 subjects (Table 1.2) (Theron, 2007; Theron, 2010; Stagni, 2012; de Seze, 2013; Bellini, 2013). The only side effects reported to date with this procedure are a slight discomfort during the beginning of the injection, which the author attributed to rate of injection and one patient having an increase in pain, which receded after the first post-operative week, which may have been suggestive of a radiculitis (Theron, 2007; de Seze, 2013). While these results are encouraging, there is currently a lack of validation for gelified ethanol mechanism of action and a paucity of information about potential side effects on adjacent tissues. It is unclear if these or other factors are responsible for the lack of adoption of this technique in North American centers.

Table 1.2: Summary of literature on the use of radiopaque gelified ethanol for the treatment of IVD herniations in humans.

<table>
<thead>
<tr>
<th>Reference:</th>
<th>Site</th>
<th>Number</th>
<th>Success</th>
<th>Adverse Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theron, 2007</td>
<td>Lumbar</td>
<td>276</td>
<td>Good or very good outcome in 89.9%</td>
<td>None</td>
</tr>
<tr>
<td>Theron, 2010</td>
<td>Cervical</td>
<td>57</td>
<td>Good or very good outcome in 89.5%</td>
<td>None</td>
</tr>
<tr>
<td>Stagni, 2012</td>
<td>Lumbar</td>
<td>32</td>
<td>Success in 75% of patients</td>
<td>None</td>
</tr>
<tr>
<td>de Seze, 2013</td>
<td>Lumbar</td>
<td>79</td>
<td>Good or very good outcome in 76%</td>
<td>Suspected radiculitis in 1 case</td>
</tr>
<tr>
<td>Bellini, 2015</td>
<td>Lumbar and cervical</td>
<td>80</td>
<td>Improvement in 85% of lumbar and 83% of cervical IVD treated</td>
<td>None</td>
</tr>
</tbody>
</table>
Very few comparison studies between injectable therapies have been performed. In one study, radiopaque gelified ethanol was found to successfully lead to improvement or resolution of symptoms in 75% of 32 patients with sciatica and imaging findings supporting IVD herniation, which did not respond to oxygen-ozone treatment 6 months prior (Stagni, 2012).

From the veterinary perspective, since the withdrawal of chymopapain, chemonucleolysis with oxygen-ozone (discussed above), collagenase and chondroitinase ABC have been evaluated (Table 1.3) (Miyabayashi, 1992; Takahasi, 1997; Han, 2007). Collagenase is a proteolytic enzyme that has been reported to cause partial or complete lysis of the NP in dogs in conjunction with narrowing of the IVD space (Miyabayashi, 1992). Following collagenase injection in 9 normal dogs, the behavior of the dogs did not change and there were no changes between myelography performed prior to and following injection (Miyabayashi, 1992). However, 3 of 9 dogs developed hindlimb lameness following injection (Miyabayashi, 1992). Fatal hemothorax due to erosion of an intercostal artery has been reported in 1 of 5 dogs treated with chemonucleolysis by collagenase (Brisson, 2004). Chondroitinase ABC is a product of *Proteus vulgaris* that specifically degrades some glycosaminoglycans having a chemonucleolytic effect (Takahasi, 1997). Seven days following injection of the IVD in dogs with IVD herniations, 91.7% (44/48) of dogs showed improvement (Takahasi, 1997). At 59 days post-treatment of dogs with IVD disease, 75% (36/48) of the dogs were considered to have normal postural reactions, spinal reflexes and no back pain (Takahasi, 1997). There were no adverse affects found following the treatment of chondroitinase ABC (Takahasi, 1997). However, in this study the majority of patients had lower clinical severity with only back pain and mild or no neurological deficits (Takahasi, 1997). This study also lacked control dogs and it is suspected that many of the mildly affected dogs would have improved with rest alone (Takahasi, 1997).
Table 1.3: Summary of literature for multiple chemonucleolytic agents studied in healthy dogs or used for the treatment of IVD herniations in dogs.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Summary</th>
<th>Adverse reactions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chymopapain</td>
<td>- 64% (14/22) of dogs with IVDH improved.</td>
<td>- No adverse reaction reported</td>
<td>Smith, 1967; Widdowson, 1967; Garvin, 1973; Atilola, 1988; Melrose, 1996</td>
</tr>
<tr>
<td></td>
<td>- Majority of studies are descriptive in normal dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagenase</td>
<td>- 9 normal dogs in one study</td>
<td>- 3/9 dogs developed transient hindlimb lameness without pain</td>
<td>Miyabayashi, 1992; Brisson, 2004</td>
</tr>
<tr>
<td></td>
<td>- 12 dogs with IVDH treated (no description of outcome)</td>
<td>- 1/12 dogs developed fatal hemothorax*</td>
<td></td>
</tr>
<tr>
<td>Chondroitinase ABC</td>
<td>- 91.7% (44/48) of dogs with IVDH improved within 7 days</td>
<td>- No adverse reaction reported</td>
<td>Takahasi, 1997</td>
</tr>
<tr>
<td>Ozone</td>
<td>- 4/5 dogs treated for IVDH had normal ambulation within 12 days</td>
<td>- No adverse reaction reported</td>
<td>Han, 2007</td>
</tr>
</tbody>
</table>

IVDH= intervertebral disc herniation
* Fatal hemothorax was secondary to erosion of the intercostal artery

Another alternative technique in dogs is prophylactic percutaneous laser disk ablation (Dickey, 1996; Bartels, 2003). This treatment involves fenestration of the IVD by use of a laser, typically a holmium yttrium aluminum garnet laser (Dickey, 1996; Bartels, 2003). The laser is placed into the NP of an IVD through a percutaneously placed spinal needle with use of fluoroscopy for confirmation of appropriate positioning (Dickey, 1996; Bartels, 2003). The mechanism of action is related to a decrease in intradiscal pressure related to a decrease in disc volume (Choy, 1992). In a retrospective study of 277 dogs treated with percutaneous laser disk ablation, 75.6% improved and 24.4% continued to have signs of pain according to a telephone survey performed at a mean follow up time of 22 months (Bartels, 2003). Of all dogs treated, 1.8% had some form of perioperative complication including pneumothorax, small abscess at a needle site and development of neurological abnormalities, including proprioceptive deficits, paresis and paralysis (Bartels, 2003).
Image Guided Access to the Intervertebral Disc

There is uncertainty about the most accurate method of delivery of injectable therapeutics. Ensuring accurate placement of the needle used for intradiscal therapeutics is essential for optimal efficacy of these treatments (Dickey, 1996; Kluner, 2006; Kelekis, 2010). Historically, fluoroscopy has been used most commonly to perform discography and guide percutaneous intradiscal therapeutics, although the use of CT guidance has been reported more recently (Muto, 2004; Kelekis, 2005; Oder, 2008; La Tessa, 2009; Guarnieri, 2014). Computed tomography has a higher contrast resolution than fluoroscopy and allows better anatomical detail with lack of superimposition to allow more precise placement of a needle with avoidance of certain anatomical structures such as adjacent vasculature, spinal cord and nerve roots (La Tessa, 2009; Guarnieri, 2014). It also provides adequate monitoring of the distribution of any injected material, including any dispersion into the epidural space (Muto, 2004; La Tessa, 2009). However CT does not provide the temporal resolution that fluoroscopy does. The use of CT, fluoroscopy or a combination of both is usually related to personal preference with some authors stating there is likely no significant difference between modalities (Kelekis, 2005; La Tessa, 2009; Guarnieri, 2014). Ultrasound is commonly used for guiding fine needle aspirates and biopsies in veterinary medicine, and although the appearance of the IVD has been reported, ultrasound has not been described as a modality to facilitate intradiscal therapeutics (Naish, 2003). To the author’s knowledge, no study has compared the ability of different modalities to delivery intradiscal therapeutic accurately.
RATIONALE:

Intervertebral disc herniation is a relatively common condition in dogs, yet surgical treatment of certain forms of IVD disease has less than adequate success rates. The possibility of a minimally invasive treatment that has good success rates and lower risks than surgery is attractive, but until now has not been widely available because of lack of a suitable therapeutic agent. Furthermore, in order for such a treatment to be effective, it must be accurately and ideally non-invasively delivered to the IVD. Although image guided percutaneous needle placement into the IVD has been described using fluoroscopy, the accuracy of this technique has not been validated. The use of CT or ultrasound to access the IVD has not been described, and there is no current evaluation of which modality would be most accurate. Once the most accurate means of guiding percutaneous needle placement is established, it can be used for evaluation of the newly available radiopaque gelified ethanol. To date, the use of gelified ethanol for the percutaneous chemonucleolysis of the NP of dogs has not been explored. Based on the results in human patients, this preparation may hold promise in the treatment of IVD disease and especially chronic protrusion associated with fibrous metaplasia in dogs. Establishing the safety and long-term effects of this product is considered foundational in establishing this as a new treatment for IVD disease in canine patients.
OBJECTIVES:

Regarding the use of gelified ethanol preparation for the percutaneous injection of IVDs in normal dogs we have the following objectives:

1) To establish the most accurate injection method for each anatomical segment of the vertebral column.
2) To apply the injection method to normal dogs in order to evaluate the accuracy and distribution of the injected preparation.
3) To establish a preliminary understanding of the safety of the procedure and to document the occurrence of side effects in normal dogs using both short and long term follow up.
4) To document any alteration in disc volume in normal dogs following the procedure.

HYPOTHESES:

Regarding the use of a gelified ethanol preparation for the percutaneous injection of IVDs in dogs we hypothesize:

1) Minimally invasive image guided injection of the NP is feasible in dogs using multiple modalities and the most accurate approach may differ by anatomic region.
2) Delivery of radiopaque gelified ethanol into the NP of normal dogs would be achieved with high accuracy.
3) Injection of gelified ethanol into the NP will not be associated with significant side effects in normal dogs.
4) The injection of gelified ethanol into the NP will result in quantifiable alteration of the size of the IVD in normal dogs during the 1 year follow up period.
CHAPTER 2:

ACCURACY OF IMAGE GUIDED PERCUTANEOUS INJECTION OF THE INTERVERTEBRAL DISCS IN CANINE CADAVERS – COMPARISON OF THREE MODALITIES

2.1 Abstract:

A minimally invasive method for delivering injectable therapeutic agents would be desirable for the treatment of IVD disease in dogs. The purpose of this study was to compare computed tomography (CT), ultrasonography (US), and fluoroscopy modalities for guiding percutaneous injection into canine IVDs. IVDs of 14 dog cadavers were injected with a gelified ethanol therapeutic agent. Successful injectate placement and injectate leakage were determined based on necropsy inspection of discs. Injection into the nucleus pulposus was successful in 55 of 78 (71%) of all injected discs. Injections guided using CT and fluoroscopy were significantly more successful than US-guided injections. Odds of successful injection without leakage were 12 times greater for CT versus ultrasound (p=0.0026) but there was no significant difference between CT and fluoroscopy (p=0.0620). Injection success rates did not differ among vertebral sites or dog cadavers of varying weights. Forty-nine (63%) of injection sites had injectate leakage outside the disc and 10 of these involved structures within the vertebral canal. The highest rate of injection success with the least amount of leakage was achieved with CT guidance. Findings indicated that CT, fluoroscopy, and ultrasound are feasible modalities for guiding percutaneous injection of a gelified ethanol therapeutic agent into the canine IVD, with moderate to high success rates for different regions of the spine. However, a moderately high rate of injectate leakage occurred outside of the disc and this should be taken into consideration for future safety and efficacy studies.
2.2 Introduction:

A minimally invasive method for delivering injectable therapeutic agents would be desirable for the treatment of IVD disease in dogs. Potential advantages include reduced cost, shorter anesthetic time, and avoidance of surgery. Avoidance of surgery would be especially advantageous for dogs with chronic protrusions of the IVD, as surgical intervention has been reported to have lower success rates for these cases versus cases with extrusion of NP material (Macias, 2002; Brisson, 2010). Injectable therapeutic agents that have been previously investigated for treating IVD disease include chymopapain, ozone, ethanol, and more recently a radiopaque gelified ethanol product (Fraser, 1984; Riquelme, 2001; Andreula, 2003; Theron, 2007; Theron, 2010; Stagni, 2012; Bellini, 2013; de Seze, 2013). Radiopaque gelified ethanol has been reported to offer advantages in that the material is sufficiently liquid to allow targeted injection in a precise manner, but sufficiently viscous to remain at the exact site of deposition without diffusion to adjacent nerves or spinal cord tissues (Theron, 2007).

