Research Study
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Incidence and Heritability of Osteosarcoma in the Irish Wolfhound

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Summary

Study Aim
Osteosarcoma is the primary bone tumor that occurs most frequently in dogs. It is the tumor type in about 85% of all primary bone tumors and affects mainly giant breed dogs of middle age. The Irish Wolfhound as a giant breed falls perfectly into the category at risk for developing osteosarcoma, and has been shown to have an increased risk in previous studies. Incidence and the heritability of this trait in the Irish Wolfhound had not yet been calculated. The aim of this study is to collect information on a group of Irish Wolfhounds to determine the incidence and estimate heritability in order to evaluate whether a follow-up association study would prove useful.
Materials and Methods A test group was formed of 532 dogs whose blood samples were collected. The owners or breeders of these dogs were contacted by telephone and information was collected on osteosarcoma status and other relevant facts (age of occurrence, location of the tumor, diagnostic imaging performed). Radiographic imaging was set as the diagnostic method minimally required to assign a dog with a positive bone cancer status. This data was used to calculate the incidence and estimate heritability using linear and logistic models in ASReml.
Results With the data collected an incidence of 6.34% (17 out of 268 deceased dogs) was calculated using the cases in which the diagnostic requirements had been met. The estimated heritability was 0.15 (± 0.09) on the liability scale (logistic model), and 0.33 (± 0.12) on the observed scale (linear model). The linear model showed a result that different significantly from zero. There appeared to be a sex predisposition, with female dogs at a 2.33 higher risk of developing bone cancer than males.
Conclusion These results indicate that an associations study using DNA markers might be fruitful.

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Canine Osteosarcoma

Introduction

Of all bone tumors in dogs, about 98% of them are malignant. There are three main groups, namely primary bone tumors, tumors that have metastasized to the bone and tumors of surrounding tissues that have invaded into the bone (Withrow et al, 2007).

Osteosarcoma is the primary bone tumor that occurs most frequently in dogs. It is the tumor type in about 85% of all primary bone tumors (mostly in large breed dogs, but even in small breeds (<15kg) osteosarcomas account for around 45% of all primary bone tumors). Osteosarcoma is a tumor of mesenchymal origin, originating from the primitive bone cells that produce osteoid. It's a highly aggressive tumor, causing severe damage and malformation locally by bone lysis and/or new bone formation. This also causes soft tissue swelling, and may result in pathological fractures. The tumors can develop spontaneously, which is seen in most of the cases, but can also be the result of radiation therapy, fractures or implants (Withrow et al, 2007). Metastatic disease is extremely common, with around 90% of affected dogs having micrometastases at the time of diagnosis, even if only 10% of the dogs show clinical signs (Eberle et al, 2010).

There are three main groups as far as tumor location is concerned: appendicular tumors, axial tumors and extra-skeletal tumors. In large breed dogs, appendicular osteosarcomas are the most common, at around 75%. They are often found at the metaphyseal region and more often on fore limbs than hind limbs. Prime locations are the distal radius/ulna (40% of all appendicular osteosarcomas), the proximal humerus, the distal femur and the proximal tibia. Because of these spots, osteosarcoma is often said to occur 'away from the elbow and around the knee'. The axial tumors are far less common, with most of them on the skull or on the ribs. Finally, there are the extra-skeletal osteosarcomas - these are very rare but extremely aggressive malignant tumors. In small breed dogs, the distribution is different, with tumors occurring as frequently on the appendicular as they do on the axial skeleton. Prime locations in the small dogs are the femur and tibia (Withrow et al, 2007).

Risk factors for developing osteosarcoma include increased height and weight. The tumor usually appears once the dog is middle-aged and older (Ru et al, 1998). Neutered dogs also seem more at risk than intact dogs, and though not precisely determined there seems to be an connection between duration of endogenous gonadal exposure (determined by the age at which the dog was neutered) and an increased incidence of osteosarcoma (Ru et al, 1998; Cooley et al, 2002). Because of these risk factors, large to giant breed dogs are most often affected, with 90% of all osteosarcomas developing in dogs over 20 kg in weight. As such, the dogs most often seen with osteosarcoma are 7.5 yrs of median age and mainly large breeds such as Rottweilers, Great Danes, St. Bernards, Irish Wolfhounds and Greyhounds (Withrow et al, 2007; Rosenberger et al, 2007).

