Department/University Clinics for Small Animals and Horses

University of Veterinary Medicine Vienna

Clinical Unit of Anaesthesiology and Perioperative Intensive-Care Medicine

(Head: Priv.-Doz. Dr.med.vet. Ulrike Auer)

Ultrasound-Guided Sciatic Nerve Block in Chicken Cadavers: A Descriptive Study

Diploma Thesis

University of Veterinary Medicine Vienna

submitted by

Eibl, Timna

Vienna, July 2022

First supervisor:

Priv.-Doz. Dr.med.vet. Ulrike Auer
Department/University Clinics for Small Animals and Horses
University of Veterinary Medicine Vienna
Clinical Unit of Anaesthesiology and Perioperative Intensive-Care Medicine
Veterinärplatz 1
1210 Vienna

Second supervisor:

DVM Robert Trujanovic
Department/University Clinics for Small Animals and Horses
University of Veterinary Medicine Vienna
Clinical Unit of Anaesthesiology and Perioperative Intensive-Care Medicine
Veterinärplatz 1
1210 Vienna

Reviewer:

Priv.-Doz. Dr.med.vet. Kirsti Witter
Institute of Morphology
Department for Pathobiology
University of Veterinary Medicine Vienna
Veterinärplatz 1
1210 Vienna

Content

Abbreviations

B	Base
BH+	Charged Base
CNS	Central Nervous System
DMSO	Dimethylsulfoxide
M	Musculus
Mm	Musculi
N	Nervus
NaCl	Sodium Chloride
NEMPs	Nerve Evoked Muscle Potentials
Nn	Nervi
US	Ultrasound

1) Introduction and Hypothesis

Analgesia management plays a critical role in successful medical treatment of all species. Different anaesthetics and pain control methods were developed in different eras, avian analgesia specifically, was not studied until the begging of the 1960's. Nowadays it is widely accepted that all species that feature specific anatomical, physiological, and biochemical traits are bound to experience pain. The inability of expressing it does not make it less relevant to the individual's welfare.

As pet bird ownership becomes more common, approved pain management for each species is required. It is not just a necessity for supporting the bird during its convalescence, but also during general anaesthesia for diagnostic or therapeutic reasons.

When performing a general anaesthesia in any individual, the risk of side effects, such as hypothermia or hypotension, occurring rise. These can develop during all phases of anaesthesia; premedication, induction, or maintenance of the anaesthetic status. They can also indirectly occur in the sedated patient by depression of autoregulatory functions. A very common side effect, during anaesthesia, in avian patients is respiratory depression leading to hypercapnia and hypothermia. Other problems that need to be addressed are caused by the drugs themselves that are used for local anaesthesia.

Birds seem to react more sensitively towards local anaesthetic agents; hence some authors discourage clinicians from utilising them in birds completely. By combining the more thoroughly researched positive effects of deep sedation with locally injectable analgesia over general anaesthesia in birds, it is possible to achieve a safer surgical intervention. Even when using general anaesthesia, the additional use of local anaesthetics is always recommended, as they contribute to more stable anaesthetic depths of the patient.

The aim of this study is to develop a technique for a regional sciatic anaesthesia in birds. This is conducted by using a Methylene-blue in physiological saline, which is injected under sonographic control in the proximity of the sciatic nerve in chicken cadavers. The success of this method is determined by analysing the nerves staining behaviour following dissection and will be summarised using descriptive statistics.

The hypothesis of this study is that with the described technique a successful ultrasound (US)-guided regional sciatic nerve block in chickens is possible in an efficient and safe manner.

2) Literature Overview

2.1) Local Anaesthesia in Avian Patients: Background and General Considerations

Local anaesthesia allows clinicians to numb specific nerves and the correlating regions they supply. Since the beginnings of its application a lot has changed, such as the choice of drugs, indications, and the species that receive them, ranging from humans to pets to livestock (Garcia 2015, Schroeder 2012).

The history of avian local anaesthesia is short. Detailed pain protocols have only been studied and analysed since the last 30 years (Lierz and Korbel 2012). Yet these have become more elaborate over the years. Some of the first papers covering avian pain management, postulated that birds experience less cutaneous sensitivity, and therefore minor defects could be sutured without anaesthesia. The authors emphasised that stress is a more prominent aspect compared to pain, making general anaesthesia inevitable (Linn 1986).

Regional anaesthetic techniques allow the patients a conscious, and therefore less risky, but pain-free surgery. Alternatively, it can be used in addition to general anaesthesia, resulting in better cardiorespiratory stability and a decreased required dosage of narcotics due to less pain-induced reactions (Skarda and Tranquilli 2011). Especially during the repair of multiple fractured bones raptors show bradycardic events intraoperatively, which seem to occur in fewer cases when using local anaesthetics (Hawkins and Griffenhagen 2022).

A retrospective case series consisting of 352 birds found that the mortality rate for inhalation anaesthesia during and after the operative procedure added up to 14 %, which is higher than in dogs and cats (Seamon et al. 2017). Common intraoperative problems in bird anaesthesia are hypothermia, especially in smaller individuals (Lierz and Korbel 2012), and cardiopulmonary depression. The possibility of desensitising merely small areas of interest in a fully conscious or slightly sedated bird therefore seems highly promising (Guzman and Beaufrère 2021, Lee and Lennox 2016). However, also acute stress occurring during manipulation of a non-sedated bird can have fatal consequences (Abou-Madi 2001). Certain avian species link manual restraint directly with predation (Doss and Mans 2021). Activation of the sympathetic “fight – or flight” leads to a rapid rise in catecholamine levels, causing tachycardia, hypertension, hyperthermia, and tachypnoea (Doss and Mans 2021, Figueiredo et al. 2008, Paul-Murphy and Ludders 2001). More long lasting effects are decreased fertility and a compromised immunological response, possibly leading to delayed healing or secondary infections (Cīrule et al. 2012, Doss and Mans 2021, Harvey et al. 1984, McRee et al. 2018).

Similar effects can be observed in a bird experiencing pain (Guzman and Beaufrère 2021), making a multimodal pain management essential.

The number of studies conducted in the highly relevant field of ultrasound-guided avian nerve blocks has risen over the past couple of years. Especially the regional brachial plexus block earned its place in contemporary research, but information about regional techniques on the hindlimbs is still scarce and would certainly benefit from fundamental research.

Therefore, the aims of the study are to 1) describe a technique for an ultrasound-guided sciatic nerve locoregional block in chickens (*Gallus gallus domesticus*), 2) identify practical landmarks for locating the injection site and the ultrasound window, 3) examine the intrafascial distribution and sciatic nerve staining following an injection of a 0.4 ml 3:1 (NaCl:Methyleneblue) dye solution.

2.2) Fundamentals of Local Anaesthesia and Mechanism of Action

The technique of local anaesthesia describes a procedure of injecting a local anaesthetic drug in the perineural space to achieve an altered nerve function, resulting in sensory or motoric elimination in the supplied area behind the injection site (Garcia 2015, Skarda and Tranquilli 2011). This method can be conducted either “blind”, landmark-guided, or if utilising advanced electronic devices; ultrasound-, or neurostimulation-guided. When choosing the landmark-guided method, the anaesthesiologist has a three-dimensional orientation via palpation of the area of interest and orient themselves on anatomic landmarks such as bone protrusions, tendons, muscle bellies and accompanying pulsating arteries (Gadsden 2021). The ultrasound-guided approach is usually applied in combination with the landmark-guided method (Gadsden 2021) and offering a detailed two-dimensional display of the surrounding tissue underneath the probe. Nerve-stimulators are devices that utilise electrical stimuli, just as nerves do. The needles are arranged in a manner that forces the supplied muscles to contract, making the manipulation by the anaesthesiologist of the nerve visible in an expectable physical reaction of the patient, meaning the flexion of the stifle when stimulating the sciatic nerve. Subsequently a lowered threshold of electrical stimuli that can still elicit a muscle contraction, indicates the correct proximity of the needle with the local anaesthetic to the nerve (Gadsden 2021).

This approach plays an important role in multimodal pain management and shares the same underlying biophysiological actions in different species that operate on saltatory nerve conduction (Hawkins and Paul-Murphy 2011). Fascinating studies about clinically applicable local anaesthesia methods in a wide range of species have therefore been published in the still growing field (Monticelli et al. 2016, Ravasio et al. 2020).

The biochemical process originates from nerval conduction inhibition (Kubiak 2016, Raymond et al. 1989, Skarda and Tranquilli 2011). Anaesthetic drugs block sodium channels, that are located within the nerval cell's membrane, a physiological base being equivalent in all vertebrates (Paul-Murphy and Ludders 2001). The exact process is still not fully understood, but a combination of membrane-expansion and specific-receptor theory is mostly agreed upon (Skarda and Tranquilli 2011). It is supposed that the amide-type drug passes with its quaternary ammonium compound, and the ester-type drug passes as an uncharged Base (B) through the cell membrane. Inside, the molecules are pronated, and through this become positively charged (BH+). A charged base can bind to the sodium channel and block it. By doing so, the influx of sodium is inhibited, resulting in a steady negative cellular charge of -270mV. Consequently, no depolarisation takes place, no action potential can be transmitted, and no pain can be felt (Paul-Murphy and Ludders 2001, 2001, Skarda and Tranquilli 2011).

This process of action makes the local anaesthetic drug one of a kind. Other analgesics work farther from the origin of the nociceptive signal, not entirely prohibiting long term effects that might occur; such as central sensitisation and maladaptive or pathological pain (Kehlet et al. 2006, Paul-Murphy and Ludders 2001, 2001, Skarda and Tranquilli 2011).

2.3) Local Anaesthesia in Avian Patients: Relevant Studies

The studies on avian local anaesthesia conducted in a clinical setting are gaining relevance, especially pharmacological and pharmacokinetic studies are preferably done in chickens and their embryos as they are easily available and cheap models (Bjørnstad et al. 2015). One of the oldest articles on gallid local anaesthesia was done in 1992. In this study, chickens underwent a beak-trimming procedure, while the control group received a topical administration of Bupivacaine and Dimethylsulfoxide (DMSO) on the freshly exposed bone, resulting in normal continuing feed intake as opposed to the non-treated group (Glatz et al. 1992). Other studies also show the applicability of topical pain management through local anaesthetics in skin-related issues, such as the amputation of supernumerous limbs, that were attached to the torso solely through connective tissue, in a conscious Fayoumi chicken (Abu-Seida 2014). Chickens seem to be representative models for the avian spinal anaesthesia to desensitise the cloacal area under radiographic control, as demonstrated in six individuals (Kazemi-Darabadi et al. 2019). A brachial plexus blockade was conducted in another six chickens using Bupivacaine, to produce anaesthesia distal to the upper arm and elbow, but no effective sensitive blockade could be provided (Figueiredo et al. 2008). In another brachial plexus blockade study six chickens received Ropivacaine injections, which seemed to result in a satisfactory effect (Cardozo et al. 2009).