Image-guided percutaneous approaches have been previously described in dogs and humans for treating IVD disease, for obtaining tissue samples for microbiological culture, cytology, and histopathology, and for performing diagnostic discography (Garrick, 1964; Smith, 1967; Wrigley, 1984; Wrigley, 1984; Attilola, 1988; Barthez, 1994; Gangi, 1996; Dickey, 1996; Fischer, 1997; Chew, 1996; Bartels, 2003; Deen, 2003; Kinzel, 2005; Singh, 2005; Han, 2007; Raj, 2008; Guarnieri, 2009). Accurate needle placement is essential for optimal efficacy of therapeutics, to ensure acquisition of an appropriate sample for diagnostic purposes, and to reduce the risk of potential side effects, including leakage of structures outside the IVD (Dickey, 1996; Kluner, 2006; Kelekis, 2010). In veterinary medicine, fluoroscopy has been the most commonly reported modality, while in human medicine CT-guided procedures or a combination of CT and fluoroscopic-guided procedures are standard approaches (Wrigley, 1984; Attilola, 1988; Gangi, 1996; Chew, 1996; Kluner, 2006; Guarnieri, 2009; Kelekis, 2010). With CT and fluoroscopy, the NP may not be identified unless it is mineralized. Accurate placement of the needle is
often based on feeling a change of resistance between the firm AF and the softer NP or by injecting a positive contrast agent. Visualization of the needle in the center of the IVD using fluoroscopy or CT is also used as evidence that the needle has been placed within the NP. The ultrasonographic appearance of the normal and degenerative IVD has previously been described in dogs and has correlated well with pathologic changes when performed on dissected spines in a water bath (Naish, 2003). To the authors’ knowledge, ultrasound has not been described as an imaging modality for guiding administration of therapeutic agents into the canine IVD. In addition, no previous reports were found comparing the accuracy of image-guided injection of the canine IVD with varying modalities and the frequency of adjacent tissue contamination by injectate leakage.

The aims of the current study were to (1) to describe the image guided percutaneous approach to the IVD in various locations of the spine using three different imaging modalities (US, fluoroscopy and CT); and (2) to compare the accuracy and distribution of gelified ethanol injectate placement into the IVDs using these modalities. We hypothesized that (1) injection of the NP would be feasible with all three modalities; (2) accuracy of injectate placement would differ among modalities, with CT being the highest; and (3) accuracy would differ among anatomic sites, with the lumbar and lumbosacral regions being the highest.

2.3 Materials and Methods:

Subjects:
Fourteen canine cadavers of unknown history and unknown age were used. There were 12 small breed dogs with a mean body weight of 8.7 kg (5.4 – 10.8 kg) and 2 large breed dogs weighing 28.5 kg and 43.4 kg. The mean body condition score was 2.2/5 (range: 1/5-4/5). Breeds included were 8 Beagles, 3 mixed breed dogs and one of each Dachshund, Pekingese, Jack Russell Terrier, and German Shepherd Dog. Some of the cadavers were used for teaching procedures prior to being used in this study, however none of these involved the vertebral column. The hair along the dorsal aspect of the
vertebral column, the ventral neck, and the ventral abdomen was clipped as needed based on the planned approach to image guided injection. Injections were performed using both ultrasound and fluoroscopic guidance for dogs 1 through 7 and CT guidance for dogs 8 through 14 over 3-day time periods. Dog positioning was optimized for accessing the different anatomical regions and was determined by path of easiest access to the IVD, avoidance of other structures, and based on previous clinical experience and reports of IVD injection (Smith, 1967; Atilola, 1988; Fischer, 1997; Han, 2007). The body weights, body condition scores, breed, and disc spaces injected by modality for each cadaver are listed in Appendix I and II.

**Disc space selection:**
In all cadavers, the vertebral column was divided into four anatomical regions based on common locations of IVD disease in dogs; cervical (C2-3 to C6-7), thoracolumbar (T10-11 to L1-2), lumbar (L2-3 to L6-7) and lumbosacral (L7-S1). For dogs 1 to 7, each dog was injected in two different randomly selected disc spaces (drawn from a hat) from each anatomic region; one using ultrasound and one using fluoroscopic guidance while assuring that each disc in each of the anatomical regions was injected in at least one dog. If the randomly chosen anatomical site was the same or adjacent to the first site selected, another anatomical site was randomly chosen. Thus there was at least 1 non treated disc space between injection sites, except for those dogs injected at L6-7 because L7-S1 was always injected. For the lumbosacral site, each dog was injected once and the modality (US versus fluoroscopy) was randomly chosen. For dogs 8 to 14 in which CT guidance was used, the same procedures for selection of disc spaces were followed as those for dogs 1-7 and each dog in the second group was also injected in one disc space from each anatomic region.

**Radiopaque gelified ethanol preparation:**
A radiopaque gelified ethanol\(^a\) therapeutic was used as the injected product. As manufactured, the product contains tungsten as an inert radiopaque marker. In order to facilitate visual identification of the radiopaque gelified ethanol material during gross specimen dissection, 0.5mLs of methylene blue\(^b\)

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\(^a\) A radiopaque gelified ethanol therapeutic was used as the injected product. As manufactured, the product contains tungsten as an inert radiopaque marker. In order to facilitate visual identification of the radiopaque gelified ethanol material during gross specimen dissection, 0.5mLs of methylene blue\(^b\)
was mixed into the 3.0 mL vial of radiopaque gelified ethanol and the preparation was agitated by an agitator\(^2\) for 2-5 minutes at the maximum speed (3200 rpm), until the vial was uniformly black in colour, as per the manufacturers recommendations. Prior to utilizing the radiopaque gelified ethanol for injection, the agitation procedure was repeated for a minimum of 5 minutes as per manufacturer’s recommendations.

**IVD injections:**
All injections were performed by a diagnostic imaging resident (SM) or a board certified radiologist (HC). Twenty-two gauge spinal needles\(^d\) and 1ml polycarbonate syringes\(^e\) were used to administer the radiopaque gelified ethanol-methylene blue preparation in each injection. The operator chose the spinal needle length based on the size and body condition of the dog. A target dose of 0.5mls was administered under steady digital pressure on the plunger until either the entire dose was given or maximum digital resistance was reached. The operator, duration of the injection procedure (including imaging of landmarks), patient position, needle direction, administered dose, and needle size were recorded. For each injection, the operator recorded if the proper anatomical landmarks were identified to their satisfaction, the ability to visualize the needle in the nucleus pulposus, and the amount of resistance during the injection. The operator subjectively assessed the resistance to injection on a scale of 1 to 4, with 1 representing no resistance, 2= mild resistance, 3= moderate resistance, and 4=marked resistance. Additionally, a qualitative assessment of the level of satisfaction with the injection was assigned by the operator from 0-4 based on subjective criteria including identification of the landmarks and the confidence that the needle was placed within the nucleus pulposus (Table 2.1).

**Ultrasonographic guidance:**
All ultrasound-guided injections were performed using a GE LOGIQ 5\(^f\) ultrasound system and a 8-5 MHz curvilinear array electronic transducer. B-mode imaging was used for all injections. An attempt was made to identify the components of the IVD as previously described, with the AF being mildly hyperechoic to the NP, and showing a parallel, linear echo pattern (Naish,
Once the disc was identified the needle was guided into the central region and an attempt was made to image the needle within the disc in two, orthogonal planes. Identification of the desired IVD space was achieved by counting from the following known anatomical landmarks: C6 transverse processes and/or C1 vertebral body in the cervical region; the 13th rib in the thoracolumbar region; and the lumbosacral space for the lumbar and lumbosacral regions. For any given disc site, once the appropriate anatomical landmark was identified, the disc spaces were counted as the US probe was advanced to the desired location. The total time of procedure was recorded.

**Fluoroscopic guidance:**
All fluoroscopic guided procedures were performed using a Phillips BV Endura C-Arm fluoroscopy unit. The fluoroscopic guided IVD injections were performed following previously described techniques for IVD injections (Garrick, 1964; Wrigley, 1984; Kahanovitz, 1986; Barthez, 1994; Ramirez, 1998). The disc was identified using anatomical landmarks and counting vertebrae cranially or caudally as needed. The spinal needle was inserted under fluoroscopic guidance to the AF, which was identified by the needle resistance. The fluoroscopic tube and image intensifier were then rotated 90 degrees to allow visualization and alignment of the needle in the orthogonal plane. Once adequate alignment was achieved on orthogonal views, the fluoroscopic tube and image intensifier were rotated to their original position and the needle was advanced into the region of the NP, which was recognized by a distinctive decrease in needle resistance. When needed, the fluoroscopic imaging was repeated in the orthogonal planes to confirm central placement of the needle in the IVD. The total time of the procedure and presence and distribution of the radiopaque gelified ethanol was recorded.

**CT guided injection:**
All CT guided injection was performed using the same 16-slice helical scanner. Scout images of the anatomical region of interest were performed first. Based on the scout images, the appropriate IVD was identified and axial CT images of the disc were acquired (section collimation thickness: 1.25mm, tube rotation time: 1.0s, mA: 200-240, kV: 120). All images were acquired with
a large field of view (50cm) and a 512 x 512 matrix using a bone algorithm. All images were reconstructed at 2.5mm thick slices with a bone algorithm and were reviewed during the procedure by two authors (SM and HC). Window width and level were adjusted at will by the image reviewers. The most appropriate image slice to allow injection of the IVD was determined and 3 to 5 axial slices (section collimation thickness: 1.25mm, tube rotation time: 1.0s, mA: 200-240, kV: 120) were performed centered on the region of interest. The laser positioning markers on the CT scanner were then used to indicate the position of the disc and used for placement of the spinal needle into the region adjacent to the IVD. A CT slice was then performed to verify needle placement prior to penetrating the IVD. Once confirmed, the needle was advanced through the AF to the NP based on resistance and based on depth measurements projected from the CT scan. During the needle placement process, the number of adjustments was recorded. When satisfied with the placement of the spinal needle in the nucleus pulposus based on CT images, the preparation was injected. Following injection, 3-5 axial slices were repeated. The dose injected, location of the bevel of the needle, presence of radiopaque gelified ethanol following injection, the number of needle adjustments and total time were recorded.

**Necropsy examination:**
Following injection of the radiopaque gelified ethanol-methylene blue preparation, a modified necropsy examination was performed to facilitate dissection of the entire vertebral column and adjacent soft tissues en bloc. From this en bloc preparation, targeted dissection was performed at each site using a modified laminectomy type approach. The trunk muscles including the epaxial spinal musculature at each injection site were dissected with a combination of blunt and sharp dissection and any visible contamination, identified as methylene blue staining of the tissues, was recorded. Following dissection of the soft tissues, the dorsal aspect of the vertebrae including the arch and spinous process were removed by first doing a precise band saw cut which removed the majority of bone and then using bone cutting forceps to remove remaining vertebral arch tissue. The vertebral canal was inspected along the entire length and any contamination was recorded as being located...
within the epidural space, within the dura mater, or within the soft tissues surrounding the vertebrae. Additionally, the ventral aspects of the vertebral column, including the ventral aspect of the IVDs, were blunt-sharp dissected and any contamination and its location was recorded. This was followed by a sagittal section of the entire spine using the band saw, which allowed evaluation of the AF and NP of all IVDs and the location of the injected mixture was recorded as being within the NP, AF, both, or not present in the disc. Following the sagittal dissection, each injected IVD was dissected transversely to evaluate the overall distribution of the injected radiopaque gelified ethanol-methylene blue preparation. A successful injection was considered to be one in which there was radiopaque gelified ethanol-methylene blue preparation within the NP (Figure 2.1). Since the clinical importance of adjacent tissue leakage is likely different depending on the tissue, the leakage data was grouped into two categories. Any contamination within the vertebral canal, staining of the dura, or within the spinal cord was categorized as potentially clinically significant leakage. Leakage into the epaxial/hypaxial muscles or other tissues was categorized as potentially nonclinically significant leakage.