Canine osteosarcoma closely resembles the osteosarcomas that develop in human patients, and as such has been used extensively for further research and treatments in both species. The only main differences between the tumors are the age at which they develop (in dogs it is late in life, whereas in humans it develops in adolescence), the prime locations (in humans mainly the stifle) and some aspects of the current treatment methods (Martano et al, 2011).

Diagnostics

Clinical Signs

Patients suffering from osteosarcoma usually present themselves with chronic but variable lameness, sometimes acutely manifesting itself after exercise. Initially, they can respond well to treatment with

non-steroidal anti-inflammatory drugs (NSAIDs). In some cases the owner notices a swelling of the leg. If there is substantial pulmonary metastasis, signs of metastatic disease can be seen, such as inappetence and malaise. Respiratory signs are uncommon (Withrow et al, 2007).

During the physical examination a hard swelling on some part of the affected leg can be found in many cases - this swelling can be painful, with or without significant soft tissue swelling. If the soft tissue swelling is not yet obvious - as is often the case in early stages - a pain response might still be elicited by deep palpation of the bone. The lameness, as stated, is chronic but extremely variable in presentation, from simply favoring the affected leg to completely non-weight bearing lameness. The degree of lameness seems to hold very little correlation with the degree of abnormalities found on radiographic images (Withrow et al, 2007).

Radiographic Imaging

In case of persistent lameness and/or a hard swelling on a limb, radiographic images of the affected leg should be made. Osteosarcoma displays one or all of the following signs on radiograph: loss of fine trabeculair bone detail in the metaphyseal region due to bone lysis; a discontinuity of the cortex; periosteal new bone formation (so called Codman's Triangle); mineralisation perpendiculair to the bone shaft (sunburst effect); the mass can extend into adjacent soft tissue; a blurry transitional zone between affected and normal bone; inappropriate areas of sclerosis and pathological fractures. (Withrow et al, 2007) There are, however, more differential diagnoses that can show some of the same abnormalities on radiographs, e.g. other bone tumors (primary or metastatic), an infection/osteomyelitis (bacterial, fungal) or bone cysts, though the last case is very rare. So while the lytic and proliferative lesions are indicative of osteosarcoma, it cannot be diagnosed as such with a reliable certainty on radiographs alone. Additional histopathological examination will be necessary (Withrow et al, 2007; Endicott et al, 2003).

Biopsies

It is extremely important to correctly diagnose the tumor down to its subtype. Just knowing it's a sarcoma is not enough, since the prognosis of the various subtypes differ. Other sarcoma options are chondrosarcoma (less metastatic tendency than osteosarcoma), hemangiosarcoma (biologically extremely aggressive) and fibrosarcoma (curable by amptation) (Neihaus et al, 2011). Therefore, biopsy of the lesion is necessary.

Histopathology

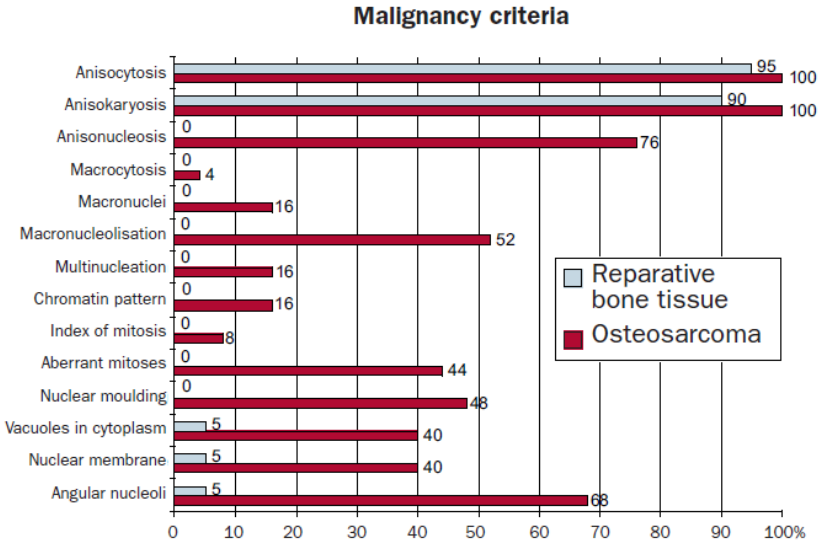
Histopathology remains the golden standard for the definitive diagnosis osteosarcoma. There are a few options for a histological biopsy pre-surgery, such as the open method, the closed method (with a Jamshidi needle) or limited open (Michele trephine). The biopsy and incision should be executed at site of optimal bone abnormalities, and in such a way that the biopsy canal will be completely removed during the following amputation (Withrow et al, 2007).