Interesting studies have also been published pertaining to falcons as they are often hospitalised as wildlife casualties or are held in close human interaction such as falconries. A wild Prairie falcon *(Falco mexicanus*) exhibited signs of neuropathic pain following a healed metacarpal fracture. It received a perineural radial and medianoulnar nerve block, using Bupivacaine and Medetomidine, which was deemed successful, as the patient discontinued the self-harming behaviour afterwards (Shaver et al. 2009). A typical falconry-training associated trauma is the gunshot wound, which caused a radial fracture with subsequent osteomyelitis in a Peregrine falcon (*Falco peregrinus).* Under neurostimulation Lidocaine and Fentanyl were injected, and as no reaction to surgical stimulation was detected, the authors were content with the efficacy of the block (d'Ovidio et al. 2011). Another five pet Peregrine falcons (*Falco peregrinus)* were scheduled for pododermatitis surgery and therefore received sciatic and femoral nerve blocks with Lidocaine, using a nerve locator. As the intraoperative status of the patient was stable upon usually painful manipulation, the authors deemed the procedure successful (d'Ovidio et al. 2015). Another pet Peregrine falcon (*Falco peregrinus*) with a chronic malunion humeral fracture needed a total wing amputation. This was facilitated though injections in the perineural shaft of the cranio-pectoral, axillary, and radial nerves prior to transection injections with Lidocaine and Bupivacaine. A splash block upon closure has been added and seemed to have contributed to a successful analgesic effect (Latney et al. 2018).

As mentioned before, wildlife patients often endure profound injury, resulting in the loss of the individuals' ability to fly and make rehabilitation impossible. A Golden eagle (*Aquila chrysaetos*) suffered an old open fracture and was luckily provided with an adequate aviary for disabled individuals. Therefore, the chosen therapy was a wing amputation. The patient received a visually localised intraoperative radial and medioulnar nerve block with Lidocaine. A proper healing process and absent self-harm was counted as analgesic success (Javanmardi et al. 2017). Another wing amputation with a brachial plexus block had to be done in a wild Striped owl (*Asio clamator*), also suffering an old open fracture. By axillary approach the injection site was palpated and Ropivacaine was instilled using a neurostimulation-guided technique (Do Nascimento et al. 2019).

Often more complicated patients are birds from the order of Psittaciformes, as they are particularly curious and sometimes deliberately destructive with their strong beaks and intelligent behaviour. These characteristics and their environmental requirements make them high maintenance pets. The right condition might allow them to grow old, which can come along with geriatric diseases, such as neoplasm (Lawton 1999, Orosz and Lichtenberger 2011). In an Umbrella cockatoo (*Cacatua alba*) and a Moluccan cockatoo (*Cacatua moluccensis*), both with a humeral air sac adenocarcinoma, a total wing amputation was carried out under the same method as described above with the Peregrine falcon suffering a chronic malunion humeral fracture (Latney et al. 2018). A thoroughly performed trial of brachial plexus blockades with Lidocaine from the axillary approach has been done in eighteen Hispaniolan Amazon parrots (*Amazona ventralis*). The goal was to evaluate the analgesic effect of the chosen method which could be used for painful lesions of the wing, intraoperative anaesthesia, central pain sensitisation, and chronic pain. The study was carried out in two groups: one under sonographic control, and the other with palpatory localisation only. Neither groups were deemed effective in the chosen dose (Da Cunha et al. 2013).

Growing numbers of livestock held as pets led to poultry being presented to veterinary clinics more frequently than ever. Eight mallard ducks (*Anas platyrhynchos*) were therefore used to evaluate the brachial plexus blockade with either Bupivacaine or Lidocaine with Epinephrine in order to desensitise the wing for surgical procedures. Both the axillar and the dorsal approach were tested and resulted in a satisfactory localisation via palpation and a neurolocator, although the motoric and sensory results were mixed (Brenner et al. 2010). Recently one pet Mallard duck with a tibiotarsal fracture received an ultrasound- and neurostimulator-guided femoral and sciatic nerve block with Lidocaine. The absence of nociceptive reactions perioperatively made the authors conclude that the block was effective (Trujanovic et al. 2021).

2.4) Chickens as Specimens

2.4.1) Broilers, Layers, and Backyard Chickens

As the commonly held pet chicken breeds are vastly different, ranging from broiler-like Jersey Giants (5.5kg) to layer-like dwarf types as Serama hens (0.4kg adult and a juvenile displayed in Fig.1), there is no typical constitutional type in chickens that are more likely to need a sciatic nerve block. Therefore, the importance of discussing those differences is required.

Figure 1: Serama juvenile size

https://cdn.backyardpoultry.iamcountryside.com/wp-content/uploads/sites/3/2019/03/Serama-BYP-AprilMay06-8.jpg, 23.02.2022, 18:09h

Through genetic selection between broilers and layer hens their metabolism works differently (Buzała et al. 2015, Jackson and Diamond 1996, Jones et al. 1986, Saito et al. 2004). This results in different behavioural tendencies (Keer-Keer et al. 1996), growth rate and even nutritional needs during embryonic stages (Muramatsu et al. 1990, Nangsuay et al. 2015, Ohta et al. 2004) and after hatching (Cooke et al. 2003).

But also when fully grown, backyard chickens and layer breeds suffer from different health issues than broiler lines do. Problems that frequently concern layer lines are connected to the management leading to emaciation and cannibalism, or to the process of egg production, such as egg yolk peritonitis, hypocalcaemia, or salpingitis (Fulton 2017).

In contrast broilers rather suffer from the outcome of a high oxygen demanding metabolism and compromised respiratory system (Hassanzadeh et al. 2005), which might lead to cardiac valvular insufficiency, right ventricular dilatation, or hypertrophy due to pulmonary hypertension ultimately leading to right ventricular failure (Julian et al. 1987, Julian 1998, Lund et al. 2013). Another aspect is emphasised by (Olkowski 2007), stating cardiac arrhythmias can cause sudden heart death, which occurs especially in hypercapnic states (Korte et al. 1999). This might be linked to their catecholamine metabolism, with elevated dopamine and dihydroxyphenyl acetic acid concentrations in their whole brain compared to layer chickens (Saito et al. 2004). Additionally due to the genetically created hypertrophic muscles (Berri et al. 2007, Knowles et al. 2008, Moghadam et al. 2005) the chickens tend to decompensate at certain stages of growth (Shim et al. 2012), leading to tibial dyschondroplasia, rickets, angular bone deformities, spondylolisthesis, curled toes, femoral head necrosis, and rupture of the gastrocnemius tendon (Angel 2007).

Often seen in backyard chickens are reproductive problems, such as described in layer chickens. Some are linked to management or housing mistakes (Huang et al. 2019) but increased welfare can bring forth older age and therefore, a higher incidence of neoplasia. Considering that the most common neoplasm-connected disease in Backyard poultry is caused by the Gallid Alphaherpesvirus Type 2, namely Marek's Disease, these deaths are not due to a higher life span but unfortunately of infectious origin in rather young aged individuals (Cadmus et al. 2019, Greenacre 2015, Mete et al. 2013, Pohjola et al. 2015, Trott et al. 2014).

2.4.2) Anatomy of the hind limbs of chickens (*Gallus gallus domesticus*)

The sciatic nerve originates in the lumbosacral plexus, consisting of the 2nd to the 9th Nervus (N.) synsacralis, specifically the first four roots. (Fig.2) It then crosses the Musculus (M.) caudoiliofemoralis and continues between the M. iliofibularis and M. puboischiofemoralis towards the popliteal fossa. The sciatic artery and vein accompany the sciatic nerve cranially and caudally, respectively. Proximal to the knee the nerve separates into the tibial and the fibular nerve. Already before the diversion of the epineurium the nerves run separately within the epineural sheath (Frewein 1992, Martinez-Pereira and Zancan 2015)

The sciatic nerve and its branches are responsible for most of the motoric and sensitive supply of the limb distally to the stifle. (Fig.3) Locoregional anaesthesia of the sciatic nerve at the demonstrated location therefore can ease surgical interventions distally; f. e. bumblefoot surgery, orthopaedic interventions such as tarsometatarsal fractures, toe amputation after injury, or profound wound management along the tibiotarsus and distally (d'Ovidio et al. 2011, d'Ovidio et al. 2015, Frewein 1992, Trujanovic et al. 2021).

The nomenclature of anatomic structures was taken from the cited literature "Avian Anatomy" (König, Horst Erich et al. 2016), and additionally from the originally German "Anatomie der Vögel" translated to English or adopting the used Latin terminology (Schummer 1992).

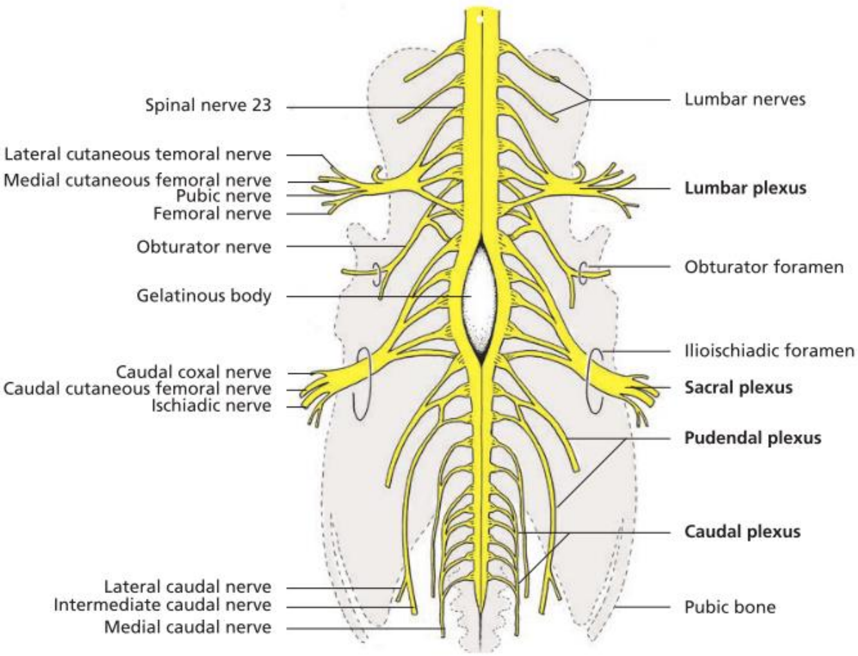

Figure 2: Image from (König et al. 2016) Page 203, Image 13.17, adapted from Ghetie, 1976. Schematic dorsal view on the spine and Nervi (Nn.) spinales at the level of the synsacrum and ossa coxae.

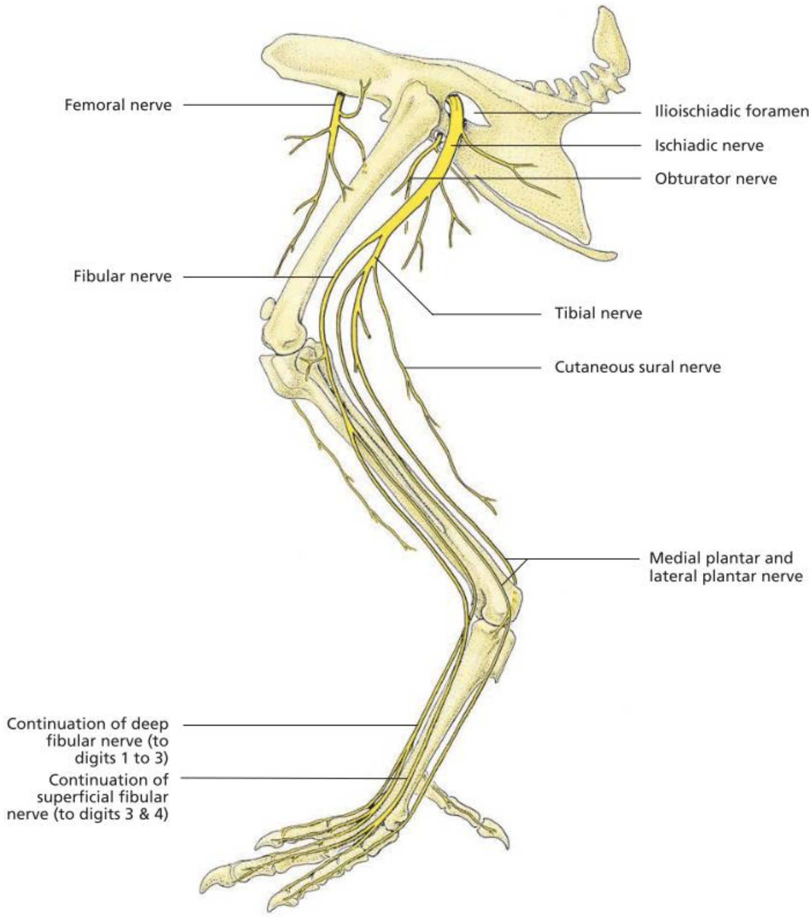

Figure 3: Image from (König et al. 2016) Page 203, Image 13.17, adapted from Ghetie, 1976. Schematic presentation of the femoral and sciatic nerve and its distal branches.