**Statistics:**
All statistical tests were selected in consultation with a statistician. For all analyses a licensed program was used. For the outcomes operator satisfaction with injection, time of the procedure, and number of adjustments when using CT guidance, analysis of covariance analysis using a generalized linear-mixed model was performed that included the variables site, method, weight, body condition score and their interactions. Model assumptions were tested using residual analysis and Shapiro-Wilk test and where needed appropriate transforms were applied. For the binary outcomes of contamination, successful injection, significant contamination and successful injection without significant contamination, a generalized linear mixed model was performed using exact p-values and included variables: method, body weight, body condition score, operator satisfaction with injection and resistance to injection. For all analyses significance was set as at p<0.05.
2.4 Results:
There was no significant difference between body condition scores (p=0.1896) and weights (p=0.7254) for dogs 1–7 (fluoroscopy and US-guided injection) versus dogs 8–14 (CT-guided injection). Positioning used for injection procedures is summarized in Tables 2.2 and 2.3. With ultrasound guidance, the IVD was more easily recognizable in a longitudinal direction as a less echogenic region between two adjacent hyperechoic vertebral endplates (Figure 2.2). Once located, the probe was rotated 90 degrees to allow visualization of the IVD in a transverse plane. A superficial linear hyperechoic structure consistent with the previously described lamella of the AF was identified; the AF could not be reliably distinguished from the NP. The radiopaque gelified ethanol preparation was not seen during the injection using US. With fluoroscopic guidance, placement of the needle into the center of the IVD was facilitated by the use of orthogonal fluoroscopic images (Figure 2.3). The NP was not visualized as a separate structure from the AF using fluoroscopy. During the injection, positive contrast (tungsten) was not visible at the injection site. With CT, serial transverse images accommodated placement of the needle into the center of the IVD (Figure 2.4). The mean number of needle adjustments performed under CT guidance to facilitate correct placement of the bevel of the needle was 6.8 (range 2–20). There was no significant difference in the number of adjustments between the sites (p=0.4797). In most cases, it was not possible to accurately differentiate the NP from the AF with CT. The injected preparation was evident at the injected site with CT and confirmed with necropsy examination in all but one dog, where the needle was placed into the LS IVD and injected preparation could not be delivered under maximal digital palpation.

A total of 78 IVDs were injected (21 injections each in the cervical and thoracolumbar, 22 lumbar region, and 14 injections in the lumbosacral IVDs) and each IVD in each region was injected at least once with all three modalities (Appendix III). The administered volume of the radiopaque gelified ethanol-methylene blue preparation ranged from 0.05–0.4ml. Agitation time for injectate preparation was 2 min during the first portion of the study and 5 minutes during the second portion of the study. The injected volume was
delivered with maximal digital pressure being met within seconds. The needle and syringe were left in place for 2 min as per recommendations (Theron, 2007; Theron, 2010).

The success and leakage rates of injections are summarized in Tables 2.4 and 2.5. Of the total 78 injections, 55 (71%) were considered successful based on postmortem identification of radiopaque gelified ethanol within the NP, 49 (63%) had evidence of leakage, 10 (13%) had evidence of what was classified as clinically significant leakage (leakage within the vertebral canal, staining the dura, or within the spinal cord) and 48 (62%) were considered successful injections with no evidence of clinically significant leakage. A total of three injections were made into the wrong IVD site and one of these occurred with each injection method. Within that site, injections were successfully made into the nucleus pulposus and were therefore classified as successful for all analyses.

The odds of a successful injection and the odds of leakage by modality are presented in table 2.6. The odds of a successful injection and the odds of leakage based on operator level of satisfaction and resistance score are presented in Table 2.7. Overall, the odds of successful injection without leakage were greatest with CT guidance, which was significantly better than ultrasound (p=0.0026). The odds of having a successful injection with CT guidance were 20 times greater than ultrasound (p=0.0014). The odds of having a successful injection without significant leakage with CT guidance were 12 times greater than ultrasound (=0.0026). Additionally, increased operator level of satisfaction significantly improved the odds of a successful injection (p=0.0314), as did decreasing levels of resistance (p=0.0026). There was an overall significant association between the time to complete the injection and the method of image guidance (p<0.0001). The mean time for the injection procedure for each modality was as follows: US 8.28 minutes (3–13), fluoroscopy 10.40 min (4–30), and CT 21.57 min (8–47). These differences were significant between CT and fluoroscopy (p< 0.0001) and CT and US (p< 0.0001) but were not significant between fluoroscopy and US (p=0.1494). There were no significant differences in the time to complete the
injection procedure between the different anatomic sites (p=0.5532).

The method of image guidance was a significant predictor of the operator level of satisfaction (p=0.0041). Using the 0–4 scale, the mean level of operator level of satisfaction with the injection for each modality was; US 2.4 (0–4), fluoroscopy 3.2 (1–4), and CT 3.2 (2–4). These differences were significantly different between CT and US (p=0.0253), and fluoroscopy versus US (p=0.0022), but were not different between fluoroscopy and CT (p=0.7741). There were no significant associations between the level of operator satisfaction and the different anatomic sites (p=0.2290). The method of image guidance was a significant predictor of successful injection (p=0.0026) and successful injection without clinically significant leakage (p=0.0098). The level of satisfaction was a significant predictor of the degree of leakage (p=0.0072). When method was considered in the model, this was still significant (p=0.0083). The anatomical site did not have a significant effect on the rate of successful injection (p=0.9337), the rate of successful injections without clinically significant leakage (p=0.9472), or on the rate of leakage (p=0.5530).

Of the total injections, 10/78 (13%) had what was classified as clinically significant leakage with evidence of radiopaque gelified ethanol-methylene blue preparation within the vertebral canal or staining the dura mater or within the spinal cord (Table 2.5). Neither method (p=0.7164) nor site (p=0.6122) was significantly associated with the outcome of clinically significant leakage. Neither resistance to injection (p=0.7675) nor operator level of satisfaction (p=0.4945) was predictors of injection with clinically significant leakage.

2.5 Discussion:

This study compared three imaging techniques that are available in most veterinary referral centers (US, fluoroscopy, and CT). Successful image-guided injection, characterized as injection of material into the nucleus pulposus, was achieved in 55 of 78 (71%) of all discs. We had hypothesized
that CT would yield more successful injections compared to fluoroscopy and ultrasound because of an improved ability to plan the injection direction, ability to measure the distance the needle must travel on the CT images, lack of superimposition of soft tissues, ability to visualize needle placement in a cross-sectional manner, and ability to visualize the injected preparation following administration. Although there was no significant difference between CT and fluoroscopy in association with any outcomes, CT did have the highest rate of success. However, authors acknowledge that the study may have been underpowered, to detect small differences in accuracy between CT and fluoroscopy.

To our knowledge, this is the first detailed description of a US-guided percutaneous IVD injection technique in the veterinary literature. Ultrasound guidance was found to have the lowest rate of success, lower operator satisfaction with the injection procedure, and the highest rate of leakage in this study. The low success of ultrasound in all regions was unexpected, as ultrasound is commonly used for guidance during fine needle aspirations, biopsies, and injections. A reason for the limited success with ultrasound may be the inability to reliably differentiate the NP from the AF with ultrasound. Although previous reports have described being able to distinguish the NP from AF, these were performed intra-operatively or following dissection of the soft tissues (Naish, 2003). In our study, ultrasound was performed from a percutaneous approach; therefore, distinguishing the features of the IVD may have been more challenging due to increased ultrasound beam attenuation and reflection, and increased depth of imaging. This was subjectively more difficult in smaller dogs, in which the IVD space was very small and differentiation of AF and NP was difficult in that it required a precise angle of insonation. Postmortem changes to the IVD may also have contributed to the decreased visualization of the NP. The oblique angles from which the US-guided needles were typically directed also likely contributed to the significantly lower successful injections when compared to CT and fluoroscopy. Ultrasound guidance was especially difficult in the thoracolumbar region, due to the anatomic orientation of the ribs. This difference between ultrasound and other modalities was also reflected in the operator satisfaction
score analyses, which included the operator’s ability to confidently identify appropriate anatomical landmarks and the needle within the disc. The level of satisfaction with ultrasound was significantly lower compared to fluoroscopy and CT. Since many primary care veterinary hospitals currently lack CT and/or fluoroscopic capability, the use of ultrasound for guidance would be desirable but cannot be recommended at this time.

The patient positioning and direction of needle placement used in the current study were determined based on previous publications and clinical experience performing fine needle aspirates or biopsies of discs and tissue in similar locations. The positioning and needle placement described here are not meant to be exclusive and authors acknowledge that different patient positioning or needle direction methods may be preferred by different operators. These injections were performed in canine cadavers and therefore blood vessels in the regions of the injections were not always visible, as they were not distended with blood. In clinical cases it may be critical to identify adjacent vasculature in order to avoid this while placing the needle. The use of CT or ultrasound to guide the placement of the needle should provide adequate guidance around the vasculature in live patients.

With fluoroscopic guidance, the placement of the needle into the center of the IVD was facilitated by orthogonal images in the current study. This likely contributed to fluoroscopy being significantly more successful than US, where typically only one visualization plane could be reliably achieved. The therapeutic agent that we elected to inject has an added radiopaque substance (tungsten) that reportedly allows visualization of the injected material on radiographs, fluoroscopy, or CT. However, in our study, this characteristic was only appreciated with CT. During the fluoroscopic-guided injections the injected preparation was not visualized. Initially, it was thought that this could have been due to the resolution of the fluoroscopic unit and/or the small volume of material injected. In human patients, the reported dose of radiopaque gelified ethanol for diseased IVD is similar to the dose used here (ranging from 0.2–0.8mls) and is reportedly visualized with comparable fluoroscopic equipment. Therefore, we suspected that the inability to see
radiopaque gelified ethanol solution with fluoroscopy was more likely because it was not agitated enough prior to the injection. This could have resulted in residual tungsten within the bottle and insufficient tungsten in the injected preparation. In order to address this (and with the aim of developing a procedure for application to clinical patients), we elected to change the agitation times for injectate preparation to 5 minutes for the CT injections. This resulted in successful visualization of the preparation with CT in all cases. Authors acknowledge that changing the agitation time during the course of the study could have introduced an additional variable and could have affected our final results.

In this cadaver study, we elected to use radiopaque gelified ethanol as an injection agent with the long-range goal of helping to develop a clinically appropriate method of administration. Ethanol acts by causing a molecular split of the remaining proteoglycans of the NP in hopes of diminishing the nuclear volume resulting indirectly in regression of IVD protrusion (Riquelme, 2001). The major concern with the use of pure ethanol as a treatment for IVD herniation was the lack of control of the distribution of the liquid ethanol. Dissolution into the tissues surrounding the IVD, especially the adjacent nervous tissue, was proposed to contribute to an increase in adverse reactions to the injections (Riquelme, 2001; Theron, 2007). To create the gelified ethanol product, ethylcellulose has been added. This increases the viscosity and allows for smaller volumes to be administered and a more restricted distribution. It is anticipated that this preparation will allow a more concentrated effect and remain isolated at the appropriate location (Theron, 2007). Clinical studies using radiopaque gelified ethanol have been reported in 436 human patients and five publications, with excellent success rates (Theron, 2007; Theron, 2010; de Seze, 2013; Stagni, 2012; Bellini, 2013). Between 75% and 91.4% of treated patients experienced improvement of symptoms without side effects. One of the earliest described agents used in disc ablation was chymopapain. Although there was good success reported in selected human and veterinary patients with this treatment, chymopapain was associated with anaphylaxis, discitis, and postoperative back spasms, all of which has led to a withdrawal of the drug (Sussman, 1975; Hall, 1983; Fraser,
1984; Le Goff, 2002; Raj, 2008; Guarnieri, 2009). While the current study investigated a newly available radiopaque gelified ethanol preparation, other injectable therapeutic agents including growth factors, inflammatory inhibitors, ozone, and stem cells have all been investigated in experimental models as possible future treatment for IVD disease (Riquelme, 2001; Deen, 2003; Andreula, 2003; Guarnieri, 2009; Lotz, 2012). Additionally, the results of this study can be extrapolated to the accurate aspiration of IVD material, which may be indicated in the diagnosis of discospondylitis (Fischer, 1997; Chew, 1996).

The maximum dose that could be administered in our cadaver studies was 0.4mls due to a high level of resistance. This high resistance may have been due to the cadaver dogs having normal IVDs, for which the maximum injection volume has been reported to be 0.3mls for discograms (Barthez, 1994). This could also have been related to the small size of our cadavers compared to human patients. In dogs with degenerative IVDs, it is expected that the dose administered may be higher and/or more easily administered than in this study. For one of the injections, the injected preparation was not located on the post injection CT or at necropsy. This may be due to an extremely high back pressure during injection and therefore an inability to injectate the preparation.

There was no significant difference in injection success for varying regions of the spine. Our hypothesis that injection in the lumbar and lumbosacral region would be the most successful was not statistically supported, although the percentage of successful injections in these regions was the highest. We had hypothesized that these areas would be the easiest because there tends to be better visualization of the IVD with ultrasound and fluoroscopy. Additionally, the orientation of the IVD in the lumbar and lumbosacral region, may have allowed an easier approach to the IVD compared to the cervical and thoracolumbar region. In these two regions, the more pronounced transverse processes and the ribs, respectively, as well as the angles of the IVD were expected to contribute to a more challenging placement of the needle. The injection of the cervical IVD space had lower success rates with
ultrasound and fluoroscopic guidance than expected. Injections in these locations may have had a decreased success rate because of the larger ventral AF and therefore greater resistance of the needle over a greater length.