- Jamshidi - 2-3 biopsies are taken from the centre of the lesion, using the Jamshidi needle and a single incision. The diagnostic accuracy for a specific tumor is 82%, the accuracy in differentiating a tumor from other differential diagnosis is 92%
- The open method gives you a larger biopsy, but also more complications.
- The limited open method has a diagnostic accuracy of 94%, but more risk of a pathological fracture (Withrow et al, 2007).

Of course after surgery the amputated limb can and should always be sent for histopathological evaluation, to confirm the diagnosis.
There are, as already indicated above, risks involved with a histopathological biopsy, such as causing a hematoma, causing infection or manual spread of the tumor cells, as well as pathological fractures (Neihaus et al, 2011).

Cytology

Cytology has some advantages over histopathology, such as less bone disruption, a shorter procedure and a rapid diagnosis. It is, however, very difficult to differentiate a tumor from reactive bone tissue, and even more difficult to differentiate between the subtypes of sarcoma's (Neihaus et al, 2011; Reinhardt et al, 2005). A study from 2005 lists some cytologic criteria after comparing samples of cases of osteosarcoma with those of cases of reactive bone (callus formation) to help differentiate between the two. In the graph below (Fig.1) it is detailed how often certain malignancy characteristics were noted in the osteoblasts in the samples of both groups (Reinhardt et al, 2005).



[Fig.1 – Cytological criteria for malignancy (Reinhardt et al, 2005)]

After differentiating between reactive and tumor tissue, there is still the issue of correctly diagnosing the subtype of the sarcoma. There is a method emerging that involves staining for the alkaline phosphatase (ALP) present in the osteoblasts. The bone isoform is a membrane-bound enzyme and the staining appears cytoplasmatic. In a study from 2012 almost all (15/17) of the osteosarcoma samples stained positive, with 4/66 of the samples from other tumors proving positive as well. The sensitivity and specificity of the particular staining method used were 88% and 94% respectively. This method might prove helpful in determining osteosarcoma from other sarcoma types (Ryseff et al, 2012).

Cytology still remains a seldomly used tool in the diagnosis plan, and it is easy to see why in a comparison with result gained from histopathology. In a study comparing positive histopathology result for osteosarcoma with cytology results, in 11% of the cases the cytology was inconclusive, in 19% of the cases it differed so significantly from the histopathology it was considered misleading, and in 22% of the cases cytology showed the same results. However, in 70% of all the cases cytology was at least able to differentiate between a malignancy and non-malignancy (despite being unable to positively diagnose the tumor type) so in cases where histopathology is not an option, this differentiation might still be made (Loukopoulos et al, 2005).

Advanced Imaging Techniques

For diagnosing osteosarcoma in the dog, radiographic imaging followed by biopsies and histopathological evaluation are sufficient. Advanced imaging techniques such as computed tomography (CT) can, however, help in evaluating the extent of the tumor accurately, which is

important for selecting the right surgery type and by that a successful outcome (Withrow et al, 2007; Davis et al, 2002).

Additional CT imaging is definitely useful in determining the presence of metastatic disease. A study comparing the ability of both thoracic radiographs and CT to diagnose pulmonary nodules in dogs with osteosarcoma showed that CT is significantly better to diagnose metastases. Pulmonary nodules were demonstrated in 5% of the cases on radiographs, and in 28% of the cases in CT (Eberle et al, 2010).