2.5) Ultrasound-guided regional anaesthesia

2.5.1) Background

The US transducer contains piezoelectrical elements, which respond to an incoming electrical signal with vibration, and thereby creating US waves. These waves are then transmitted through the patient's body, causing typical changes of the waves, due to reflection, refraction, or being scattered after passing through various types of tissue consisting of different densities (Marhofer and Chan 2007). These waves return to the transducer, and are again changed into an electrical signal, creating a visible image of the incoming information on the monitor. If more waves are being sent back towards the transducer, the colour in the US monitor becomes lighter, "hyperechogenic," as in bones. If more waves are passing through the tissue and are not reflected onto the probe, the darker, "hypoechogenic" the structures are in the image, as in fluids (Marhofer and Chan 2007).

US-guided regional anaesthesia allows the clinician to elide anatomic variances and confirming expected nerve location in a non-invasive way, as the location of the nerve can be visually confirmed (Comolli et al. 2019, Neal et al. 2010, Seco et al. 2012). Other advantages are the confirmation of needle position in relation to the nerves and vessels, of the process of injection and following local reaction such as the doughnut sign, and thereby higher chances of preventing complications, f. e. intrafascial or intravascular injection (Comolli et al. 2019, Marhofer et al. 2005, Marhofer and Chan 2007, Sites and Antonakakis 2009).

2.5.2) Artefacts in Birds

Most studies have been carried out in mammals, mostly humans, followed by pets, such as dogs, and horses due to size of the individual (Krautwald-Junghanns et al. 2011) and the patient's cooperation. Some avian anatomic differences cause problems that may not be apparent in these well-studied animals. Birds with their typical light-weight construction are covered in feathers, which are designed to enable flight, but create transmission errors in the sonographic picture (Krautwald-Junghanns et al. 2011). Most areas of the bird's bodies are covered in feathers, "Pterylae", but some are physiologically feather-free, "Apteria", as seen in Figure 4 (Vollmerhaus and Sinowatz 1992a)

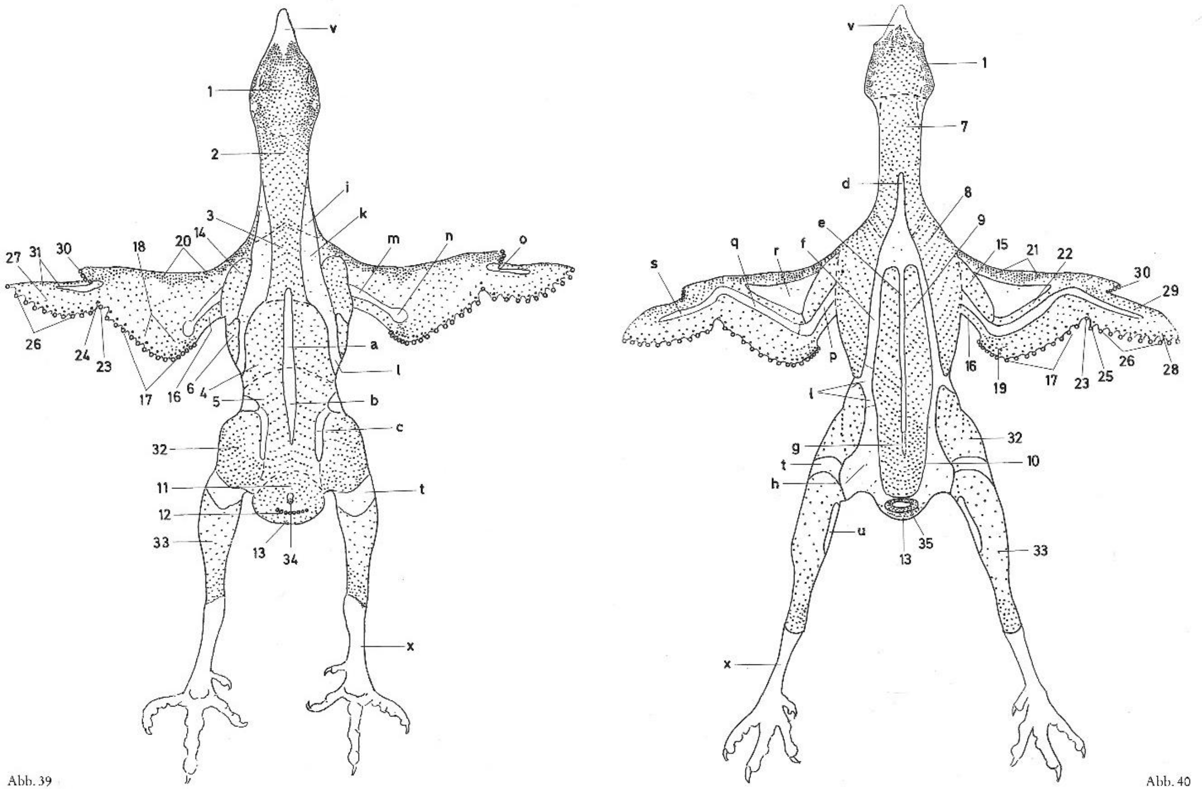

Figure 4: Feather-containing (Pterylae) and feather-free areas (Apteria) on the body of a Japanese quail (Coturnix japonica) in dorsal (Abb.39) and ventral (Abb. 40) view. Taken from (Schummer 1992), Page 40f.

Most of the feather-free areas cannot be used, as bone is found directly underneath. This enables the stability required for the bird's flight-constructed bodies, to obtain a better force transmission from muscle to bone to propel the entire body with less resistance forward (Vollmerhaus 1992). So, the clinician's job is to find a feather-free window which depends heavily on the species of interest.

The main avian coupling sites for the ultrasound probe are the ventromedial, which is found behind the sternum, and the parasternal, which is located lateral, behind the last rib. The ventromedial site is more frequently used, lying between the xyphoid process and the pelvis, as anatomic variations are not as prominent in different species. Opposed to the lateral parasternal site, which is only accessible if the species, that offer enough space between the last rib and the pelvis, such as pigeons and some raptors. Another possibility is the brood patch which is found in some female birds, depending on the species and brood activity. Individuals often seen in avian clinics that might develop a brood patch are chickens, ducks, geese, pigeons, falcons, parrots, and many more (Dr. Hans Frey, personal communication, 10.07.2022) making this temporary coupling site an eligible option if needed and if applicable in the particular species.

In chickens, birds of prey and pigeons some feathers usually have to be plucked, which is supposed to be done under general anaesthesia, as this is painful and emotionally riles up the patient (Krautwald-Junghanns et al. 2011). In psittacines on the other hand, separating the feathers often is sufficient. In waterfowl plucking is always necessary (Krautwald-Junghanns et al. 2011) since they are fully covered in dunes to prevent soaking when in contact with water. This should be done carefully so they would not lose the ability to swim (Krautwald-Junghanns et al. 2011, Vollmerhaus and Sinowatz 1992a). Even after locating an acoustic window underneath the feathers and between the bones, the intraabdominal air sacs need to be compressed in order to visualise the patients' internal organs (Hochleithner 2006).

Air sacs are expansions of the respiratory system in birds that do not participate in gas exchange, but are found in various locations, some of them being intraosseous within the humerus or the femur, subcutaneous cervical, and intracoelomic as in the caudal abdomen (König, H. E. et al. 2016, Vollmerhaus and Sinowatz 1992b). These air sacs limit the usage of ultrasound techniques to the specific windows mentioned above (Hochleithner 2006, Krautwald-Junghanns et al. 2011). High quality pictures can still be created through homogenic organs, as for example the liver (Krautwald-Junghanns et al. 2011, Pees et al. 2008).

A small additional alternation in the composition of the ultrasound image is caused by the diverging skin-anatomy, as the avian skin is thinner than the mammal's, regarding the additional protection layer that the feathers provide (Vollmerhaus and Sinowatz 1992a). Due to this, the ultrasound waves are less likely to be filtered in this layer after the feathers have been plucked or parted, letting more waves pass, causing a more hyperechogenic picture compared to mammal.

3) Material and Methods

3.1) Animals

Five plucked and gutted chicken cadavers with the mean weight of 1.65 (1.6-1.7) kg, that were bought in local supermarket and originally intended for consumption, were used in this pilot study. The approximal live weight would vary between 2.4-2.6 kg. One pelvic limb had been used for preliminary anatomic dissection of the sciatic nerve and the surrounding tissue. Nine pelvic limbs were therefore left for US-guided injection, followed by dissection. The tissue visualised in the anatomic dissection was then compared with the structures identified within the sonographic picture (Linear array probe, 13-6 MHz; MicroMaxx, Sonosite, WA, USA) and used for orientation.

3.2) Study Design and Procedures

After lateral positioning of the cadavers (Fig.5), the lateral condyle of the femur, the dorsolateral iliac crest, and the trochanter femoris were used as anatomical landmarks. For better skin-to-transducer-contact, an alcohol-gel was applied. A linear transducer was placed 2 cm distal to the Trochanter major femoris and with a transverse orientation to the long axis of the femur, with the marker of the probe orientated cranially. Then, the transducer was moved slightly caudally to have the region of interest in the central part of the screen, and the depth was adjusted. When the sciatic nerve was visualized a 50 mm long, 22G, hyperechoic/insulated needle was introduced at the caudal border of the transducer (Fig. 6), and US-guided advanced in-plane in a cranio-medial direction to the proximity of the sciatic nerve, laying between the laterocranial M. iliotibialis lateralis and the mediocaudal M. flexor cruris lateralis.

A 1:3 mixture of 1 % methylene blue solution (Methylene Blue 1 % w/v aq. soln., Alfa Aesar, ThermoFischer GmbH, Germany) and physiological saline (NaCl 0.9 %, B.Braun, Germany) was applied, resulting in a total volume of 0.4 ml which was injected under sonographic control. The correct position of the tip of the needle was verified by interfascial spread of the injectate which surrounded and slightly displaced the sciatic nerve. Directly after injection, the extent of nerve staining was quantified by measuring the length and width of stained nerval tissue with a digital ruler following dissection (Fig.18). This was deemed successful if more than 10 mm of continuous longitudinal staining and full width of the sciatic nerve was achieved.

Figure 5: The cadaver is placed in dextrolateral recumbency in lateral view.