The majority of injections had evidence of leakage (49/78, 63%) and the majority of this leakage occurred along the needle tracts into the surrounding soft tissue. This was unexpected, as the viscosity of this preparation was designed to reduce retrograde leakage along the needle tract. The addition of methylene blue to the preparation could have reduced the viscosity and the large amount of backpressure required for injecting the non-degenerative IVDs in our cadaver dogs may have contributed to high incidence of soft tissue leakage. Radiopaque gelified ethanol did not have any morphostructural effects when injected into soft tissue or along neurologic tissue in a porcine model at 48 h post-injection (Guarnieri, 2010). Another possible explanation for the high rate of leakage was that the injections were performed on cadavers that were frozen and thawed multiple times, this may have altered the integrity of the IVDs. Since our long-range goal is to develop the most accurate and safe technique for future application in clinical cases, we considered any injection that resulted in extension of radiopaque gelified ethanol into the vertebral canal, nerve roots, spinal cord, and/or epidural space to be clinically significant. In the current study, 10/78 (13%) of the injections had evidence of leakage that met these criteria. While there was no difference among modalities for the number of injections with clinically significant leakage, this may have been due to low number of injections included in this portion of the statistical analyses. Since CT allows immediate visualization of the distribution of radiopaque gelified ethanol, we expect that this modality would have advantages in clinical patients, as it would allow rapid quantification and localization of clinically significant leakage.

When comparing whether an injection was successful or not we elected to use necropsy examination to characterize the presence of the injected preparation. Authors acknowledge that necropsy examination may not necessarily be the ideal gold standard as it is possible that a small amount of
the injected preparation could be missed. Since the gelified ethanol product has an added radiopaque material, CT may be more sensitive than necropsy examination for detecting the presence of small amounts of the gelified ethanol product.

Three injections were made at the incorrect disc locations, with the injection occurring in one disc space cranial or caudal to the intended IVD. This was attributed to incorrect counting of the IVD spaces. This error can easily be minimized in future studies by increasing the scan length in CT to ensure that definitive anatomic landmarks are acquired for confirmation. With ultrasound guidance, because of the small probe size, only a few vertebrae are visualized at a time and the operator must count from known anatomic reference points. With fluoroscopy this was attributed to an error in counting the last rib while the patient was in an oblique position. Use of orthogonal fluoroscopic evaluation for counting ribs would prevent this. For all modalities, care must be taken to ensure that the correct site is accessed through the rigorous use of anatomical landmarks.

Computed tomography-guided injections took significantly longer compared to fluoroscopy and US-guided injections. Much of the time with CT-guided injections was spent in the setup of the study and making small adjustments of the needle, having to leave the room, move the table and scan the region of interest again. It is anticipated that the time to perform CT-guided injections would decrease with increasing experience and furthermore the magnitude of this difference is not sufficiently long to deter its use.

A more clinically appropriate way of classifying a successful injection may be considering one that has the preparation visualized within the NP with no evidence of clinically significant leakage. When this was considered, CT guidance remained significantly associated with successful injections without clinically significant leakage compared to US and there was a trend towards CT having a statistically significant relationship with this outcome compared to fluoroscopy. This, combined with the advantage of being able to visualize leakage in this study, leads us to recommend CT for use in the clinical setting.
Where CT is not available or practical, fluoroscopy should be considered an appropriate alternative.

In conclusion, findings from this canine cadaver study indicate that CT, fluoroscopy, and US-guided percutaneous injection of radiopaque gelified ethanol into the NP are feasible at the cervical, thoracolumbar, lumbar, and lumbosacral regions. Highest rates of success with the least amount of clinically significant leakage were achieved with CT guidance, and this modality also facilitated visualization of the radiopaque gelified ethanol preparation. However, there was a high rate of leakage within the soft tissues along the needle tract, affecting 49/78 discs (63%). The clinical significance of such leakage warrants further investigation prior to introducing this technique for clinical patients.

Acknowledgements
The authors wish to thank Gabrielle Monteith for her help with statistical analysis and the OVC Pet Trust Fund for their financial support.

Footnotes:
a - Discogel®, Gelscom, France
b – Methylene blue, Omega, Montreal, QC, Canada
c – Vortex-Genie 2, Scientific Industries, Inc., New York, USA
d - Spinal needles, BD Medical, Franklin Lakes, NJ, USA
e – Polycarbonate syringes, Merit Medical, South Jordan, UT, USA
f – GE LOGIQ 5, General Electric Healthcare, Milwaukee, WI, USA
g – Philips BV Endura C-Arm, Phillips, Bothell, WA, USA
h - GE Bright Speed, General Electric Healthcare, Milwaukee, Wi, USA
i - SAS version 9.2, Toronto, ON, Canada
2.6 Table and figures:

**Table 2.1:** Subjective Grading of Level of Operator Satisfaction with 78 Injections into the IVDs in 14 Canine Cadavers.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Improper injection; dissatisfied with anatomical landmarks and lacked confidence in location and/or positioning of needle</td>
</tr>
<tr>
<td>1</td>
<td>Landmarks were identified but the injection localization within the nucleus pulposus was highly uncertain</td>
</tr>
<tr>
<td>2</td>
<td>Landmarks were identified and the operator was somewhat confident of injection localization within the nucleus pulposus</td>
</tr>
<tr>
<td>3</td>
<td>Landmarks were identified and the operator was moderately confident of injection localization within the nucleus pulposus</td>
</tr>
<tr>
<td>4</td>
<td>Landmarks were identified and the operator was certain of injection localization within the nucleus pulposus</td>
</tr>
</tbody>
</table>
### Table 2.2: Cadaver Positioning for Fluoroscopy and Ultrasound Injections

<table>
<thead>
<tr>
<th>Anatomic Region</th>
<th>Dog Positioning</th>
<th>Injection direction</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cervical</strong> (C2-3 to C6-7)</td>
<td>Dorsal</td>
<td>Dorsal</td>
<td>Ventrolateral</td>
</tr>
<tr>
<td><strong>TL</strong> (T10-11 to L2-3)</td>
<td>Oblique sternal</td>
<td>Oblique sternal</td>
<td>Lateral</td>
</tr>
<tr>
<td><strong>Lumbar</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2-3 to L5-6</td>
<td>Lateral</td>
<td>Dorsal</td>
<td>Lateral</td>
</tr>
<tr>
<td>L6-7</td>
<td>Sternal</td>
<td>Dorsal</td>
<td>Dorsal</td>
</tr>
<tr>
<td><strong>LS</strong></td>
<td>Sternal</td>
<td>Dorsal</td>
<td>Dorsal</td>
</tr>
</tbody>
</table>

Fluoro=fluoroscopy. Cadaver positioning and the needle direction for injection of the 50 IVD with fluoroscopic and ultrasonographic guidance at each of the four anatomical regions (cervical, thoracolumbar (TL), lumbar and lumbosacral (LS)) in 7 canine cadavers. Needle direction is in regards to the originating direction and the aspect of the IVD first contacted by the needle.
**Table 2.3: Cadaver Positioning for Computed Tomography Injections**

<table>
<thead>
<tr>
<th></th>
<th>Dog Positioning</th>
<th>Injection direction</th>
<th>Landmarks:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cervical</strong></td>
<td>Dorsal with oblique to right</td>
<td>Left ventrolateral</td>
<td>Ventral aspect of left transverse process</td>
</tr>
<tr>
<td>(C2-3 to C6-7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TL</strong></td>
<td>Right lateral</td>
<td>Left lateral</td>
<td></td>
</tr>
<tr>
<td>(T10-11 to L1-2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lumbar</strong></td>
<td>Right lateral</td>
<td>Left lateral</td>
<td>Ventral aspect of left transverse process</td>
</tr>
<tr>
<td>L2-3 to L5-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L6-7</td>
<td>Sternal</td>
<td>Dorsal</td>
<td></td>
</tr>
<tr>
<td><strong>LS</strong></td>
<td>Sternal</td>
<td>Dorsal</td>
<td></td>
</tr>
</tbody>
</table>

Cadaver positioning and needle direction for injection of 28 IVDs using computed tomography guidance at each of the four anatomical regions (cervical, thoracolumbar (TL), lumbar and lumbosacral (LS)) in 7 dogs. Needle direction is in regards to the originating direction and the aspect of the IVD first contacted by the needle.
**Table 2.4:** Number and Frequency of Successful Intervertebral Disc Injections in 14 Canine Cadavers

<table>
<thead>
<tr>
<th>Site</th>
<th>Fluoroscopy Overall</th>
<th>Fluoroscopy Without leakage</th>
<th>Ultrasound Overall</th>
<th>Ultrasound Without leakage</th>
<th>CT Overall</th>
<th>CT Without leakage</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (C2-2 to C6-7)</td>
<td>4/7 (56%)</td>
<td>3/7 (43%)</td>
<td>3/7 (43%)</td>
<td>3/7 (43%)</td>
<td>7/7 (100%)</td>
<td>7/7 (100%)</td>
</tr>
<tr>
<td>TL (T10-11 to L1-2)</td>
<td>5/7 (71%)</td>
<td>4/7 (57%)</td>
<td>1/7 (14%)</td>
<td>1/7 (14%)</td>
<td>7/7 (100%)</td>
<td>7/7 (100%)</td>
</tr>
<tr>
<td>L (L2-3 to L6-7)</td>
<td>6/7 (86%)</td>
<td>6/7 (86%)</td>
<td>4/8 (50%)</td>
<td>3/8 (38%)</td>
<td>6/7 (86%)</td>
<td>5/7 (71%)</td>
</tr>
<tr>
<td>LS</td>
<td>4/4 (100%)</td>
<td>2/4 (50%)</td>
<td>2/3 (67%)</td>
<td>2/3 (67%)</td>
<td>6/7 (86%)</td>
<td>5/7 (71%)</td>
</tr>
<tr>
<td>Total</td>
<td>19/25 (76%)</td>
<td>15/25 (60%)</td>
<td>10/25 (40%)</td>
<td>9/25 (36%)</td>
<td>26/28 (93%)</td>
<td>24/28 (86%)</td>
</tr>
</tbody>
</table>

Fluoroscopy and ultrasound guided injections were performed in a total of 25 intervertebral discs in dogs 1-7. CT guided injections were performed in a total of 25 intervertebral discs in dogs 8-14. Injections were considered successful if the preparation of Discogel® and methylene blue was found within the nucleus pulposus. Injections were considered to have leakage if the preparation of Discogel® and methylene blue was found outside the nucleus pulposus including within the annulus fibrosus.
Table 2.5: The Rate of Leakage and Clinically Significant Leakage Following 78 Intervertebral Disc Injections in 14 Canine Cadavers Using Fluoroscopy, Ultrasound and CT Guidance

<table>
<thead>
<tr>
<th>Site</th>
<th>Fluoroscopy Leakage</th>
<th>Fluoroscopy Sig Leakage</th>
<th>Ultrasound Leakage</th>
<th>Ultrasound Sig leakage</th>
<th>CT Leakage</th>
<th>CT Sig Leakage</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (C2-3 to C6-7)</td>
<td>6/7 (86%)</td>
<td>1/7 (14%)</td>
<td>5/7 (71%)</td>
<td>1/7 (14%)</td>
<td>3/7 (43%)</td>
<td>0/7 (0%)</td>
</tr>
<tr>
<td>TL (T10-11 to L1-2)</td>
<td>4/7 (57%)</td>
<td>1/7 (14%)</td>
<td>7/7 (100%)</td>
<td>2/7 (29%)</td>
<td>4/7 (57%)</td>
<td>0/7 (0%)</td>
</tr>
<tr>
<td>L (L2-3 to L6-7)</td>
<td>4/7 (57%)</td>
<td>0/7 (0%)</td>
<td>6/8 (75%)</td>
<td>1/8 (13%)</td>
<td>4/7 (57%)</td>
<td>1/7 (14%)</td>
</tr>
<tr>
<td>LS</td>
<td>3/4 (75%)</td>
<td>2/4 (50%)</td>
<td>1/3 (33%)</td>
<td>0/3 (0%)</td>
<td>2/7 (29%)</td>
<td>1/7 (14%)</td>
</tr>
<tr>
<td>Total</td>
<td>17/25 (68%)</td>
<td>4/25 (16%)</td>
<td>19/25 (76%)</td>
<td>4/25 (16%)</td>
<td>13/28 (46%)</td>
<td>2/28 (7%)</td>
</tr>
</tbody>
</table>

Fluoroscopy and ultrasound guided injections were performed in a total of 25 intervertebral discs in dogs 1-7. CT guided injections were performed in a total of 25 intervertebral discs in dogs 8-14. Injections were considered to have leakage if the preparation of Discogel® and methylene blue was found outside the nucleus pulposus including within the annulus fibrosus. Sig Leakage – clinically significant leakage. Any injection with evidence of Discogel®-methylene blue preparation within the vertebral canal or staining the dura mater or within the spinal cord was considered to be an injection with significant leakage.
Table 2.6: Odds Ratios for Comparing Outcomes of Injection of the Intervertebral Disc In Canine Cadavers Between Three Imaging Modalities

<table>
<thead>
<tr>
<th></th>
<th>Successful injections</th>
<th>Successful injections with no significant leakage</th>
<th>Injections with leakage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>CI</td>
<td>p-value</td>
</tr>
<tr>
<td>CT vs US</td>
<td>20.135</td>
<td>3.356-120.798</td>
<td>p=0.0014*</td>
</tr>
<tr>
<td>CT vs F</td>
<td>4.102</td>
<td>0.676-24.887</td>
<td>p=0.1227</td>
</tr>
<tr>
<td>US vs F</td>
<td>0.204</td>
<td>0.056-0.740</td>
<td>p=0.0165*</td>
</tr>
</tbody>
</table>

CT, computed tomography, US, ultrasonography, F, fluoroscopy

OR, odds ratio; CI, 95% confidence interval

Successful injection (an injection in the nucleus pulposus), successful injection with no significant contamination (injection within the nucleus pulposus with no evidence of leakage within the vertebral canal, staining the dura, or within the spinal cord) and injections with leakage.