Therapy and prognosis

When a dog is diagnosed with osteosarcoma, there are a variety of treatment options available. It should be noted that none of these treatments offer a curative solution. Because of its high metastatic tendency removing the tumor will not offer a cure. Treatments following amputation are meant to treat metastatic disease and/or to provide analgesia.

Analgesia

Sufficient analgesia for patients with bone cancer is most definitely required for a good quality of life. In human bone cancer patients, two types of pain are described, namely ongoing pain, and breakthrough pain. The ongoing pain is a dull, constant ache, while breakthrough pain is episodes of extreme pain occurring spontaneously or brought on by movement or weight-bearing of the limb. Since canine osteosarcoma has similarities on many fronts with human osteosarcoma, it is very likely that dogs also experience these two types of pain. This has been observed in affected dogs (Mayer et al, 2006).

Pain relief, other than the following treatment methods, include the use of NSAIDs, opioids, biphosphonates and drugs that treat neurogenic pain (Mayer et al, 2006). The survival time in dogs post-diagnosis who are only treated with analgesics is rarely longer than three months (Withrow et al, 2007).

One of the relatively new methods of pain relief in bone cancer are the biphosphonates. These drugs counteract one of the main mechanisms that cause pain in these patients - namely an excessive or irregulated osteoclast activity. A study determining the efficacy of one such drug - pamidronate - concluded that - because of reductions in pain index scores and increased in bone mass density (as a result of lower osteoclast activity) in the testgroup - use of single agent pamidronate administered intra-venously could prove a valuable addition to an analgesic protocol (Fan et al, 2007). However, when this drug was tested in a double-blind placebo-controlled study as an additional method of pain relief in a standardized treatment plan (radiotherapy and chemotherapy) it was found that while the drug is safe to use, it did not clearly improve pain alleviation (Fan et al, 2009). So while the mechanisms support its usage, the practical application is still very limited.

Pathological fracture repair

Pathological fractures may occur due to the altered and essentially weakened bone structure, though the incidence of these fractures is reportedly very low. The fractures are not frequently repaired, as in most cases of osteosarcoma the therapy of choice is amputation. There are however patients where amputation is not an option, and the existence of a pathological fracture could cause such significant loss of quality of life that euthanasia would be the option of choice (Boston et al, 2011). There are of course considerations to be made before deciding to repair a pathological fracture, such as location and formation of the fracture, degree of bone lysis and grade of metastasis. Fractures due to osteosarcoma are most commonly found in the metaphyseal area of the bone and, while it is not the optimal location for external fixation, it can still be attempted if the conformation of the fracture is repairable. Evaluating the amount of pulmonary metastasis is important, as the result might change the owner's decision. While no significant difference could be found in the survival time with

and without adjunctive therapy post-repair, it is still recommended as it may improve quality of life during that survival time (Boston et al, 2011).

Amputation

Amputation of the affected limb is regarded as the standard therapy of osteosarcoma, with or without adjunctive chemotherapy. There are a few contraindications for amputation, such as serious concurrent neurological or orthopedic disease, which causes the other limbs to be unable to support the added weight if one of the limbs is amputated. In dogs with tumors of the front limb, the whole limb in amputated, including the scapula. With hind limb amputation, it's either a disarticulation of the femoral joint, or the former with an added hemipelvectomy if it concerns a proximal femur tumor (Withrow et al, 2007).
This therapy removes the pain and lameness caused by the tumor, but - if there is no adjunctive treatment - does nothing for the already existing metastases. That is why, while the quality of life is excellent, the life expectancy post-surgery is fairly low (Fossum et al, 2007). Mean survival time with amputation alone is 119-175 days, with a 12-month and 24-month survival percentage of 11-21% and 0-4%, respectively. Adjunctive chemotherapy is recommended to treat metastatic disease, and can prolong the mean survival time to 262-540 days, with a 12-month survival percentage of 33-69%. It is highly variable due to the numerous chemotherapy protocols combined with the location of the primary tumor (Withrow et al, 2007).