Figure 6: The location of probe and needle, offering a tangential dorsoventral view.

3.3) Data Analysis

A descriptive analysis of the obtained information has been realised and is comparatively shown in Table 1.

4) Results

4.1) The preliminary anatomical part

The first part of the study revealed the morphology of the sciatic nerve, accompanying vessels, and muscular surrounding structures (Fig.8-15). Additionally, orientation points for the injection site (Fig. 7), could be reassured within the sonographic picture in the second part of the study (Fig.7 and 8). The measurements of the unstained nerves are shown in detail in Table 1, just as the results of the subsequent dye-injections.

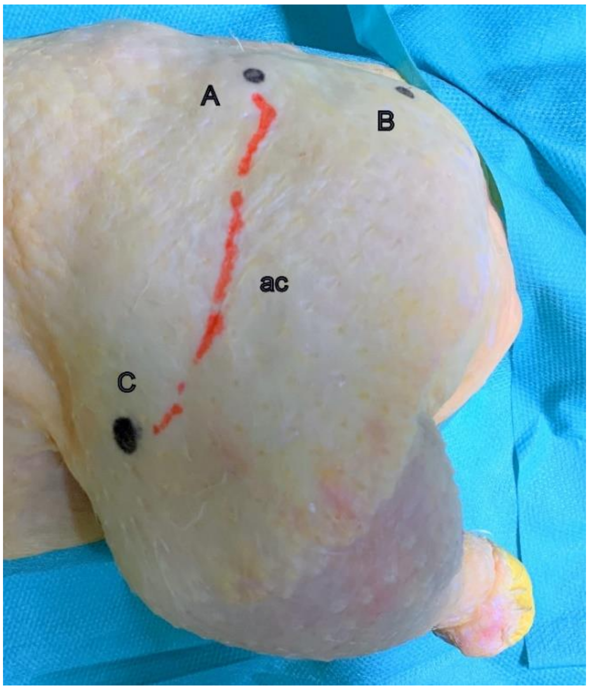

Figure 7: The palpated anatomical landmarks are accentuated with black dots and labelled in letters: trochanter femoris (A), dorsolateral iliac crest (B), lateral condyle of femur (C), femur (ac)

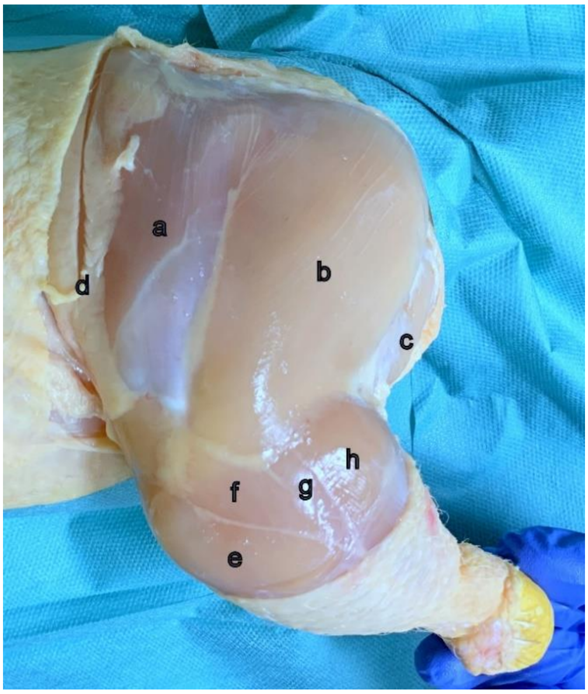

Figure 8: After dissection of the skin.

M. iliotibialis lateralis, pars praeacetabularis (a), pars postacetabularis (b), M. flexor cruris lateralis with its Pars pelvica (c), Subcutaneous fat tissue and underlying M. iliotibialis cranialis (d), M. gastrocnemius, pars medials (e), M. fibularis longus (f), M. flexor perforans et perforatorus digiti III (g), M. gastrocnemius, pars lateralis (h)

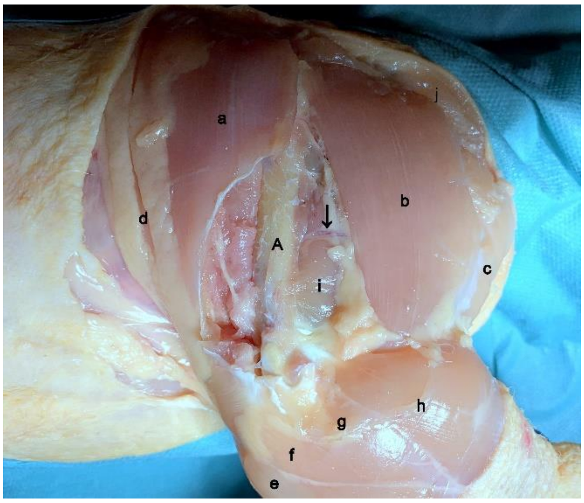

Figure 95: Resected pars postacetabularis of M. iliotibialis lateralis, section (j).

M. iliotibialis lateralis, pars praeacetabularis (a), M. iliofibularis (b), M. flexor cruris lateralis with its pars pelvica (c), subcutaneous fat tissue and underlying M. iliotibialis cranialis (d), M. gastrocnemius, pars medials (e), M. fibularis longus (f), M. flexor perforans et perforatorus digiti III (g), M. gastrocnemius, pars lateralis (h), M. puboischiofemoralis (i), resected M. iliotibialis lateralis, pars postacetabularis (j), circumflex femoral artery and vine (arrow), femur (A)

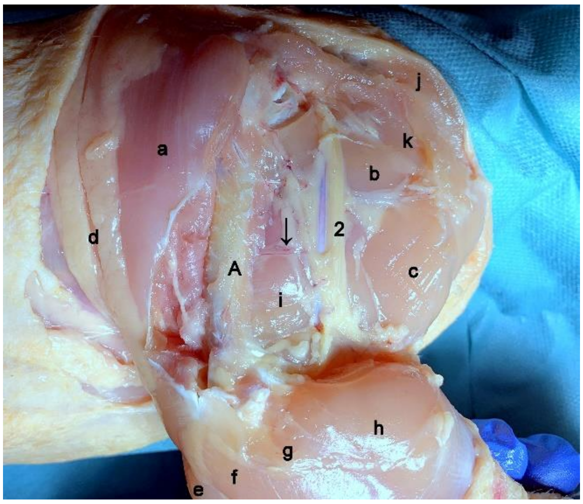

Figure 10: Resected pars postacetabularis of M. iliotibialis lateralis, section (j) and resected M. iliofibularis (k).

M. iliotibialis lateralis, pars praeacetabularis (a), M. ischiofemoralis (b), M. flexor cruris lateralis, pars pelvica (c), subcutaneous fat tissue and underlying M. iliotibialis cranialis (d), M. gastrocnemius, pars medials (e), M. fibularis longus (f), M. flexor perforans et perforatorus digiti III (g), M. gastrocnemius, pars lateralis (h), M. flexor cruris lateralis, pars accessoria (i), resected M. iliotibialis lateralis, pars postacetabularis (j), resected M. iliofibularis (k), circumflex femoral artery and vein (arrow), sciatic artery next to sciatic nerve in fat (2), femur (A)

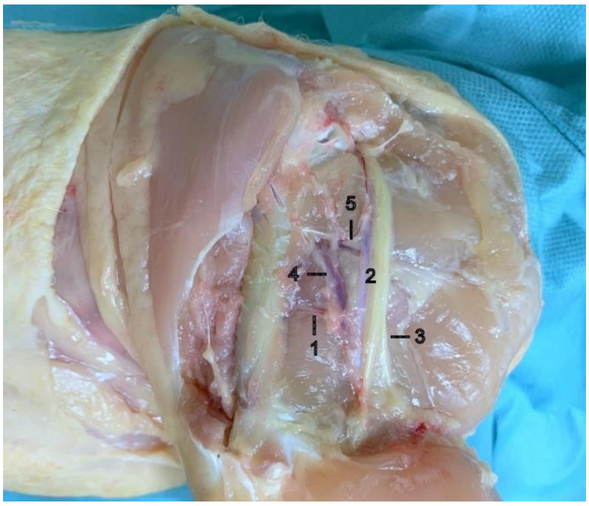

Figure 6: Removal of fatty tissue surrounding nerves and vessels.

Circumflex femoral lateral artery (1), Sciatic nerve and accompanying sciatic artery (2), division of sciatic nerve into tibial nerve (caud.) and fibular nerve (cran.) (3), femoral vein (4), femoral caudal vein (5).

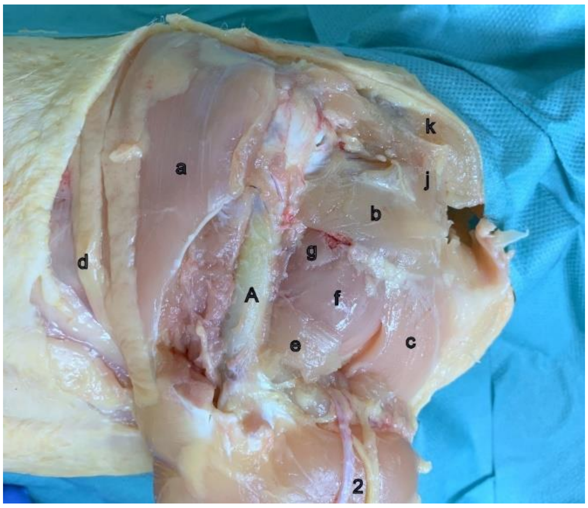

Figure 12: Proximal division of neural and vascular structures and hinging them down distally to obtain a better view on muscular tissue.

M. iliotibialis lateralis, pars praeacetabularis (a), M. ischiofemoralis (b), M. flexor cruris lateralis, pars pelvica (c), subcutaneous fat tissue and underlying M. iliotibialis cranialis (d), M. flexor cruris lateralis, pars accessoria (e), M. puboischiofemoralis, pars lateralis (f) and pars medialis (g), resected M. iliofibularis (j), resected M. iliotibialis lateralis, pars postacetabularis (k), femur (A), sciatic nerve and accompanying sciatic artery (2)

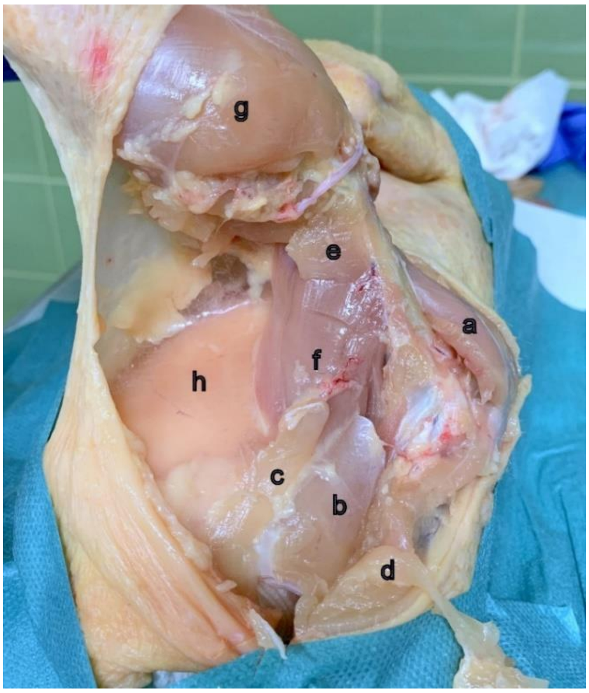

Figure 13: Caudal view of the specimen lying on its right side with limb pulled upwards after dissection of M. ischiofemoralis and M. flexor cruris lateralis, pars pelvica on the caudal upper limb.