* a significant difference was detected between the odds of an outcome in regards to one modality over the other.

**There was a trend, p<0.10, towards the odds of an outcome in regards to one modality over the other.
Table 2.7: Odds Ratio for the Injection Success and Presence of Contamination Based on Resistance to Injection and Operator level of satisfaction when Injecting the Nucleus Pulposus in 78 Cadaver IVDs.

<table>
<thead>
<tr>
<th></th>
<th>Successful injections</th>
<th>Successful injections with no significant leakage</th>
<th>Injections with leakage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>CI</td>
<td>p-value</td>
</tr>
<tr>
<td>Resistance</td>
<td>2.272</td>
<td>1.303-4.174</td>
<td>p=0.0026*</td>
</tr>
<tr>
<td>Satisfaction</td>
<td>1.731</td>
<td>1.047-2.969</td>
<td>p=0.0314**</td>
</tr>
</tbody>
</table>

Resistance to injection (Resistance) was subjectively scored on a scale of 1-4 (lowest to highest degree of resistance). The operator level of satisfaction (Satisfaction) was based on a subjective score of 0-4 (lowest to highest degree of satisfaction with the injection) based on identification of the landmarks and the confidence that the needle was placed within the nucleus pulposus.

* a significant difference was detected.
Figure 2.1 – A- Sagittal dissection of the lumbar spine showing injected preparation confined to nucleus pulposus at injection sites. B- Transverse dissection of a lumbar IVD following injection of preparation showing preparation within the IVD.
Figure 2.2 – A – Sagittal (cranial to caudal) ultrasound image of the ventral aspect of the L6-7 IVD from a ventral approach of a Dachshund (dog 3) prior to needle placement. The ventral aspect of the IVD (arrow) is seen as a triangular shaped structure between the adjacent hyperechoic vertebral endplates. B – Sagittal (cranial-caudal) ultrasound image of the ventral aspect of the L3-4 IVD space of a mixed breed dog (dog 5) with a needle (arrow) placed through the ventral aspect of the L3-4 IVD during injection.
Figure 2.3 – A- lateral and B- dorsoventral fluoroscopic images of a percutaneously placed spinal needle into the C2-3 IVD of a Beagle dog (dog 1). The needle was placed percutaneously in a left ventrolateral direction.
**Figure 2.4** – Serial Axial CT images of the lumbosacral (L7-S1) IVD space of dog 8, performed with a sharp algorithm and presented in a bone window. A – a 22 gauge spinal needle is placed into the center of the IVD from a dorsal to ventral percutaneous approach. B – Within the center of the IVD space there is an amorphous distribution of dense positive contrast consistent with Discogel® injection.
CHAPTER 3:

DISTRIBUTION, SHORT AND LONG-TERM EFFECTS OF INJECTED GELIFIED ETHANOL INTO THE LUMBOSACRAL INTERVERTEBRAL DISC IN HEALTHY DOGS

3.1 Abstract:

Radiopaque gelified ethanol preparation has been described as a useful agent for treatment of humans with IVD herniations. The material is injected into the NP under image guidance with intention to cause the protruded IVD material to recede. Because treatment options for dogs with chronic protrusions are limited, new and minimally invasive treatments are desirable. The aim of this study was to assess the feasibility and safety of percutaneous injection of gelified ethanol into the LS IVD of dogs. The lumbosacral IVDs of normal dogs (n=10) were imaged with magnetic resonance imaging and then injected with gelified ethanol using image guidance. The accuracy of gelified ethanol placement in the NP and presence of leakage of the injected material were documented. Post-injection computed tomography (CT) findings (n=10), short (n=10) and long-term (n=5) follow up MRI and CT findings were compared to document the distribution of the injected preparation and identify effects on adjacent tissues. Percutaneous injection of the IVD was successful in delivering radiopaque gelified ethanol to the NP in all dogs. Leakage of the injected material into the vertebral canal was present in 5 dogs immediately following injection and in an additional dog at 1 year following injection. All dogs tolerated the injection well and had no clinical adverse reactions within the study period. Findings indicate that injection of the NP of healthy dogs is well tolerated, even in the presence of mild leakage of material from the IVD.
3.2 Introduction

Intervertebral disc herniation is a common and debilitating disease in dogs. Two types of IVD herniation associated with degeneration have been described in veterinary medicine: a nuclear extrusion and an annular protrusion (Hansen, 1951; Hansen, 1952; Bray, 1998b; Mckee, 2000b; Brisson, 2010; Bergknut, 2013; Bergknut, Smolders, et al., 2013; Smolders, 2013). Surgery is a common treatment for acute nuclear extrusion in dogs with significant spinal cord compression, with excellent success rates in dogs with intact deep nociception; however dogs with annular protrusion treated surgically tend to have a poorer overall recovery rate with reported success rates ranging between 22-89% (Brisson, 2010; Mckee, 2000b; Macias, 2002; Cherrone, 2004; Moissonnier, 2004; Kinzel, 2005; Downes, 2009; Scmeid, 2011). Given the degree of invasiveness of surgical therapies for protrusion of the AF coupled with the poor success rate, further treatment options should be investigated. In general, minimally invasive procedures are associated with lower complication rates, shorter hospitalization times and less patient stress (Atilola, 1988; Muto, 2004).

Intervertebral disc degeneration in dogs has been shown to be similar to the disease process in humans (Singh, 2005; Bergknut, 2012). In human medicine, multiple minimally invasive procedures have been evaluated for the treatment of protrusions of the IVD (Singh, 2005; Smith, 1964; Smith, 1967; David, 1980; Benoist, 1982; Riquelme, 2001; Andreula, 2003; Deen, 2003; Muto, 2004; Theron, 2007; Guarnieri, 2009; Theron, 2010). These procedures include chemical, ultrasonic, laser and thermal methods of nucleolysis, which all share the same goal of ablation of the degenerative NP (Guarnieri, 2009; Kalekis, 2010). The aim of these techniques is either to decrease in the intradiscal pressure or to dehydrate the NP causing the herniated portion of the IVD to recede (Guarnieri, 2009; Kalekis, 2010). Few reports have been published on minimally invasive injectable therapy for IVD herniation in dogs (Smith, 1967; Widdowson, 1967; Gavin, 1973; Takaharski, 1997; Han, 2002).
A radiopaque gelified ethanol product\(^a\) has recently been developed for percutaneous injectable treatment of IVD herniation in human patients (Theron, 2007). In preliminary clinical studies involving over 400 humans, 75-91\% of patients reported improvement of symptoms with no adverse effects following treatment with radiopaque gelified ethanol (Theron, 2007; Theron, 2010; Stagni, 2012; Bellini, 2013; de Seze, 2013). It is theorized that the ethanol results in molecular splitting of the proteoglycans in the NP, causing a reduction in the nuclear volume and regression of the protrusion (Riquelme, 2001). Ethylcellulose is added to the ethanol to increase the viscosity, allowing better control of the injection and less dispersion of the material post injection (Theron, 2007). Additionally, the ethylcellulose may cause dehydration of the disc due to its hydrophilic properties, potentially contributing to further regression of the protruded AF (de Seze, 2013). As described in Chapter 2, injection of radiopaque gelified ethanol into the IVD of dog cadavers can be accomplished by fluoroscopy, ultrasound and computed tomography (CT) but CT guidance achieved the highest rates of success with the least amount of adjacent tissue contamination (Mackenzie, 2014). However, a high rate of contamination of the soft tissues along the needle tract was reported (63\%) (Mackenzie, 2014). The clinical significance of this contamination in live dogs is unknown. A previous study found that intentional injection of gelified ethanol into the intervertebral foramina, epidural space, and paravertebral musculature of a pig did not result in clinical or morphologic tissue alterations at 48 hours (Guarnieri, 2010). The long-term effects, if any, remain unexamined. Further evaluation of the safety and potential side effects of this compound in dogs is warranted prior to its application to clinical patients.

The aims of the current study were to 1) evaluate the safety, 2) the distribution, and 3) the effect of injection of radiopaque gelified ethanol into the LS IVD of normal dogs. The hypotheses are that 1) the administration of gelified ethanol will be well tolerated by normal dogs with no clinically observable adverse effects, 2) that delivery of the radiopaque gelified ethanol to the NP can be achieved using CT guidance in all dogs with the material being contained within the NP, and 3) that short and long term follow-
up imaging with CT and MRI will not reveal substantial regional tissue changes. The findings in this study will establish a basis to begin a prospective clinical trial in dogs with IVD protrusion.

3.3 Materials and methods
Healthy dogs from a research colony were used. The inclusion criteria included dogs with normal physical and neurological examination, normal complete blood count and biochemical profile and a pre-injection MRI examination of the LS region with no evidence of LS disease or other clinically significant abnormalities. Equivocal findings included the presences of mild IVD protrusion (less than 25% of height of the vertebral canal), presence of transitional vertebrae, unfused sacral vertebrae or spondylosis deformans. Protocols were approved by the Animal Care and Use Committee of the University of Guelph, operating under the guidelines of the Canadian Council on Animal Care. The study design timeline for the dogs receiving injection of radiopaque gelified ethanol into the LS IVD is presented in Figure 3.1.

_Pilot study (n=1)_

The first dog was a 4.5-year old male neutered Walker Hound that was used as a trial dog, as requested by the animal care committee, prior to initiating the full study. The protocol was slightly different from other dogs; MRI assessment, fluoroscopic guided injection, and a post-injection CT scan of the LS IVD were all performed on the same day. Fluoroscopic guided injection of the LS IVD was performed under general anesthesia using a Phillips Endura C-Arm fluoroscopy unit from a dorsal approach (Mackenzie, 2014). Physical and neurological examinations were performed daily for 7 days and at 6 months following injection. A post-injection MRI was performed at 3 days following injection. Long-term follow-up MRI, CT and neurological and physical examinations were performed 2 years after injection of the LS IVD. Because of the difference in technique and follow-up time periods, this dog was left out of statistical
analysis. Based on the successful injection in this patient and lack of side effects or complications, it was decided to proceed with the full trial using an additional 9 dogs as described below.

*Full study (n=9)*

*Pre-injection MRI*

Magnetic resonance images of the LS IVD were acquired with a 1.5 Tesla scanner under general anesthesia. A receive only, phased array, 12 element, 8-channel radiofrequency coil was used for all studies. Magnetic resonance imaging sequences were performed in three standard planes, including dorsal, sagittal, and transverse relative to the long axis of the vertebral column in order to thoroughly assess the region for any abnormalities. Sequences acquired for images included: T2*-weighted single shot fast-spin echo in a sagittal plane (TR 3000ms, TE 11000ms, 2.0mm, NEX 1); T2-weighted (TR 3200-5200ms, TE 85ms, 2.00mm, NEX 4) fast spin echo in sagittal and transverse plans; T1-weighted (TR 416ms, TE 11ms, 3.00mm, NEX 4) fast-spin echo in transverse plane; and dorsal short tau inversion recovery (TR 4150ms, TE 49ms, 3.00mm, inversion time 150ms, NEX 4). Additionally sagittal T2*-weighted single shot fast-spin echo images of the cervical and thoracic spine were performed in all dogs prior to injection. For the T2*-weighted single shot fast-spin echo myelogram images a large field of view was used and for all other images a small field of view was used. A 256 x 256 matrix was used. The time for MR examination was recorded. The images were reviewed by a board certified radiologist and radiology resident independently. The degree of degenerative changes in the LS IVD were scored according to previously validated Pfirrmann grading scheme using sagittal T2 weighted images (Table 1.1) (Pfirrmann, 2001; Bergknut, Auriemma, et al., 2011; Bergknut, Grinwis, et al., 2011). When the Pfirrmann score of the IVD varied between categories, the higher grade was chosen. Any discrepancies between the two observers were discussed and a consensus was made. Any other findings in the LS region were recorded by the
observer as for a clinical case review. Measurements of lumbosacral IVD size were performed by one observer and included: dorsal-ventral height on sagittal and transverse images, cranial-caudal thickness on sagittal images and right-left width on transverse images (Figure 3.2). The cranial-caudal thickness was performed at two locations of the IVD on the sagittal images by dividing the IVD into thirds and measuring height at the dorsal and ventral thirds. The angle between the center of the sacral vertebrae and the 7th lumbar vertebrae was measured as a reference for any change in position.