Limb-sparing operation

In a limb-sparing operation, the tumor is completely removed and the remaining gap in the bone filled with either a graft (allo or auto) or a prothesis to keep the limb functional. This therapy can be used when amputation is not an option, either due to choices of the owners or because of concurrent neurological or orthopedic disease. There are certain criteria that have to be met. The tumor has to be clinically and radiographically confined to one bone, and must involve less than 50% of that bone. Advanced imaging techniques such as CT can be used to evaluate the spread of the tumor (Withrow et al, 2007). There are certain considerations to keep in mind. The existence of a pathological fracture may prove contra-indicatory for a limb-sparing operation, as the tumor may have spread to the surrounding soft tissue and cause a quick local recurrence of the tumor. During the operation, the joint nearest to the tumor will be constructed into an arthrodesis. Because of the limited motion in that joint, certain locations are ill-suited for limb-sparing operations, such as the scapulo-humeral, coxo-femoral and tarsal joint regions. Complications post-surgery are the local recurrence of the disease already stated earlier - which occurs in 21-28% of the cases - and local infection. Survival time with this treatment option is the same as with amputation (Endicott et al, 2003).

Radiation therapy

In dogs where amputation might not be feasible due to the extent or location of the tumor, or in cases where the owner does not want to select amputation, radiation therapy might prove a valuable treatment option as part of a palliative treatment plan. In cases of appendicular osteosarcoma, palliative radiation therapy can provide sufficiently reliable (50-93% response rate) analgesia for approximately 100 days (53-180 days) (Coomer et al, 2009). This decrease in pain results from direct killing of the tumor and inflammatory cells and by reducing the destruction of healthy bone by osteoclasts. Lytic bone lesions respond to irradiation with the formation of mature organized bone. The existence of pulmonary metastasis form no contraindication for radiation therapy, as long as there are no clinical signs (Mayer et al, 2006).

During the period of treatment, quality of life is improved and adverse clinical side effects are usually rare when using a large fraction time-dose fractionation protocol (8-10 Gy in each fraction, once weekly for 3-4 treatments) (Coomer et al, 2009; Weinstein et al, 2009). Acute skin-related local side effects include desquamation, erythrema and alopecia, which do not impair quality of life (Coomer et

al, 2009). There is a wide variation in the time of onset and the duration of improvement and care should be taken to adjust treatment protocols to fit the patient (Weinstein et al, 2009).

Chemotherapy

Chemotherapy can be added to any of the treatment choices already discussed, and can be used to treat the metastases already existing at the time of diagnosis. There are a few patient criteria: the patient must have a neutrophil count of >3000/ul, and platelets of >150.000/ul. Also, if a platinum-based chemodrug is used, urea and creatinine levels should be checked beforehand, as these drugs are nephrotoxic (Endicott et al, 2003).
There are a few drugs frequently used in protocols treating osteosarcoma:

Cis-platinum (Cisplatin)

Cisplatin is a platinum-based cytostatic drug, and has been drug of choice for many years before the toxicity and the emergence of suitable alternatives have made it much less desirable. It is, as all platinum-based cytostatic drugs, nephrotoxic, but the risk of toxicity in cisplatin is so severe that it requires aggressive diuresis pre- and post-administration. Other adverse effects including vomiting, which occurs most frequently after administration. Dogs with pre-existing renal disease should not be treated with this drug. As it is renally excreted, urine should be treated as chemotherapeutic waste for 24 to 48 hours after dosing. That dose is around 60 to 70 mg/m2. With this treatment, medial survival time is prolonged to 262 to 283 days (Endicott et al, 2003).

Carboplatin

Carboplatin is a platinum-based cytostatic drug, and a suitable replacement for cisplatin, which has been used in the past. They are both nephrotoxic, but carboplatin is decidedly less toxic, and can be used as a single agent or in a alternate drug protocol with doxorubicin. The recommended dose is 300 mg/m^2 every three weeks. (Endicott et al, 2003) The mean survival time is almost comparable to that when using cisplatin, around 307 days overall with a mean disease free interval of 256 days (Philips et al, 2009).