M. iliotibialis lateralis, pars praeacetabularis (a), M. ischiofemoralis (b), resected M. flexor cruris lateralis, pars pelvica (c), resected M. iliofibularis (d), M. flexor cruris lateralis, pars accessoria (e), M. puboischiofemoralis (f), M. gastrocnemius, pars lateralis (g), M. obliquus externus abdominis (h)

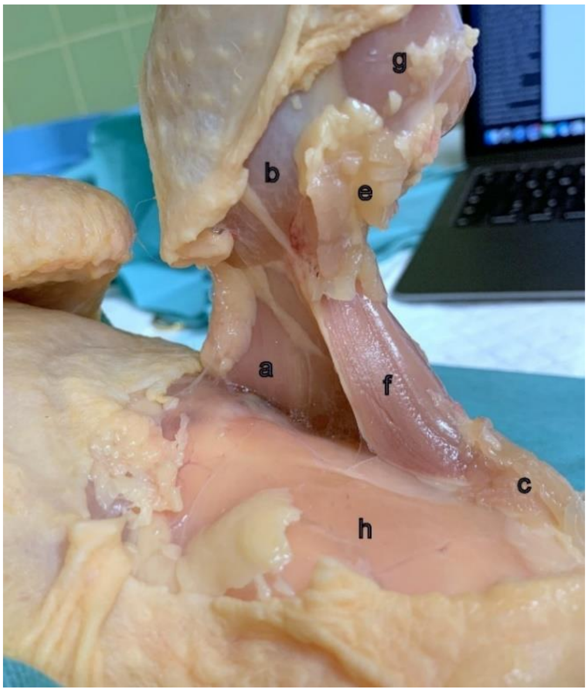

Figure 14: Ventral view with abducted hind limb.

M. iliotibialis cranialis (a), M. gastrocnemius, pars medialis (b), resected M. flexor cruris lateralis, pars pelvica (c), resected M. flexor cruris lateralis, pars accessoria (e), M. puboischiofemoralis (f), M. gastrocnemius, pars lateralis (g), M. obliquus externus abdominis (h)

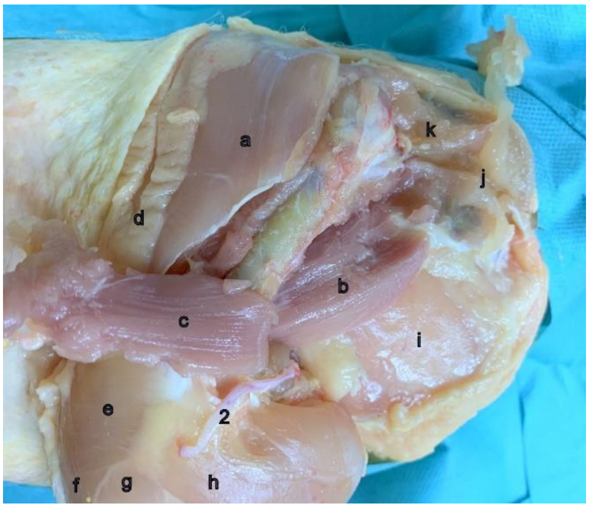

Figure 15: Dissection of M. puboischiofemoralis, pars lateralis in lateral view.

M. iliotibialis lateralis, pars praeacetabularis (a), M. puboischiofemoralis, pars medialis (b), and lateralis (c), subcutaneous fat tissue and underlying M. iliotibialis cranialis (d), M. gastrocnemius, pars medials (e), M. fibularis longus (f), M. flexor perforans et perforatorus digiti III (g), M. gastrocnemius, pars lateralis (h), M. obliquus externus abdominis (i), resected M. iliotibialis lateralis, pars postacetabularis (k), resected M. iliofibularis (j), sciatic nerve and accompanying sciatic artery (2)

4.2) The main part including injection and dissection

The aforementioned anatomic landmarks were intuitively found, and injection site located quickly in the plucked cadavers. The nerve could be visualised effortless via ultrasound once the femur appeared in the cranial edge of the display. (Fig.16) The needle entered through the M. iliotibialis lateralis and advanced through the M. iliofibularis in all specimens. (Fig.6) Needle identification by the semi-experienced author was prompt. No resistance occurred during injection and the spread of the hypoechogenic injectate could be sonographically monitored. The dissection was uneventful, resulting in a homogenous dissection field and reproducible measurement in all cadavers. Compared to the anatomical example all nerves could be stained successful (Fig. 18). The length and width are provided in table 1.

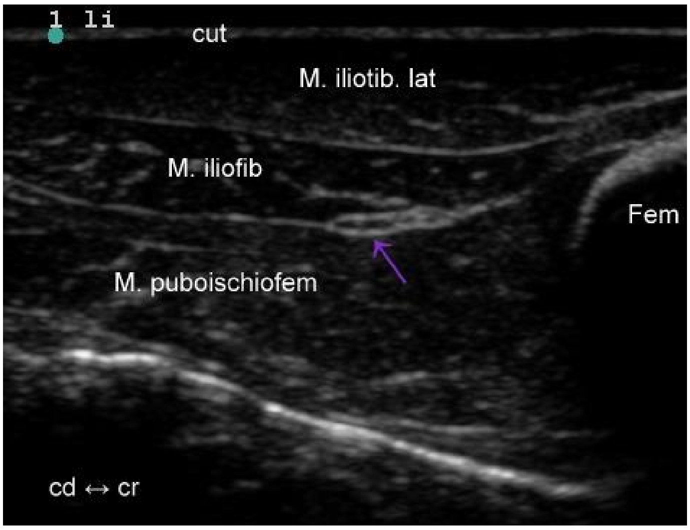

Figure 16: Ultrasonographic picture, revealing the layers of muscle and intrafascial location of the sciatic nerve. Two hypo-echogenic dots within the nerval sheath (pink arrow) show the division of the tibial and fibular nerves already at the chosen proximal injection site. Cut = skin, Fem = femur, cd = caudal, cr = cranial, abbreviations for M. iliotibialis lateralis, M. iliofibularis, M. puboischiofemoralis.

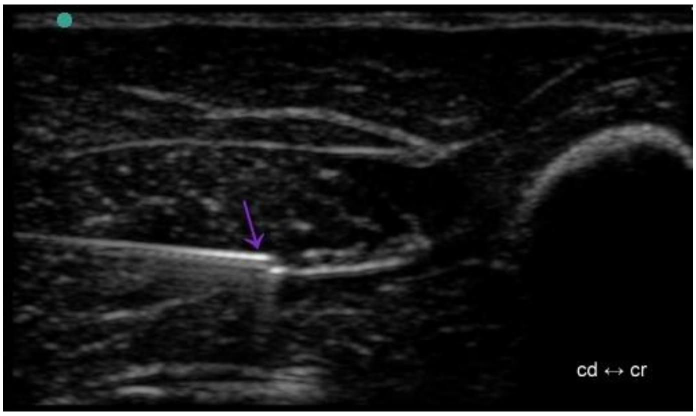

Figure 17: The hyperechogenic needle (pink arrow) enters the muscle from caudally. The tip is orientated towards the nerval sheath.

Figure 18: Cadaver 4, left limb. Result of dissection after injection.

M. iliotibialis lateralis, pars postacetabularis and M. iliofibularis have been separated proximally and hinged downwards to evaluate the dispersal of the dye, which follows the intrafascial space in every direction, depending on position of the specimen and possible lateral manual pressure during injection.

Table 1: Length of nerve in millimetres and after injection measurement of stained nerve length.

Cadaver Number	**Left / Right pelvic limb**	**Total length (mm)**	**Total width (mm)**	**Stained section (mm)**	**Successfully stained amount of visible nerve (%)**
Cadaver 1	Left	45	4	Anatomical part	Anatomical part
	Right	45	4	17	38
Cadaver 2	Left	47	3.5	36	77
	Right	47	3.5	28	60
Cadaver 3	Left	49	4	35	71
	Right	49	4	17	35
Cadaver 4	Left	43	3.5	33	77
	Right	43	3.5	28	65
Cadaver 5	Left	44	3.5	28	64
	Right	44	3.5	38	86

5) Discussion

This pilot cadaver study describes a possible method for a sciatic nerve blockade in chickens and additionally elaborates on the sciatic region, in terms of anatomy. The achieved dispersal of methylene blue dye has shown that the applied model could induce a proper block *in-vivo*. A mean longitudinal nerval length staining value of 28.9 mm can be counted as a successful block, as it is greater than the set 10 mm, which is a cut-off used in mammals (Rodrigo-Mocholi and Martinez-Taboada 2020). In order to achieve a motoric and sensory block three Nodes of Ranvier need to be covered in anaesthetic solution (Raymond et al. 1989, Skarda and Tranquilli 2011), whereas the internodal distance in myelinated fibres is about 1 mm long in amphibians and mammals (Raymond et al. 1989). Even in large mammals the internodal space is rarely wider than 2 mm (Arbuthnott et al. 1980), thus 10 mm is already a broad goal, that was fully reached in all nine specimens.

The total volume of injectate applied was 0.4 ml per pelvic limb. Standard lidocaine formulations such as “Lidor” (Richter Pharma AG, Wels, Austria) contain 20mg/ml Lidocaine. In the cited literature (d'Ovidio and Adami 2019, Kazemi-Darabadi et al. 2019), a minimum dose of 2mg/kg Lidocaine is necessary to chieve pain control. That results in a total volume of 0.26 ml for a 2.6kg chicken. A solution to the discrepancy in volumes *in-vivo* and in this cadaveric study could be to dilute the lidocaine injectate with NaCl up to 0.4 ml to be able to even out the volume-dependent factor and expect a similar intrafascial and perineural spread.

The lethal dose is estimated to be above 6mg/kg in chickens (Brandão May 2014), resulting in 0.8 ml of 2 % Lidocaine formulation used in a 2.6kg chicken. This needs to be kept in mind when using multiple injection sites to prevent exceeding the maximum recommended dosage (Hocking et al. 1997). In a recent study a mallard duck received 8mg/kg total dose in a sciatic- and femoral nerve block without negative effects (Trujanovic et al. 2021). Therefore, a dose between 2 – 8 mg/kg could be a safe amount.

The chosen landmarks for the sciatic nerve block in this study coincides with already existing studies (d'Ovidio et al. 2015) done in peregrine falcons. The great trochanter, the dorsolateral iliac crest, and the lateral condyle of the femur were identified. The puncture site was chosen in the transverse plane in the middle of the connective line between the great trochanter and the dorsolateral iliac crest, and in the dorsal plane at the cranial third on the connective line between the great trochanter and the lateral condyle of the femur. The identification of the sciatic nerve was simple in their described technique, just as it was found in this US-guided study. This confirms the sciatic nerve as an easy target to block, enabling a broad range of indications.

Within the last two years great progress in research concerning avian US-guided nerve blocks has been made, although there is still a lot of work to do to keep up with the studies conducted on other pet species. In one previously mentioned study a group of eighteen Hispaniolan amazon parrots had been assigned to either a palpation orientated or to a sonographically guided brachial plexus block group (Da Cunha et al. 2013). Results were defined by using nerve evoked muscle potentials (NEMPs) for motor response, whereas the sensory loss was not assessed. The onset time of the US-guided group was faster, but neither group showed a difference in motor function, wing droop, or muscle relaxation when compared to the non-blocked wings of the same individuals. The NEMPs showed a lower amplitude in both methods, facilitating the assumption that the US-guided block in avian patients is a feasible and successful technique.