**Radiopaque Gelified Ethanol injection**

Ten days after the pre-injection MRI, all dogs (n=9) had a CT-guided injection of the LS IVD preformed by either a radiology resident, a board certified radiologist or a board certified surgeon under general anesthesia following a previously described technique (Mackenzie, 2014). Computed tomographic examination of the LS IVD was performed with the dog in sternal recumbency with the pelvic limbs flexed using a 16-slice helical scanner. The LS IVD space was identified on scout images and axial CT images of the LS IVD were acquired (section collimation thickness: 1.25mm, tube rotation time: 1.0s, mA: 200, kV: 120). All images were acquired with a large field of view (50cm) and a 512x512 matrix using both a soft tissue and bone algorithm. The skin dorsal to the LS junction was clipped and steriley prepared. The prepared area was isolated using a fenestrated pediatric laparotomy sheet and an iodophor impregnated incise drape was applied to the skin. Palpation of the iliac crests and the spinous process of L7 was used to identify the LS space and a spinal needle was placed from a dorsal direction towards the LS disc space. The needle was guided through the spinal canal and into the center of the LS IVD to the NP using serial CT scans for guidance. When satisfied with placement of the needle in the center of the IVD, radiopaque gelified ethanol was administered. The radiopaque gelified ethanol was prepared by agitation at maximum speed (3200 rpm) using an agitator for a minimum of 5 minutes, until the preparation
was uniform in color as recommended by the manufacturer. A target volume of up to 0.8 ml/disc of radiopaque gelified ethanol was administered based on recommended volume in human patients (Theron, 2007). The syringe plunger was pressed using digital (thumb) pressure until maximum injection resistance was reached and maintained for an additional 2 minutes prior to releasing the pressure and removing the needle. The CT scan was repeated post-injection. The dogs were then recovered from anesthesia and a physical examination performed following recovery post-anesthesia. One hour post anesthetic recovery and after the physical examination, all dogs received 0.03 mg/kg of hydromorphone and 0.1 mg/kg of meloxicam subcutaneously for analgesia. The CT images were reviewed by two independently and blinded reviewers. Window width and level were adjusted at will.

Based on the CT scan, recorded data included whether radiopaque gelified ethanol was present or absent following injection, number of times the needle was repositioned, the duration of the injection procedure (including setup time), administered volume, and needle size. Injections were considered successful if the injected material was evident within the NP on the post injection CT scan images. For each injection the operator recorded if the needle was visualized within the NP of the LS IVD. Similar to a preliminary study, the operator subjectively assessed the resistance to injection and the satisfaction with the injection (Mackenzie, 2014). Resistance was recorded on a scale of 1 to 4, with 1 representing no resistance, 2= mild resistance, 3= moderate resistance, and 4=marked resistance. The level of satisfaction with the injection was assigned by the operator from 0-4 based on subjective criteria including identification of the landmarks and the confidence that the needle was placed within the NP (Table 2.1) (Mackenzie, 2014).

On the day following the injection, each dog had a repeat neurological examination performed by the same board certified neurologist as the pre-anesthetic assessment and each dog had daily physical and neurological examinations performed by one of the
authors for 7 days following injection. During physical and neurological examination specific testing for LS pain including firm dorsal palpation over the LS region, elevation of the tail (tail jack) and hyperextension of the caudal lumbar spine with LS pressure (lordosis test) were performed. Dogs were assigned a restricted exercise regime for 48 hours post-injection (leash walks only).

*Post-injection MRI examination*

For all dogs (n=9) a post-injection MRI of the LS IVD, performed under general anesthesia and using the same protocol as the pre-injection MRI, was performed at 14 days following the CT-guided injection of radiopaque gelified ethanol. The same MRI sequences as those acquired in the pre-injection MRI were performed in the same planes in order to thoroughly assess the region for any changes from the pre-injection MRI. The post-injection MRI findings recorded included: evidence of radiopaque gelified ethanol within the NP, within the IVD, spinal cord, vertebral canal or soft tissues adjacent to the vertebrae, and any other MRI changes compared to the pre-injection MRI study. Measurements of the IVD were performed in a similar manner to the initial MRI.

*Long-term follow-up examination*

Physical and neurological examinations were performed on all dogs (n=9) by a board certified neurologist at 6 months following injection. Additionally, repeat physical, neurologic, and CT and MRI examinations were performed at 1 year in 4 dogs that remained in the research colony. For these 4 subjects, CT and MR were performed under general anesthesia and protocols were identical to those used for the pre-injection and post-injection imaging. The CT and MRI were assessed by the same reviewers and using the same criteria as the pre- and post-injection studies and the findings were compared to the prior examinations. The images were assessed for persistence of and changes in the amount or distribution of the injected preparation, and
changes to the injected LS IVD. Any changes in MRI signal intensity within the IVD and within the peri-vertebral soft tissues including the spinal cord and nerve roots, as well as any bony changes evident on MRI and/or CT images were recorded. Measurements of the IVD were performed in a similar manner to the initial and post-injection MRI examinations.

Statistics
For all statistical analysis a licensed program\textsuperscript{1} was used. For assessing the distribution of the injected material within the vertebral canal, the changes seen on the imaging studies performed over the study period and any adverse reactions, a logistic regression was performed using exact p-values for continuous variables and a Fischer’s exact test was used for non-continuous variables. Variables that were assessed that may have been associated with these outcomes included: body weight, body condition score, injection order, number of needle adjustments, time for injection, volume of injection, Pfirrmann IVD grade, operator satisfaction with injection and resistance to injection. For assessment of change in the IVD size following injection, analysis of covariance analysis using a generalized linear mixed model was performed that controlled for changes in patient positioning. Model assumptions were tested for normality using Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, Anderson-Darling tests and visual inspection of residuals. Where needed, appropriate transforms were applied. For all analyses significance was set as at p<0.05. The data collected from the 4 dogs that had long-term follow-up imaging performed at 1 year following injection was treated as descriptive data because of the small sample size.

3.4 Results
Pilot study (n=1):
On MRI examination prior to fluoroscopic guided injection dog 1 had mild protrusion of the LS IVD and an equivocal finding of a partially fused LS transitional vertebrae. The Pfirrmann grade of the LS IVD was scored at 3. Fluoroscopy guided injection of the LS
IVD was successful in delivery of radiopaque gelified ethanol into the NP based on post-injection CT images. There was leakage of the radiopaque material into the vertebral canal seen on CT examination following the injection. On post-injection CT images there was mild LS spondylosis deformans. The radiopaque gelified ethanol was not visualized on fluoroscopy during injection. Dog 1 recovered uneventfully from all periods of general anesthesia. There were no changes in behaviour or in physical or neurological examinations at any of the time points assessed. On long-term follow-up CT images, 2 years following injection, there was no substantial difference in the location of the metal dense material within the IVD and the vertebral canal. There was worsening of the spondylosis deformans on the follow-up CT images. There were no changes in the long-term follow-up MRI compared to the post-injection MRI.

*Full study (n=9)*

**CT-guided injections**

Nine healthy hound dogs were used (Table 3.1). These included mixed breed hounds (3), Treeing Walker Coonhound (2), Bluetick Coonhounds (2), and one English Foxhound. Three of the dogs were neutered males, 3 were sexually intact males and 3 were spayed females and their mean weight was 23.8kg (range 17.5-28.5kg) and mean body condition score was 2.7/5 (range 2.5-3.5). The average age of the dogs was 2.9 years (range 1.6-4.3 years).

**Pre-injection MRI**

All dogs had evidence of mild LS IVD degeneration by use of the Pfirrmann scoring system, having either grade 2 (n=7) or grade 3 (n=2) changes. Five of 9 dogs had other abnormalities at the LS junction noted on MRI examination prior to injection including: mild LS IVD protrusion (5/9), unfused sacral vertebrae (2/9), mild stenosis of the LS vertebral canal (2/9), partially fused LS transitional vertebrae (1/9) and spondylosis
deformans (1/9). The average time to perform the MRI study was 38.2 min (range 33-44 min).

CT injection

The average time for performing the CT-guided injection was 30.1 minutes (range 15-45 minutes), including subject positioning, site preparation and draping of the patient, the serial CT slices to verify needle placement, the injection procedure and subsequent 2-minute waiting period. The average time of the procedure without setup was 18.4 minutes (range 6-31 minutes). For all injections, a 22-gauge 3.5" spinal needle (BD Medical, Franklin Lakes, NJ, USA) was used and could be visualized in the center of the IVD prior to the injection. A clear distinction between the NP and the AF was not seen on CT images and the center of the IVD was presumed to represent the NP. The procedure required a mean of 5.6 (range 3-11) adjustments of the needle prior to puncture of the IVD. The average volume administered was 0.18ml/disc (range 0.1-0.2ml/disc). All 9 IVD injections were deemed successful based on the presence of radiopaque material visible centrally within the IVD and shaped in a manner deemed consistent with the NP of the LS IVD on transverse CT images following injection (Figure 2a).

Three of 9 injections had leakage of the injected material into the vertebral canal evident immediately after injection based on the presence of metallic dense material within the vertebral canal (Figure 3). There was various distribution of this material within the vertebral canal, with it either being very small, focal, amount of metal dense material centered over the IVD (n=1) or dispersing in a linear fashion surrounding the spinal cord at the level of the 7th lumbar vertebra and extending caudally to the caudal aspect of the first sacral vertebra (n=2). The body weight and the body condition score of the dog were not significantly associated with the presence of injected material within the vertebral canal (p=0.86 and p=0.31 respectively). There was no significant association
between the presence of injected material in the vertebral canal and the Pfirrmann grades of the IVD (p=0.67), time of the procedure (p=0.74), number of times the needle was adjusted (p=1.00), the resistance to the injection (p=1.00) or the satisfaction with injection (p=1.00). The volume of the injected radiopaque gelified ethanol was not significantly associated with the presence of injected material in the vertebral canal (p=0.83).

Follow up physical and neurological examinations:

All dogs recovered uneventfully from all periods of general anesthesia. There were no changes in behavior or in the physical or neurological examinations at any of the time points assessed. This included no evidence of LS pain on dorsal palpation, hyperextension of the caudal lumbar spine or tail elevation. All dogs tolerated the injection well with no adverse reactions seen over the study period, which included immediate (post-recovery, 24 hours, and daily for 7 days) (n=9), 6 months (n=9) and one year (n=4/9) follow up neurological and physical examinations.

Post-injection MRI

On MRI performed within 2 weeks following injection, a focal decrease in signal intensity within the NP was seen in all dogs on T2 weighted images in a similar area that the radiopaque material was identified on the post-injection CT (Figure 2c). In dogs with Pfirrmann grades of 3 this focal decrease in signal intensity was less obvious. No other other changes were observed on the post-injection MRI compared to the initial MRI in any of the dogs. There was no significant change in the cranial-caudal, dorsal-ventral or right to left dimensions of the IVD between pre- injection and post-injection MRI (range p>0.114) (Appendix VI).

Long-term follow-up CT and MRI
Follow-up MRI and CT images were acquired 1 year following CT-guided injection for 4 dogs (Table 3.1). At this time, one dog (dog 4) had evidence of redistribution of the injected preparation as evidenced by the presence of metal density within the vertebral canal, which was not present on the immediate post-injection CT images. One dog (dog 2) had progressive protrusion of the LS IVD (Figure 4). This was accompanied by an increase in the amount of metal dense material within the vertebral canal compared to the immediate post-injection CT images (Figure 5). For all other dogs the LS IVD was similar in appearance on follow-up MR images compared to that performed within 2 weeks post-injection. On follow-up CT images, the radiopaque material was still seen in the IVD in all dogs and on follow-up MRI, a hypointense region in the center of the IVD, similar to that on immediately post-injection MR, was seen in all 4 dogs. One dog had progression of LS spondylosis deformans (dog 8), but this dog did not have any new evidence of material in the vertebral canal or worsening of the IVD protrusion. There were no other new findings on follow-up MRI or CT images, including no evidence of vertebral end plate abnormalities or changes within the soft tissues surrounding the LS vertebrae. There was no significant change in the cranial-caudal, dorsal-ventral or right to left dimensions of the IVD controlling for change in position between post-injection and follow-up MRI (range p>0.17).