Doxorubicin

In a dose of 30 mg/m^2 per treatment every 3 weeks for a total of 5 treatments. The maximum dose will be 180-240 mg/m^2. Because this drug is cardiotoxic, patients should be screened for underlying cardiac disease before treatment starts. Care should also be taken when administrating this drug, as it causes extreme tissue damage and necrosis when injected extravasally. Treatment with doxorubicin post-surgically gives a mean survival time of 350 days, with a 12-month survival rate of 50% (Endicott et al, 2003).

Combination protocols

Combination protocols of carboplatin and doxorubicin do not result in longer mean survival times. Single agent use of carboplatin is equally effective, with less toxicity (Lane et al, 2012; Bacon et al, 2008). A study comparing the combination protocol of carboplatin and gemcitabine with single-agent use of carboplatin resulted in similar mean disease-free interval times (McMahon et al, 2011).

Euthanasia

Euthanasia is the inevitable end 'treatment' for dogs with osteosarcoma, either due to loss of quality of life because of untreatable pain and lameness, or because of metastatic disease. It is however also a possibility as soon as the diagnosis is made, should the owners choose it. It is the responsibility of the treating veterinarian to properly explain what the options are and what the expectations regarding each option are, to allow owners to make a well-informed choice.

Osteosarcoma and the Irish Wolfhound

Introduction

The Irish Wolfhound as a giant breed falls perfectly into the category at risk for developing osteosarcoma. In two separate studies the Irish Wolfhound had an increased risk of 27.5 compared relatively to all other breeds examined combined (Dorn et al, 2012) and 20.7 times the risk compared to the German Shepherd (Ru et al, 1998).

There is, however, evidence that the Irish Wolfhound might be more at risk than other giant breeds such as the Newfoundlander and Saint Bernard dog. In studies examining incidence rates of osteosarcoma of dogs in Sweden (multiple breeds) and Norway (four giant breeds) the Irish Wolfhound scored top of the lists with 99 cases and 126 cases, respectively, per 10.000 DYAR (Dog Years At Risk – each case contributed its life span to a total for the population per breed). It was followed closely by other mentioned giant breeds, but at a significant distance (Saint Bernard Dog 78 cases in Sweden, Leonberger 72 cases in Norway) (Egenvall et al, 2007; Anfinsen et al, 2011).

These data suggest that there might be an additional genetic risk factor for the Irish Wolfhound besides the shared traits with a genetic basis such as increased height and weight. Since there has been no official recording of the cases of osteosarcoma seen in Irish Wolfhounds at the University Clinic for Companion Animals in Utrecht, it is unknown whether the incidence in this population mirrors the ones reported before in published studies.

Study Objectives
The aim of this study is to collect information on the health status of a group of Irish Wolfhounds in the Netherlands, Germany and Belgium through contact with the owners and breeders of the dogs. This information will then be used to gain insight in the incidence and spread of osteosarcoma in this population. The pedigree information will be used to estimate heritability of osteosarcoma in the Irish Wolfhound, and to assess whether there are any significant results to warrant a follow-up association study, in which case the breeding values will be used to assign samples to designated test groups: case or control.

Materials and Methods

INCIDENCE

Study Group

The dogs in the study group consist of Irish Wolfhounds of whom a blood sample has been collected either during the heart screening, mandatory in this breed, by Dr. Andrea Vollmar or at various treatments and inspections at the University Clinic for Companion Animals in Utrecht. DNA samples extracted from these blood samples are stored at the University Clinic for Companion Animals in Utrecht. These samples gave us a test group of 532 dogs, belonging to a total of 101 different breeders. There was additional information available for the majority of the dogs, such as sex, sire and dam and pedigree number.

Data Collection

There have been two main tiers in the data collection process, namely contacting the registered owners for the dogs and - in cases where the owner details were outdated, or incorrect, or if the owners could not be contacted, or in cases where the breeder was the designated owner - the breeders of the dogs. This was done via telephone. After an introduction of the study, the owners were asked a few questions (see protocol – Appendix A), about date of death or current health status, the cause of death and the occurrence of osteosarcoma. In the positive cases, additional information was collected such as age of occurrence, location of the tumor, which diagnostic method was used and at which clinic.
The owners that were contacted were of dogs of whom the health status was unknown, or already reported positive for osteosarcoma. Owners of known and negative health status dogs (i.e. dogs that are documented as having died from another cause of death) were not contacted again.