There is one other study examining the US-, and neurostimulator-guided sciatic nerve blocks in clinical setting in a mallard duck (Trujanovic et al. 2021), and another neurostimulator-guided one in a cockerel (Dmitrović et al. 2021). In many other species the sciatic nerve block was examined in cadaver studies in cats (Dos-Santos et al. 2021), pigs (Lee et al. 2020), rabbits (Felisberto et al. 2022), dogs (de Miguel Garcia et al. 2020, Micieli, Chiavaccini et al. 2021), rats, (Hughey et al. 2022), tortoises (Mones et al. 2021) and raptors (de Miguel Garcia et al. 2020, Dos-Santos et al. 2021, Felisberto et al. 2022, Micieli, Chiavaccini et al. 2021, Micieli, Mirra et al. 2021, Mones et al. 2021). There is a growing number of avian *in-vivo* studies conducted in various types of peripheral nerve blocks in avian patients (d’Ovidio et al. 2014, Da Cunha et al. 2013, Da Silva et al. 2021, Dmitrović et al. 2021, Do Nascimento et al. 2019, Figueiredo et al. 2008, Javanmardi et al. 2017), which have achieved variable success.

The positioning and approach play an important role in injectate dispersal. Positions other than lateral recumbency and lateral approach might lead to a different staining behaviour than shown in this study (Vloka et al. 2001).

This pilot study does have its limitations. Firstly, the clinical efficacy of the injections cannot be evaluated as the nature of the study does not involve alive animals. Similar weights of the cadavers allowed only one injection volume to be studied. Also, the same breed of cadavers with the same origin have been used, limiting both the anatomic variances that were possible to observe, as well as the dispersal of the injectate by diverging muscle masses. The metabolic, anatomic, and enzymatic configurations might differ considerably in varying chicken breeds. Local anaesthetics might show different dispersal and onset times or duration of analgesic effects regarding these differences. Also, the variable muscular tissue development may alter the sonographic picture and presentation of the sciatic nerve.

It can be summarised that the US-guided sciatic nerve block in lateral approach in chickens is a clinically applicable and fast method to enable analgesia of the pelvic limb distal to the stifle. The nerve is easy to find due to its size and anatomic consistent superficial location. The landmarks are intuitively found, and the injection site is simple to access.

6) Conclusion

The described method of ultrasonographic controlled sciatic nerve block in lateral approach in chicken cadavers was deemed successful, and the methylene blue spread would produce a proper analgesia if replaced in a clinical study by a local anaesthetic solution. Further *in-vivo* studies are recommended to confirm the clinical use of this locoregional anaesthesia method in chickens (*Gallus gallus domesticus*).

7) Summary

As the largest nerve of the body, the sciatic nerve supplies broad areas of the body distal to the stifle with motoric and sensory information. The ultrasonographic-guided block of this nerve can be used for a variety of indications below the stifle, both in superficial and profound tissue manipulation. This could enable a stabilised anaesthetic depth and lower the risk of post-surgical paraesthesia. The studies concerning chickens have mostly been of pharmacological and pharmacokinetic value, whereas only a modicum of avian locoregional block studies were executed in a clinical setting.

This pilot cadaver study was done at the University of Veterinary Medicine in Vienna, using ten whole body carcasses. One pelvic limb was used for anatomic display of the sciatic nerve, its branches, and surrounding muscles and vessels, and the other nine were included in the injection protocol. The cadavers were positioned in lateral recumbency, nerves were located via ultrasound and injected with Methylene-blue in physiological saline. After injection, the specimens were dissected, and dye spread was measured via digital calliper and compared through descriptive statistics.

This method generated a mean longitudinal nerve staining of 28.9 mm in 100 % of the cohort (nine out of nine specimens), with a threshold set at 10 mm, and thereby achieving a positive outcome.

The results show that the sciatic locoregional anaesthesia under sonographic control tested in chicken cadavers with the described method would likely generate an anaesthetic block of the pelvic limb distal to the stifle *in-vivo.*

8) Zusammenfassung

Als größter Nerv des Körpers versorgt der Nervus ischiadicus weitreichende Gebiete des Körpers sowohl motorisch als auch sensibel. Die ultraschall-kontrollierte Lokalanästhesie dieses Nervs kann für eine Vielfalt an oberflächlichen und tiefen Eingriffen unterhalb des Knies angewendet werden. Damit kann eine Stabilisierung der Narkosetiefe erzielt und die Gefahr von postoperativen Parästhesien vermindert werden. Die bislang durchgeführten Studien bei Hühnern gingen größtenteils pharmakologischen und pharmakokinetischen Fragestellungen nach, aber nur einige wenige aviäre lokalanästhetische Studien standen dabei im klinischen Kontext.

Die vorliegende Studie wurde mit zehn Hintergliedmaßen von Hühnerschlachtkörpern an der Veterinärmedizinischen Universität Wien durchgeführt. Eine Gliedmaße wurde für die anatomische Darstellung des Nervus ischiadicus, dessen Äste, und umgebende Muskeln und Gefäße verwendet, während an den anderen neun die Instillation der färbenden Injektionslösung durchgeführt wurde. Die Kadaver wurden dazu in Seitenlage positioniert. Die mittels Ultraschall lokalisierten Nerven wurden mit einer Mixtur aus einer Kochsalzlösung und Methylenblau injiziert. Anschließend wurden die eingefärbten Nerven durch eine Sektion dargestellt, und die Länge der gefärbten Nervenabschnitte mit einem elektronischen Messschieber erhoben. Die dabei gewonnenen Ergebnisse wurden im Zuge einer deskriptiven Statistik aufgearbeitet.

Die beschriebene Methode konnte eine durchschnittliche longitudinale Nervenfärbung von 28,9 mm bei 100 % der Kohorte (neun von neun Hintergliedmaßen) erzeugen, was bei einem gewählten Schwellenwert von 10 mm einem positiven Resultat entspricht.

Die Ergebnisse zeigen, dass die bei Hühnerkadavern getestete Ultraschall-kontrollierte Lokalanästhesie des Nervus ischiadicus mit großer Wahrscheinlichkeit *in-vivo* eine anästhetische Blockade distal des Knies hervorgerufen hätte.

References

Abou-Madi N. 2001. Avian anesthesia. Veterinary Clinics of North America: Exotic Animal Practice, 4 (1): 147–167. DOI 10.1016/S1094-9194(17)30055-5.

Abu-Seida AM. 2014. Amputation of polymelia in a layer chicken. Avian Diseases, 58 (2): 330–332. DOI 10.1637/10682-100413-Case.1.

Angel R. 2007. Metabolic disorders: Limitations to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. Journal of Applied Poultry Research, 16 (1): 138–149. DOI 10.1093/japr/16.1.138.

Arbuthnott ER, Boyd IA, Kalu KU. 1980. Ultrastructural dimensions of myelinated peripheral nerve fibres in the cat and their relation to conduction velocity. The Journal of Physiology, 308 (1): 125–157.

Berri C, Le Bihan-Duval E, Debut M, Santé-Lhoutellier V, Baéza E, Gigaud V, Jégo Y, Duclos MJ. 2007. Consequence of muscle hypertrophy on characteristics of pectoralis major muscle and breast meat quality of broiler chickens. Journal of Animal Science, 85 (8): 2005–2011. DOI 10.2527/jas.2006-398.

Bjørnstad S, Austdal LPE, Roald B, Glover JC, Paulsen RE. 2015. The chicken model for nonclinical safety studies of drugs. Journal of Pharmacology and Experimental Therapeutics, 355 (3): 386–396. https://doi.org/10.1124/jpet.115.227025.

Brandão JML. May 2014. Cardiovascular tolerance and safety of intravenous lidocaine in the broiler chicken (Gallus gallus domesticus) [LSU Master's Thesis]. Baton Rouge, Louisiana, United States of America: Louisiana State University and Agricultural & Mechanical College, 96.

Brenner DJ, Larsen RS, Dickinson PJ, Wack RF, Williams DC, Pascoe PJ. 2010. Development of an avian brachial plexus nerve block technique for perioperative analgesia in mallard ducks (Anas platyrhynchos). Journal of Avian Medicine and Surgery, 24 (1): 24–34. DOI 10.1647/1082-6742-24.1.24.

Buzała M, Janicki B, Czarnecki R. 2015. Consequences of different growth rates in broiler breeder and layer hens on embryogenesis, metabolism and metabolic rate: A review. Poultry Science, 94 (4): 728–733. DOI 10.3382/ps/pev015.

Cadmus KJ, Mete A, Harris M, Anderson D, Davison S, Sato Y, Helm J, Boger L, Odani J, Ficken MD, Pabilonia KL. 2019. Causes of mortality in backyard poultry in eight states in the United States. Journal of Veterinary Diagnostic Investigation, 31 (3): 318–326. DOI 10.1177/1040638719848718.

Cardozo LB, Almeida RM, Fiúza LC, Galera PD. 2009. Brachial plexus blockade in chickens with 0.75% ropivacaine. Veterinary Anaesthesia and Analgesia, 36 (4): 396–400. DOI 10.1111/j.1467-2995.2009.00467.x.

Cīrule D, Krama T, Vrublevska J, Rantala MJ, Krams I. 2012. A rapid effect of handling on counts of white blood cells in a wintering passerine bird: a more practical measure of stress? Journal of Ornithology, 153 (1): 161–166. DOI 10.1007/s10336-011-0719-9.

Comolli J, d'Ovidio D, Adami C, Schnellbacher R. 2019. Technological advances in exotic pet anesthesia and analgesia. The Veterinary Clinics of North America: Exotic Animal Practice, 22 (3): 419–439. DOI 10.1016/j.cvex.2019.06.003.

Cooke VE, Gilpin S, Mahon M, Sandercock DA, Mitchell MA. 2003. A comparison of skeletal muscle fibre growth in broiler and layer chickens. British Poultry Science, 44 (S1): 33–34. DOI 10.1080/713655294.

d’Ovidio D, Rota S, Noviello E, Briganti A, Adami C. 2014. Nerve stimulator–guided sciatic-femoral block in pet rabbits (Oryctolagus cuniculus) undergoing hind limb surgery: A case series. Journal of Exotic Pet Medicine, 23 (1): 91–95. DOI 10.1053/j.jepm.2013.11.014.

Da Cunha AF, Strain GM, Rademacher N, Schnellbacher R, Tully TN. 2013. Palpation- and ultrasound-guided brachial plexus blockade in hispaniolan amazon parrots (Amazona ventralis). Veterinary Anaesthesia and Analgesia, 40 (1): 96–102. DOI 10.1111/j.1467-2995.2012.00783.x.

Da Silva HRA, Nunes N, Gering AP, Souza PGA, Salgado KPA, Ferreira OR, Nakamura AJ, Da Guimarães AKS. 2021. Hypersensitivity in chicken (Gallus gallus domesticus) due to the association of lidocaine and bupivacaine in neural-guided femoral and sciatic nerve block. Acta Scientiae Veterinariae, 49 (Suppl.1): 1–4. DOI 10.22456/1679-9216.109636.

de Miguel Garcia C, Whyte M, St James M, Ferreira TH. 2020. Effect of contrast and local anesthetic on dye spread following transversus abdominis plane injection in dog cadavers. Veterinary Anaesthesia and Analgesia, 47 (3): 391–395. DOI 10.1016/j.vaa.2020.01.003.