3.5 Discussion:
Radiopaque gelified ethanol has shown promise in human patients but has not yet been evaluated in veterinary patients (Theron, 2007; Theron, 2010; Stagni, 2012; Bellini, 2013; de Seze, 2013). Successful image-guided injection of the radiopaque gelified ethanol into the NP of the LS IVD was achieved in all dogs (n=10) in this study. The distribution of the injected material could be documented immediately post-injection and up to 2 years following injection using CT. On MRI the injected material corresponded to a hypointense region within the NP on T2 weighted images. Overall, in clinically normal dogs, image guided percutaneous administration of radiopaque gelified ethanol was well tolerated with no dogs having adverse reactions to the injection or alterations of
their neurological status despite the presence of the injected material within the vertebral canal in 5 of 10 dogs over the study period.

Injections were performed on a single IVD site so that any adverse reactions could be attributed to this site with no confusion between injected sites. Further, this site is the only disc space approached via the vertebral canal (the other discs are approached either ventrally or laterally (Mackenzie, 2014)), so this maximized the likelihood of observing adverse reactions associated with leakage into the epidural space. Finally, IVD protrusion plays a major role in dogs with degenerative LS stenosis (Suwankong, 2008), so the choice of this site is relevant to clinical applications. Along with this potential application for treatment of LS IVD protrusion, radiopaque gelified ethanol may also be useful for the treatment of IVD protrusions in other regions of the spine. In human patients radiopaque gelified ethanol has been used to treat IVD herniations in both cervical and lumbar regions with high success (Theron, 2007; Theron, 2010; Stagni, 2012; Bellini, 2013; de Seze, 2013). As outlined in Chapter 2, percutaneous CT-guided injection of the cervical, thoracolumbar and lumbar regions of the spine were performed in canine cadavers with success rates ranging from 86% to 100% (Mackenzie, 2014).

To maximize the sensitivity for any changes to the IVD or adjacent soft tissues we elected to perform post-injection MRI examinations as the high contrast resolution of MRI makes it a better modality for detection of soft tissue changes and it is the preferred modality for imaging myelitis, early discospondylitis and empyema in veterinary patients (da Costa, 2010). Additionally, MRI allows better visualization of the NP and therefore permits detection of changes in signal intensity of the NP following injection.

On follow-up MRI examination the extent of the radiopaque gelified ethanol could not be fully quantified in comparison to the CT examination, however in a similar region of the NP in which the radiopaque gelified ethanol was deposited there was a focal,
hypointense, region seen in the majority of dogs. We attributed this to the injected material although this was not examined histologically. Alternatively, this focal hypointense region could represent a change in the NP secondary to the placement of the needle. Following placement of a needle or a stab incision into the IVD, histological changes and corresponding changes to the MR signal intensity and size of the IVD have been reported, however MRI detection of these changes may not be apparent until 3 weeks after injection (Sobajima, 2005; Carragee, 2009). We considered that the injection process may have introduced gas, but the fact that none was observed in the IVD on the post-injection CT makes this unlikely. Since the injected preparation was noted to create a hypointense region in the NP on MRI, this made follow-up assessment of degeneration of the NP and Pfirrmann grading more difficult since some of the altered intensity of the NP could be attributed to the injected product and may not represent further degeneration.

Three of 9 dogs that had CT-guided injection had radiopaque gelified ethanol present within the vertebral canal immediately following injection and 1 additional dog had radiopaque material present within the vertebral canal at 1 year following injection. No dog had adverse clinical reactions to the presence of the material within the vertebral canal. Physical and neurological examination of these dogs continued to be normal over the study period and MRI did not reveal tissue changes associated with the presence of this material outside the NP. We cannot completely rule out the possibility of mild inflammation or other tissue changes associated with the presence of this material in the vertebral canal, but the lack of abnormal physical and neurologic findings indicates that any such tissue responses were not of clinical significance in these dogs. The gelified nature of the ethanol restricts spreading of the material and therefore should limit the leakage of the material into the vertebral canal (Theron, 2007). In healthy individuals the intact NP and AF may also act to restrict the diffusion of the injected material but movement of the material into the vertebral canal may be more likely in animals with IVD degeneration. In fact, the procedure is contraindicated in
human patients with extruded IVD material due to the increased risk of leakage of material into the vertebral canal (Guarnieri, 2009; Kalekis, 2010). Nevertheless, we did not find an association with the Pfirrmann grade of IVD degeneration and the rate of leakage of the material into the vertebral canal, perhaps because of the small population or the fact that most had only a low grade of disc degeneration.

The material that was seen within the vertebral canal was suspected to be within the epidural space however leakage of the material into the subdural space cannot be excluded. In human patients, complications following percutaneous IVD injections are rare with the most commonly reported being diskitis, radiculitis, and epidural abscesses (Kalekis, 2010; de Seze, 2013; Fraser, 1987; Zeidman, 1995; Bouillet, 1990). Another potential complication is discospondylitis. There was no evidence of any of these complications in our study.

A different distribution of the injected material was seen in 2 of 4 dogs on the 1-year follow-up CT images (this included metal dense material within the vertebral canal that was not present immediately following injection in one dog and an increased amount of metal dense material in the vertebral canal in another). These findings are concerning for the possibility of delayed movement of the injected preparation over time. We hypothesize this may be due to movement of the material through the needle tract or progression of the degenerative changes with progressive tearing or rupture of the AF. It is possible that only the tungsten portion of the preparation eventually tracked into the vertebral canal and that the ethanol component of the injected material remained in the IVD or was absorbed prior to leakage. This could not be confirmed in the study but suggests that the injected material may herniate from the center of the IVD into the vertebral canal over time. The impact of migration of the injected preparation on the efficacy of this treatment has not been elucidated. However, no dog in this study had adverse reaction to the injection including in those two cases where the material migrated over time.
One dog had worsening of the LS IVD protrusion at the 1-year follow-up period, suggesting progression of intervertebral degeneration. Two dogs, including the trial dog, had worsening of the spondylosis deformans, a condition that has also been associated with IVD degeneration (Hansen, 1951; Levine 2006). Whether these represent natural progression or an effect of the procedure is unknown, although IVD puncture using a 22 or 25 gauge needle has been shown to increase the rate of disc degeneration in humans (Carragee, 2009). Although this study used healthy dogs, 6 of 10 dogs had findings we considered equivocal such as mild IVD protrusion or transitional vertebrae that may be associated with further LS degeneration independently of the injection (Morgan, 1993; Fluckiger, 2006).

Over our study period there was no evidence of a change in the size of the IVDs based on our measurements. Our hypothesis that a change would be appreciated was based on humans treated with radiopaque gelified ethanol where follow up MR examinations at 1 year or more have revealed a reduction in the IVD herniation (Theron, 2007). However in shorter follow-up time periods there was discordance between the reported improved clinical signs and static imaging findings (Theron, 2007). Although the lack of significant changes may suggest that the product may not reduce the IVD volume in dogs, this should be interpreted with caution, as the power of our study was low due to only 5 dogs being available for a long-term (1-2year) follow-up. Additionally measurements of the intervertebral discs have not been validated and therefore subtle changes in IVD size may have been overlooked. In our opinion there is likely a large level of inter- and intra-observer variability of the measurements, however this was not tested. Furthermore, the discs injected in the current study were not clinically diseased, and the product’s efficacy may be more notable in diseased populations.

This study used a small number of dogs (n=10, including the pilot dog) and therefore rare reactions may have been missed, but nonetheless supports that the injection is
tolerated in healthy patients. The dogs in this study were healthy individuals with only mild Pfirrmann grades of IVD degeneration. The response to the injection may be different in various disease populations. Thus, the potential for adverse reactions should be carefully considered when the procedure is applied to clinical cases of more severe disease, including follow-up at or beyond the 1 year used in this study. Another limitation is the limited long-term (1 to 2 year) follow-up, which was only performed in 5 dogs that remained in the research colony.

This study established that injection of a radiopaque gelified ethanol into the LS IVD was successful in a population of clinically healthy individuals, and that even with evidence of injected material in the vertebral canal in some individuals, there were no clinically apparent adverse reactions. Further evaluation in patients with clinically significant intervertebral disc protrusion at the LS and also cervical, thoracolumbar and lumbar regions of the spine is recommended.

**Acknowledgements**
The authors wish to thank Gabrielle Monteith for her help with statistical analysis, and the anesthesia, radiology and research technicians for the help with anesthesia and acquiring images.

**Footnotes:**
a – Discogel, Gelscom, France
b- Phillips Endura C-Arm fluoroscopy unit, Phillips, Bothell, WA, USA
c - GE Signa Excite II, General Electric Healthcare, Milwaukee, Wi, USA
d - Premier 9000 8-Channel CTL Coil, USA Instruments Inc. Aurora, OH, USA
e - GE Bright Speed, General Electric Healthcare, Milwaukee, Wi, USA
f - Ioban, 3M healthcare, London, Ontario, Canada
g – Vortex-Genie 2, Scientific Industries, Inc., New York, USA
h – Hydromorphone, Sandoz, Quebec, Canada
### 3.6 Tables and Figures

Table 3.1: Summary data for successful CT guided percutaneous injection of radiopaque gelified ethanol into the lumbosacral intervertebral disc in 9 healthy dogs.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Wt. (kg)</th>
<th>IVD grade (1-5)</th>
<th>Number of adjustments to properly place spinal needle</th>
<th>Time of procedure without setup (minutes)</th>
<th>Res.</th>
<th>Sat.</th>
<th>Leakage of material into the vertebral canal (immediate post-injection)</th>
<th>1-year follow-up</th>
<th>Change in metal dense material in vertebral canal</th>
<th>Worsening of IVD protrusion</th>
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Wt.= weight; IVD=intervertebral disc; Res=Resistance to injection; Sat=satisfaction.

Leakage of material into the vertebral canal was evident by the presence of metal dense material within the vertebral canal. The change in metal dense material was seen as an increase in the amount in one dog (dog 2) and the presence of metal dense material in the vertebral canal that was not seen on the immediate post-injection CT (dog 4). The intervertebral disc grade was based on the Pfirrmann grading scheme with a scale of 1 to 5. The resistance to injection represents a subjective scoring of the pressure needed to inject the material on a scale of 1-4 (from least resistance to marked degree of resistance). The operator level of satisfaction was based on a subjective score of 0-4 based on identification of landmarks and the confidence the needle was placed within the nucleus pulposus.
**Figure 3.1:** Study design timeline for 9 healthy dogs receiving CT-guided injection of radiopaque gelified ethanol into the lumbosacral intervertebral disc.
Figure 3.2: Measurement of lumbosacral IVD size on MR images. On transverse images (A) measurements included: dorsal-ventral height (blue line) and right-left width (red line). On sagittal images (B) measurements included: dorsal-ventral height (blue line), cranial-caudal thickness performed at two locations of the IVD by dividing the IVD into thirds and measuring height at the dorsal and ventral thirds (green lines), and the angle between the center of the sacral vertebrae and the 7th lumbar vertebrae as a reference for any change in position (red lines).
**Figure 3.3:** Transverse CT images of the lumbosacral intervertebral disc of Dog 3 following injection of radiopaque gelified ethanol. CT was performed with a sharp algorithm and presented in a bone window (A). Within the center of the intervertebral disc space there is an amorphous distribution of dense positive contrast consistent with radiopaque gelified ethanol (black arrow). Transverse T2-weighted MR images at the same level of the lumbosacral intervertebral disc prior to (B) and after injection (C) are shown. Within the center of the intervertebral disc a region of decreased T2 signal intensity is seen (white arrowhead) in a similar region as the positive contrast material seen on CT. This was attributed to the presence of radiopaque gelified ethanol.
Figure 3.4: Transverse CT images of the lumbosacral intervertebral disc of Dog 7 following injection of radiopaque gelified ethanol. CT was performed with a sharp algorithm and presented in a bone window. Within the intervertebral disc and also within the vertebral canal (arrow) there is amorphous distribution of metallic dense material consistent with radiopaque gelified ethanol.
Figure 3.5: Sagittal T2 weighted MR images centered on the lumbosacral disc of Dog 2 - 1 week (A) and 1 year (B) following CT-guided injection of the lumbosacral disc. There is a decrease in the intervertebral disc T2 signal intensity and worsening of the dorsal protrusion of the lumbosacral intervertebral disc. The findings are consistent with progression of intervertebral disc degeneration. The first and second sacral vertebrae are unfused.
Figure 3.6: Transverse CT images of the lumbosacral intervertebral disc of Dog 2. Immediately (A) following CT-guided injection of the lumbosacral disc, there is a small, focal, metallic dense region is seen within the right ventrolateral aspect of the vertebral canal (arrow). On 1 year follow-up images (B) there is increased amount of metallic dense material in the vertebral canal, predominately on the left side (arrowhead). Sagittal multiplanar reconstruction of the lumbosacral vertebrae 1 year following injection (C) shows the extent of the metallic material in the vertebral canal. The first and second sacral vertebrae are unfused.
Chapter 4: Summary, Conclusions and Recommendations

Compensatory hypertrophy and cyclic damage to the AF secondary to maturation can lead to thickening and tearing of the AF and protrusion of the IVD causing neurological dysfunction, spinal or nerve root compression, and pain (Hansen, 1952; Macias, 2002; Jeffery, 2013). Treatment options for dogs with IVD protrusions are equivocal compared to treatments for other forms of IVD herniation (Schmid, 1993; Macias, 2002; Moissonnier, 2004; Kinzel, 2005; Downes, 2009). Since IVD herniation in dogs shares many similarities with that seen in human patients (Bergknut, 2012), it was of interest to evaluate the suitability of treatments used for human IVD herniation in dogs. Specifically a percutaneous image guided injected therapeutic, radiopaque gelified ethanol (Discogel®), is studied here.

Our work accepts the following hypotheses: minimally invasive image guided injection of the NP is feasible in dogs using CT, fluoroscopy and ultrasound; delivery of radiopaque gelified ethanol into the NP of normal dogs would be achieved with high accuracy; and injection of gelified ethanol into the NP will not be associated with significant side effects in normal dogs. Our work rejects the hypothesis that injection of gelified ethanol into the NP will result in quantifiable alteration of the size of the IVD in normal dogs during the 1 year follow up period. Our work found that successful injection of the IVD was better achieved using CT or fluoroscopy compared to ultrasound. Using fluoroscopy (n=1) and CT-guided (n=9) injection of the LS IVD, the radiopaque gelified ethanol therapeutic was delivered successfully in 10 live dogs with no short-term (2 weeks) or long-term (1 year) adverse clinical effects. This is the first study to evaluate the use of this product in dogs.

The radiopaque gelified ethanol product was developed to allow injection of ethanol with increased control and less dispersion of the ethanol (Theron, 2007).
Our work found leakage of the injected material from the IVD was not uncommon following injection in both canine cadavers and live dogs. In our pilot cadaver study 49 of 78 injections (63%) had leakage of material into the soft tissues adjacent to the vertebrae, most commonly along the needle tract. Leakage of material within the soft tissues along the needle tract was not seen on our second study involving live dogs. This suggests that tissue integrity is important in containing the radiopaque gelified ethanol at the site of deposition and that the distribution in cadaver dogs should be interpreted cautiously in comparison to that seen in live dogs. Four of 10 (40%) LS IVD injections had evidence of leakage of the injected material into the vertebral canal in live dogs immediately following injection. Despite this, no dog in our study had clinical evidence of adverse reaction to the injected material. This is consistent with very minimal rates of adverse reactions reported in human patients, with only one patient having signs consistent with post-injection radiculitis being reported in over 400 subjects treated with gelified ethanol treatment (Theron, 2007; Theron, 2010; Stagni, 2012; de Seze, 2013; Bellini, 2013). This also supports the conclusions of a previous study finding no morphological tissue alterations in the intradiscal and intervertebral foramina soft tissues, epidural space and paravertebral musculature of a pig at 48 hours post injection (Guarnieri, 2010).

In our study, the lack of complications in association with injection of radiopaque gelified ethanol (n=10) is in agreement with similar studies reporting no complications following injection of other agents including chondroitinase ABC (n=59), chymopapain (n=38), and oxygen-ozone (n=5) (Smith, 1967; Atila, 1988; Takahasi, 1997; Han, 2007). In contrast, injection of collagenase in 9 dogs was associated with a higher rate of post-injection adverse reactions compared to our study (Miyabayashi, 1992). Taken together, these findings support that gelified ethanol is likely as safe as most other injectable agents that have been assessed in dogs and safer than collagenase. Extrapolation of the current findings for comparison of the safety of gelified ethanol to other percutaneous procedures, such as laser disc ablation, is more difficult. In a large retrospective
study of percutaneous laser disc ablation in dogs (n=277) reported a 1.8% complication rate associated with the procedure for prophylactic ablation of thoracolumbar IVDs (Bartels, 2003). Although the approach to the IVD differs between percutaneous laser disc ablation procedure and our LS injection (Dickey, 1996, Bartels 2003), complication rates for both procedures may be similar and a larger sample size would have been needed to detect similarly low complication rates.

Although our work supports the low risk of adverse effects of the injected material, there is still a need for further histological and/or immunohistochemical evaluation of the IVDs and adjacent soft tissues following injected with gelified ethanol over a longer period of time to determine the direct effects on the disc and the significance of any reaction to the injected material within the vertebral canal, which we found occurred occasionally. In our long-term follow up of the injected IVDs of 5 live dogs, one dog had worsening of the IVD protrusion with no adverse clinical signs or abnormal findings on physical or neurological examinations. Further histological and/or immunohistochemical analysis of treated IVDs may aid in understanding this change over time. Additionally other more sensitive testing for adverse reactions including electrodiagnostics may be warranted in the future to detect subclinical adverse effects related to the use of Discogel.

Although no clinically significant adverse reactions were detected in our study population, the leakage of the injected material into the vertebral canal is still concerning. Adverse reactions that occur at a low rate may not have been detected due to the small number of dogs in our study and the limitations of examining veterinary patients (versus human patients who can report minor and intermittent pain during and following the procedure). Additionally the impact of epidural contamination adjacent to the cauda equina may be different than the impact of the epidural material adjacent to other spinal cord segments. Therefore, this technique should be applied to the more cranial IVDs with some caution, as
any concurrent epidural contamination could be more impactful. The LS IVD was accessed from a dorsal approach, through the spinal canal. This was based on limited access from a lateral approach due to obstruction by the ilial wings and a similar approach to commonly performed epidural or intrathecal injections (myelography). This approach may have increased the risk for leakage of contrast material through the needle tract and into the vertebral epidural space. Approaching other IVDs from a lateral or ventral approach should reduce the risk of epidural contamination. Treatment of cervical and lumbar IVD herniations with gelified ethanol has been reported in human patients from an anteriolateral approach (Theron, 2007; Theron, 2010). In our cadaveric study the cervical, caudal thoracic and portions of the lumbar spine were approached from lateral and ventrolateral approaches. Despite this, occurrences of leakage of material into the vertebral canal were still present at all sites with no significant differences in the rate of contamination between sites noted (p=0.5530) (Table 2.5). It is uncertain if this is due to differences in tissue integrity of cadavers or if this is a complication that may also be expected in live dogs. Further evaluation may include injection of IVDs at other sites in live dogs using a different approach to determine if there is reduction in the rate of significant contamination.

Puncture of the dorsal aspect of the IVD has been used to create IVD degeneration models in animals and it is possible that IVD puncture with the spinal needle lead to worsening of the IVD protrusion, as seen in 1 dog at 1 year follow up (Sobajima, 2005; Masuda, 2005; Rousseau, 2007). We hypothesize that an injection performed from a lateral or ventrolateral approach may be more likely associated with lateral or ventrolateral herniation of the IVD and thus be less clinically significant if it occurred ventral to the vertebral bodies or nerve roots.

Over our study period there was no evidence of a change in the cranial-caudal dimensions of the injected IVD and the only IVD change seen was an increase in the IVD protrusion. The lack of change of IVD size in our study is intriguing
because of the chemonucleolytic effects of ethanol that have been documented (Riquelme, 2001). In human patients treated with gelified ethanol there are few reports of imaging to assess the changes to the IVD over time. Discordance has been reported between the MRI findings a few weeks following injection and the resolution of clinical signs, with no change seen on MRI (Theron, 2007). On MRI acquired 1 year following injection in human patients dramatic reduction of herniated volume has been reported (Theron, 2007). Changes to the IVD size may not have been detected due to our small number of cases. It is also possible that performing injections in clinically normal dogs with degenerative changes but no or minimal evidence of IVD protrusion prior to injection may have less effects than injecting clinically affected dogs with large volume protrusions. This is because the proposed effect of ethanol on the IVD is regression of the IVD secondary to diminishing the nuclear volume and lysing the herniated material (Riquelme, 2001). Another possibility for why a change in the IVD size was not found in our study is related to the difference in the volume administered in our study population versus that in human patients. In human patients a dose of 0.3-0.8ml/disc has been reported (Theron, 2007; Theron, 2010). In our work the mean volume delivered into the LS IVD in healthy dogs was 0.18ml (range 0.1-0.25). The reduced volume injected contained less ethanol and therefore may have less nucleolytic effects. The leakage of the material into the vertebral canal in 4 of 10 injections may have further reduced the volume of ethanol contained within the NP. Further evaluation in clinical dogs including long term follow-up imaging is warranted.

Interestingly in the 5 dogs that had follow-up CT examinations at 1 to 2 years following the initial injection, the volume of metal dense material (tungsten seen in the IVD was subjectively reduced. The CT examination also included the regional draining lymph nodes (aortic lymph nodes and iliosacral lymphatic chain) and no uptake of tungsten or other abnormalities were noted. Hypotheses for the reduction of the metal dense tungsten include lymphatic drainage that was not seen on regional CT examination, contraction of the tungsten within a smaller
volume due to absorption or redistribution of other elements of the injected preparation or concentration of tungsten particles within the IVD.

The treatment of IVD protrusion in dogs is more challenging than acute forms of IVD herniation and therefore alternative treatments should be evaluated. In human patients with disc herniation, gelified ethanol has been associated with success rates between 75-91.4% in over 400 subjects (Theron, 2007; Theron, 2010; Stagni, 2012; de Seze, 2013; Bellini, 2013). In the current work, image guided injectable techniques were found to have differing accuracies for injecting the NP at various locations, with CT and fluoroscopy being significantly more accurate than ultrasound guidance (Table 2.6). Using CT guidance, the gelified ethanol was successfully delivered in all subjects and its safety and efficacy was monitored for up to 2 years. Although no perceptible changes in the IVD size were identified, even in the presence of leakage of the material into the epidural space there were no adverse reactions observed. This work establishes the basis for progression to a clinical trial using dogs with clinical IVD disease and may be especially suited for those that are poor surgical candidates.
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## APPENDIX

### I. SUBJECT INFORMATION FOR FLUOROSCOPIC AND ULTRASOUND GUIDED INJECTIONS (CADAVERS)

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**Wt= weight; BCS= Body condition score out of 5**

F = fluoroscopy guidance; US = ultrasound guidance; TL= thoracolumbar; LS=lumbosacral; GSD= German Shepherd Dog. * An additional injection site with a slightly different technique.


### II. SUBJECT INFORMATION FOR CT GUIDED INJECTIONS (CADAVERS)

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Dog number continued from Appendix I; Wt= weight; BCS= Body condition score out of 5; JRT= Jack Russell Terrier
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Dogs 1-14 are same as listed in Appendix I and II. Method = image modality used to guide injection; Time = time for entire injection including patient positioning; Ease = the ease at which the preparation could be injected into the disc on a scale of 4; Satis = operator satisfaction with the injection on a scale of 5; NP = injection was properly delivered to the nucleus pulposus; ST = there was leakage of preparation from injection site into the adjacent soft tissues; VC = there was leakage of preparation from injection site into vertebral canal. 0 = absent; 1 = present.
IV. RESULTS OF LS INTERVERTEBRAL DISC INJECTION IN 10 HEALTHY DOGS

<table>
<thead>
<tr>
<th>Dog</th>
<th>Sex</th>
<th>Weight (Kg)</th>
<th>BCS</th>
<th>Successful injection</th>
<th>Leakage of Injected material</th>
<th>Pfirrmann Grade (n=10)</th>
<th>Time without setup (minutes) (n=9)</th>
<th>Number of adjustments to properly place spinal needle (n=9)</th>
<th>Resistance to injection (n=9)</th>
<th>Satisfaction with injection (n=10)</th>
<th>Person who performed the injections</th>
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BCS = body condition score (out of 5)*. SM = Shawn Mackenzie; HC = Heather Chalmers; BB = Brigitte Brisson

Successful injection was one were the product was delivered to the nucleus pulposus of the intended intervertebral disc. Dog 1 received injection of the LS IVD was performed with fluoroscopic guidance. Dogs 2-10 received injection of the LS IVD was performed with CT guidance.


## V. INTERVERTEBRAL DISC SIZE BASED ON PRE- AND POST-INJECTION AND 1-YEAR FOLLOW-UP MRI

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<th>Cr-Cd on sagittal Ventral 1/3rd</th>
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