Dmitrović P, Dupont J, Marlier D, Monchaux M, Sandersen C. 2021. Nerve stimulator-guided sciatic nerve block in a cockerel (Gallus gallus domesticus) for a bone marrow biopsy. Vet Record Case Reports, 9 (3): 1–6. DOI 10.1002/vrc2.134.

Do Nascimento FM, Nunes TL, Silva Souza TB, Couto Andrade MDA, Barbosa VF. 2019. Brachial plexus block with use of a neurostimulator in a striped owl (Asio clamator) undergoing wing amputation. Acta Scientiae Veterinariae, 47 (Suppl 1): 1–6. DOI 10.22456/1679-9216.89521.

Doss G, Mans C. 2021. Avian sedation. Journal of Avian Medicine and Surgery, 35 (3): 253–268. DOI 10.1647/20-00045.

Dos-Santos JD, Ginja M, Alves-Pimenta S, Otero PE, Ribeiro L, Colaço B. 2021. A description of an ultrasound-guided technique for a quadratus lumborum block in the cat: A cadaver study. Veterinary Anaesthesia and Analgesia, 48 (5): 804–808. DOI 10.1016/j.vaa.2021.03.017.

d'Ovidio D, Adami C. 2019. Locoregional anesthesia in exotic pets. The Veterinary Clinics of North America: Exotic Animal Practice, 22 (2): 301–314. DOI 10.1016/j.cvex.2019.01.007.

d'Ovidio D, Noviello E, Adami C. 2015. Nerve stimulator-guided sciatic-femoral nerve block in raptors undergoing surgical treatment of pododermatitis. Veterinary Anaesthesia and Analgesia, 42 (4): 449–453. DOI 10.1111/vaa.12204.

d'Ovidio D, Noviello E, Nocerino M. 2011. Combination of fentanyl and lidocaine for brachial plexus block in a peregrine falcon. In: Samour J, Montesinos A, eds. Association of Avian Veterinarians European College of Zoological Medicine (EAAV). : 394–396.

Felisberto R, Flaherty D, Tayari H. 2022. Ultrasound-guided saphenous nerve block in rabbits (Oryctolagus cuniculus): A cadaveric study comparing two injectate volumes. Animals, 12 (624): 1–14. DOI 10.3390/ani12050624.

Figueiredo JP, Cruz ML, Mendes GM, Marucio RL, Riccó CH, Campagnol D. 2008. Assessment of brachial plexus blockade in chickens by an axillary approach. Veterinary Anaesthesia and Analgesia, 35 (6): 511–518. DOI 10.1111/j.1467-2995.2008.00410.x.

Frewein J. 1992. Lenden-,Kreuz, und Schamgeflecht, Plexus lumbalis, sacralis et pudendus. In: Schummer A, ed. Anatomie der Vögel. Second ed. Berlin und Hamburg: Paul Parey, 359–361.

Fulton RM. 2017. Causes of normal mortality in commercial egg-laying chickens. Avian Diseases, 61 (3): 289–295. https://doi.org/10.1637/11556-120816-RegR.

Gadsden JC. 2021. The role of peripheral nerve stimulation in the era of ultrasound-guided regional anaesthesia. Anaesthesia, 76 (Suppl 1): 65–73. DOI 10.1111/anae.15257.

Garcia ER. 2015. Local Anaesthetics. In: Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA, eds. Veterinary Anesthesia and Analgesia. The Fifth Edition of Lumb and Jones. Fifth ed. Ames Iowa: Wiley Blackwell, 332–354.

Glatz PC, Murphy LB, Preston AP. 1992. Analgesic therapy of beak-trimmed chickens. Australian Veterinary Journal, 69 (1): 18. DOI 10.1111/j.1751-0813.1992.tb09859.x.

Greenacre CB. 2015. Reproductive diseases of the backyard hen. Journal of Exotic Pet Medicine, 24 (2): 164–171. http://dx.doi.org/10.1053/j.jepm.2015.04.004.

Guzman DS-M, Beaufrère H. 2021. Avian pain management and anesthesia. In: Graham JE, Doss GA, Beaufrère H, eds. Exotic Animal Emergency and Critical Care Medicine. First. : John Wiley & Sons Inc, 488–502.

Harvey S, Phillips, J.G., Rees A, Hall TR. 1984. Stress and adrenal function. The Journal of Experimental Zoology, 232 (3): 633–645.

Hassanzadeh M, Gilanpour H, Charkhkar S, Buyse J, Decuypere E. 2005. Anatomical parameters of cardiopulmonary system in three different lines of chickens: further evidence for involvement in ascites syndrome. Avian Pathology, 34 (3): 188–193. DOI 10.1080/03079450500096372.

Hawkins MG, Griffenhagen GM. 2022. Raptor sedation and anesthesia. The Veterinary Clinics of North America: Exotic Animal Practice, 25 (1): 135–161. DOI 10.1016/j.cvex.2021.08.011.

Hawkins MG, Paul-Murphy J. 2011. Avian analgesia. The Veterinary Clinics of North America: Exotic Animal Practice, 14 (1): 61–80. DOI 10.1016/j.cvex.2010.09.011.

Hochleithner C. 2006. Ultrasound in pet birds. Israel Journal of Veterinary Medicine, 61 (1): 26–29.

Hocking PM, Gentle MJ, Bernard R, Dunn LN. 1997. Evaluation of a protocol for determining the effectiveness of pretreatment with local analgetics for reducing experimentally induced articular pain in domestic fowl. Research in Veterinary Science, 63 (3): 263–267.

Huang AS, Carvallo FR, Pitesky ME, Stoute S, Mete Aslı. 2019. Gastrointestinal impactions in backyard poultry. Journal of Veterinary Diagnostic Investigation, 31 (3): 368–370. DOI 10.1177/1040638719843966.

Hughey S, Campbell D, Rapp-Santos K, Cole J, Booth G, Longwell J, Stedje-Larsen E. 2022. Refining the rat sciatic nerve block: A novel ultrasound-guided technique. Laboratory Animals, 56 (2): 191–195. DOI 10.1177/00236772211034627.

Jackson S, Diamond J. 1996. Metabolic and digestive responses to artificial selection in chickens. Evolution, 50 (4): 1638–1650. DOI 10.1111/j.1558-5646.1996.tb03936.x.

Javanmardi S, Madadi MS, Abbasi MF. 2017. Wing amputation in a golden eagle with humeral fracture. Iranian Journal of Veterinary Surgery, 12 (2): 71–75.

Jones SJ, Aberle ED, Judge MD. 1986. Skeletal muscle protein turnover in broiler and layerchicks. Journal of Animal Science, 62 (6): 1576–1583.

Julian RJ. 1998. Rapid growth problems: Ascites and skeletal deformities in broilers. Poultry Science, 77 (12): 1773–1780. DOI 10.1093/ps/77.12.1773.

Julian RJ, Friars GW, French DH, Quinton M. 1987. The relationship of right ventricular hypertrophy, right ventricular failure, and ascites to weight gain in broiler and roaster chickens. Avian Diseases, 31 (1): 130–135.

Kazemi-Darabadi S, Akbari G, Shokrollahi S. 2019. Development and evaluation of a technique for spinal anaesthesia in broiler chickens. New Zealand Veterinary Journal, 67 (5):

241–248. DOI 10.1080/00480169.2019.1618223.

Keer-Keer S, Hughes BO, Hocking PM, Jones RB. 1996. Behavioural comparison of layer and broiler fowl: Measuring fear responses. Applied Animal Behaviour Science, 49 (4): 321–333.

Kehlet H, Jensen TS, Woolf CJ. 2006. Persistent postsurgical pain: Risk factors and prevention. The Lancet, 367 (9522): 1618–1625. DOI 10.1016/S0140-6736(06)68700-X.

Knowles TG, Kestin SC, Haslam SM, Brown SN, Green LE, Butterworth A, Pope SJ, Pfeiffer D, Nicol CJ. 2008. Leg disorders in broiler chickens: Prevalence, risk factors and prevention. PloS one, 3 (2): 1-5. DOI 10.1371/journal.pone.0001545.

König, Horst Erich, Korbel, Rüdiger, Liebich, Hans-Georg, Hrsg. 2016. Avian Anatomy. Textbook and Colour Atlas. Second ed. Sheffield, United Kingdom: 5m Publishing, 340.

König, H. E., Navarro, M., Zengerling, G., Korbel, R. 2016. Respiratory system (apparatus respiratorius). In: König, Horst Erich, Korbel, Rüdiger, Liebich, Hans-Georg, eds. Avian Anatomy. Textbook and Colour Atlas. Second ed. Sheffield, United Kingdom: 5m Publishing, 118–130.

Korte SM, Sgoifo A, Ruesink W, Kwakernaak C, van Voorst S, Scheele CW, Blokhuis HJ. 1999. High carbon dioxide tension (PCO2) and the incidence of cardiac arrhythmias in rapidly growing broiler chickens. The Veterinary Record, 145 (2): 40–43. DOI 10.1136/vr.145.2.40.

Krautwald-Junghanns M-E, Pees M, Böhme J. 2011. Ultrasonographic examination in birds. In: Samour J, Montesinos A, eds. Association of Avian Veterinarians European College of Zoological Medicine (EAAV). : 489–494.

Kubiak M. 2016. Avian analgesia. Companion Animal, 21 (8): 480–484. DOI 10.12968/coan.2016.21.8.480.

Latney L, Runge J, Wyre N, Larenza Menzies MP, Neville C, Briscoe J. 2018. Novel technique for scapulohumeral amputations in avian species: A case series. Israel Journal of Veterinary Medicine, 73 (1): 35–45.

Lawton MP. 1999. Management of respiratory disease in psittacine birds. In Practise, 21 (2): 76–88. https://doi.org/10.1136/inpract.21.2.76.

Lee A, Lennox A. 2016. Sedation and local anesthesia as an alternative to general anesthesia in 3 birds. Journal of Exotic Pet Medicine, 25 (2): 100–105. DOI 10.1053/j.jepm.2016.03.012.

Lee MG, Choi SU, Lim JK, Lee MJ, Hong JS, Baek MO, Yoon SZ, Park HY, Shin HJ. 2020. Ultrasound-guided sciatic nerve block at the midthigh level in a porcine model: a descriptive study. Veterinary Medicine and Science, 6 (3): 543–549. DOI 10.1002/vms3.265.

Lierz M, Korbel R. 2012. Anesthesia and Analgesia in Birds. Journal of Exotic Pet Medicine, 21 (1): 44–58. DOI 10.1053/j.jepm.2011.11.008.

Linn K. 1986. Avian Anesthesia. Cor. 84'. In: Redmond K, ed. Avian Rounds. : New York State College of Veterinary Medicine, Cornell University, 2–6.

Lund VP, Kyvsgaard NC, Christensen JP, Bisgaard M. 2013. Pathological manifestations observed in dead-on-arrival broilers at a Danish abattoir. British Poultry Science, 54 (4): 430–440. DOI 10.1080/00071668.2013.804173.

Marhofer P, Chan VWS. 2007. Ultrasound-guided regional anesthesia: Current concepts and future trends. Anesthesia and Analgesia, 104 (5): 1265-1269. DOI 10.1213/01.ane.0000260614.32794.7b.

Marhofer P, Greher M, Kapral S. 2005. Ultrasound guidance in regional anaesthesia. British

Journal of Anaesthesia, 94 (1): 7–17. DOI 10.1093/bja/aei002.

Martinez-Pereira MA, Zancan DM. 2015. Comparative anatomy of the peripheral nerves. In: Martinez-Pereira MA, Zancan DM, eds. Nerves and Nerve Injuries.: Academic Press, 55–77.

McRee AE, Tully TN, Nevarez JG, Beaufrere H, Ammersbach M, Gaunt SD, Fuller RG, Romero LM. 2018. Effect of routine handling and transportation on blood leucocyte concentrations and plasma corticosterone in captive hispaniolan amazon parrots (Amazona Ventralis). Journal of Zoo and Wildlife Medicine, 49 (2): 396–403. DOI 10.1638/2016-0100.1.

Mete A, Giannitti F, Barr B, Woods L, Anderson M. 2013. Causes of mortality in backyard chickens in northern california: 2007-2011. Avian Diseases, 57 (2): 311–315. DOI 10.1637/10382-092312-Case.1.

Micieli F, Chiavaccini L, Mennonna G, Della Valle G, Prisco F, Meomartino L, Vesce G. 2021. An ultrasound-guided subparaneural approach to the sciatic nerve in the dog: a cadaver study. Veterinary Anaesthesia and Analgesia, 48 (1): 107–115. DOI 10.1016/j.vaa.2020.06.008.

Micieli F, Mirra A, Santangelo B, Minichino A, Fuensalida SE, Milito M, Vesce G, Otero PE. 2021. Ultrasound-guided dorsal approach for the brachial plexus block in common kestrels (Falco tinnunculus): A cadaver study. Veterinary Anaesthesia and Analgesia, 48 (4): 617–621. DOI 10.1016/j.vaa.2020.12.009.

Moghadam HK, McMillan I, Chambers JR, Julian RJ, Tranchant CC. 2005. Heritability of sudden death syndrome and its associated correlations to ascites and body weight in broilers. British Poultry Science, 46 (1): 54–57. DOI 10.1080/00071660400023862.

Mones AB, Gorges MA, Santangelo SM, Lewbart GA, Harrison TM, Gerard MP. 2021. Feasibility of a blind perineural injection technique for brachial plexus blockade in eastern box turtles (Terrapene carolina carolina): A cadaver study. Veterinary Anaesthesia and Analgesia, 48 (5): 789–797. DOI 10.1016/j.vaa.2021.04.007.

Monticelli P, Campoy L, Adami C. 2016. Ultrasound-guided femoral and sciatic nerve blocks for repair of tibia and fibula fractures in a bennett's wallaby (Macropus rufogriseus). Case Reports in Anesthesiology, 2016: 1–4. DOI 10.1155/2016/8909205.

Muramatsu T, Hiramoto K, Okumura J. 1990. Strain differences in whole-body protein turnover in the chicken embryo. British Poultry Science, 31 (1): 91–99. DOI 10.1080/00071669008417234.

Nangsuay A, Molenaar R, Meijerhof R, van den Anker I, Heetkamp MJW, Kemp B, van den Brand H. 2015. Differences in egg nutrient availability, development, and nutrient metabolism of broiler and layer embryos. Poultry Science, 94 (3): 415–423. DOI 10.3382/ps/pev007.

Neal JM, Brull R, Chan VWS, Grant SA, Horn J-L, Liu SS, McCartney CJL, Narouze SN, Perlas A, Salinas FV, Sites BD, Tsui BC. 2010. The ASRA evidence-based medicine assessment of ultrasound-guided regional anesthesia and pain medicine: Executive summary. Regional Anesthesia and Pain Medicine, 35 (Suppl 1): 1-9. DOI 10.1097/AAP.0b013e3181d22fe0.

Ohta Y, Yoshida T, Tsushima N. 2004. Comparison between broilers and layers for growth and protein use by embryos. Poultry Science, 83 (5): 783–787. DOI 10.1093/ps/83.5.783.

Olkowski AA. 2007. Pathophysiology of heart failure in broiler chickens: structural, biochemical, and molecular characteristics. Poultry Science, 86 (5): 999–1005. DOI 10.1093/ps/86.5.999.

Orosz SE, Lichtenberger M. 2011. Avian respiratory distress: Etiology, diagnosis, and treatment. Veterinary Clinics of North America: Exotic Animal Practice, 14 (2): 241–255. https://doi.org/10.1016/j.cvex.2011.03.003.

Paul-Murphy J, Ludders JW. 2001. Avian Analgesia. Veterinary Clinics of North America: Exotic Animal Practice, 4 (1): 35–45. DOI 10.1016/S1094-9194(17)30049-X.

Pees M, Kiefer I, Krautwald-Junghanns M-E. 2008. The use of ultrasonography for the examination of the gastrointestinal tract and the liver in birds. In: Aschenbach JR, Gäbel G, Daugschies A, eds. LBH: Proceedings 4. Leipziger Tierärztekongress.: Universität Leipzig Pressestelle, 452–454.

Pohjola L, Rossow L, Huovilainen A, Soveri T, Hänninen M-L, Fredriksson-Ahomaa M. 2015. Questionnaire study and postmortem findings in backyard chicken flocks in Finland. Acta Veterinaria Scandinavica, 57 (1): 1–9. DOI 10.1186/s13028-015-0095-1.

Ravasio G, Brioschi FA, Rabbogliatti V, Gioeni D, Di Cesare F, Corletto F, Oltolina M, Carnevale L. 2020. Ultrasound sciatic and saphenous nerve blocks for tibial malunion surgical correction in a pediatric african leopard (Panthera pardus). Frontiers in Veterinary Science, 7 (538883): 1–7. DOI 10.3389/fvets.2020.538883.

Raymond SA, Steffensen SC, Gugino LD, Strichartz GR. 1989. The role of length of nerve exposed to local anesthetics in impulse blocking action. Anesthesia and Analgesia, 68 (5): 563-570. DOI 10.1213/00000539-198905000-00004.

Rodrigo-Mocholi D, Martinez-Taboada F. 2020. Novel ultrasound-guided lateral approach for femoral nerve block in cats: a pilot study. Journal of Feline Medicine and Surgery, 22 (4): 339–343. DOI 10.1177/1098612X19845719.

Saito S, Takagi T, Koutoku T, Saito ES, Hirakawa H, Tomonaga S, Tachibana T, Denbow DM, Furuse M. 2004. Differences in catecholamine metabolism and behaviour in neonatal broiler and layer chicks. British Poultry Science, 45 (2): 158–162. DOI 10.1080/00071660410001715740.

Schroeder K. 2012. History of regional anesthesia. In: Campoy L, Read M, eds. Small Animal Regional Anesthesia and Analgesia. Arnes, AI: Wiley, 1–9.

Schummer A, Hrsg. 1992. Anatomie der Vögel. Second ed. Berlin und Hamburg: Paul Parey.

Seamon AB, Hofmeister EH, Divers SJ. 2017. Outcome following inhalation anesthesia in birds at a veterinary referral hospital 352 cases (2004–2014). Journal of the American Veterinary Medical Association, 251 (7): 814–817.

Seco O, Zarucco L, Campoy L. 2012. Ultrasound-guided peripheral nerve blocks. In: Campoy L, Read M, eds. Small Animal Regional Anesthesia and Analgesia. Arnes, AI: Wiley, 77–86.

Shaver SL, Robinson NG, Wright BD, Kratz GE, Johnston MS. 2009. A multimodal approach to management of suspected neuropathic pain in a prairie falcon (Falco mexicanus). Journal of Avian Medicine and Surgery, 23 (3): 209–213. DOI 10.1647/2008-038.1.

Shim MY, Karnuah A, Anthony NB, Pesti GM, Aggrey SE. 2012. The effects of broiler chicken growth rate on valgus, varus, and tibial dyschondroplasia. Poultry Science, 91 (1): 62–65. DOI 10.3382/ps.2011-01599.

Sites BD, Antonakakis JG. 2009. Ultrasound guidance in regional anesthesia: state of the art review through challenging clinical scenarios. Local and Regional Anesthesia, 2: 1–14. DOI 10.2147/lra.s3444.

Skarda RT, Tranquilli WJ. 2011. Local anesthetics and regional analgesic techniques. In: Grimm KA, Tranquilli WJ, Lamont LA, eds. Essentials of Small Animal Anesthesia and Analgesia. Second ed. Oxford, UK, Iowa, USA, West Sussex, UK: John Wiley & Sons, Inc., 326–377.

Trott KA, Giannitti F, Rimoldi G, Hill A, Woods L, Barr B, Anderson M, Mete A. 2014. Fatty liver hemorrhagic syndrome in the backyard chicken: a retrospective histopathologic case series. Veterinary Pathology, 51 (4): 787–795. DOI 10.1177/0300985813503569.

Trujanovic R, Otero PE, Larenza-Menzies MP. 2021. Ultrasound- and nerve stimulation-guided femoral and sciatic nerve block in a duck (Anas platyrhynchos) undergoing surgical fixation of a tibiotarsal fracture. Veterinary Anaesthesia and Analgesia, 48 (2): 277–278. DOI 10.1016/j.vaa.2020.11.001.

Vloka JD, Hadžić A, April E, Thys DM. 2001. Anterior approach to the sciatic nerve block: The effects of leg rotation. Anesthesia and Analgesia, 92 (2): 460–462. DOI 10.1213/00000539-200102000-00034.

Vollmerhaus B. 1992. Bewegungsapparat des Stammes. In: Schummer A, ed. Anatomie der Vögel. Second ed. Berlin und Hamburg: Paul Parey, 72–73.

Vollmerhaus B, Sinowatz F. 1992a. Haut und Hautgebilde. In: Schummer A, ed. Anatomie der Vögel. Second ed. Berlin und Hamburg: Paul Parey, 16-50.

Vollmerhaus B, Sinowatz F. 1992b. Luftsäcke, Sacci pneumatici. In: Schummer A, ed. Anatomie der Vögel. Second ed. Berlin und Hamburg: Paul Parey, 172–175.

10) List of Tables

11) List of Figures

12) Acknowledgements

This endeavour would not have been possible without Priv.-Doz. Dr.med.vet. Ulrike Auer from the Clinical Unit of Anaesthesiology and Perioperative Intensive-Care Medicine of the University of Veterinary Medicine Vienna for being reliably present, on time, and the saving escort that was so urgently needed. The author would like to deeply thank Robert Trujanovic, DVM for opening previously closed doors, welcoming nomadic students, and enabling this experience although not to its full extent. And a special thanks to Univ.-Prof. Jean-Loup Rault PhD. from the Institute of Animal Welfare Science of the University of Veterinary Medicine Vienna for expanding the never expected possibilities of this thesis and assisting me in finding this highly interesting topic. Priv.-Doz. Dr.med.vet. Kirsti Witter from the institute of morphology of the University of Veterinary Medicine Vienna has shaped the author’s interest in anatomy from the early study-years on and contributed with a fast and thoroughly comprehensible evaluation of this thesis. The professional and efficient help concerning language related issues from Dr.med.univ. Casey Zachariah B.Sc. is much appreciated. Lastly many thanks to my family, friends, and pets that offered their support thoroughly and consistently and providing breaks when necessary. Without them the author would still hide in deep grass with her chickens, pigeons, cats, and ducks and hope that the last day of university would never come.