Phenotypes

Since necessary additional diagnostics to accurately diagnose osteosarcoma were hardly ever utilized, the aim of the study had to be adapted to the more general 'bone tumor' instead of 'osteosarcoma'. Since, as stated before, 85% of primary bone tumors are in fact osteosarcoma, this adaptation can still give a fairly accurate picture of the occurrence of osteosarcoma, even if the actual differentiation has only been made in one of the cases.

Because of the very variable levels of diagnostic methods used, an additional classification has been applied. As stated before when the diagnostic approach for osteosarcoma was discussed, even with the radiographic abnormalities there are still other differential diagnoses, but it will be considered the minimum necessary level of diagnostics. The results using data from other levels of diagnostics are marked as such.

There were three levels of reliability of the data, depending on its source. It either came from the owner of the dog, from the breeder of the dog, or – if neither could be reached or had information – on the information provided previously to Dr. Vollmar. The latter only included information about date of death and cause of death, whether that be bone cancer or not. No additional information, such as the diagnostics performed, age of occurrence or location of the tumor was available for these dogs, and results using data from these dogs are marked as such.

HERITABILITY

Data

Two sets of data were used to create the data set for the estimation of heritability. The first was the group of dogs used to calculate the incidence of bone cancer (268 dogs). Added to this data set was a

information on a group of dogs who had been seen by Dr. Vollmar, (266 additional dogs) but of whom there were no blood samples available, and besides a positive or negative status on bone cancer, their sex, their pedigree, date of birth and date of death, no additional information was known – which is why they were not included in the first set.

This data set was then evaluated based on their pedigree – since pedigree information needed to be available for the creation of a pedigree file, 119 dogs were excluded for their lack of the aforementioned information.

The final group consisted of 416 dogs. A datafile was created using each dogs pedigree number, age at date of death (in months), sex, pedigree numbers for his ancestors (sire, dam, sire's father and sire's mother, dam's father and dam's mother) and their bone cancer status. The pedigree information was then utilized to create a pedigree file from which a relation matrix could be calculated.

Statistical Analysis

The heritability of the occurrence of bone cancer was calculated using the ASReml statistical program (Gilmour et al, 2009), using an animal model to calculate both the environmental and genetic variance to estimate heritability. The data was evaluated using both a logistic and a linear model:

$$OS = \mu + sex + animal + e$$

where OS is the positive or negative bone cancer status and μ the general mean. Sex (male of female) was a fixed effect. A Wald F-statistic was used to test fixed effects. P≤ 0.05 was considered significant. The fixed effect of age at time of death (age in months, then divided by 12) was not significant and excluded from the model. Animal and *e* were random effects, with σ_e^2 fixed at $\pi^2/3$ for the logistic model. Random effects were considered to be normally distributed:

$$animal \sim N(0, A\sigma_a^2)$$

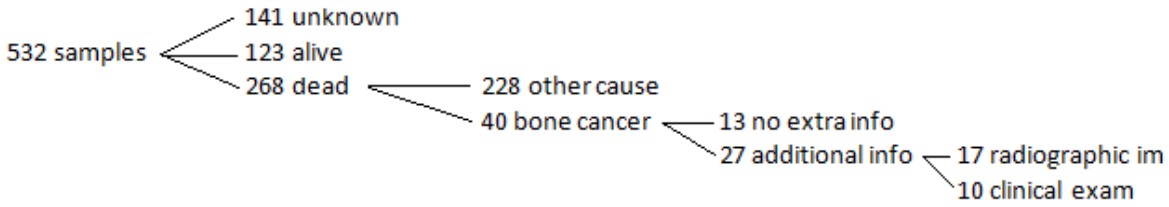
where A has the genetic relationships between the dogs. The relationship matrix was constructed using the pedigree information of 866 Irish Wolfhounds over 9 generations (with a minimum of 2 generations).

Heritability is the part of phenotypic variance that is caused by genetic variance between individuals. Variance components were estimated using the model given above. The heritability (h^2) is estimated using the following equation: $h^2 = \sigma_a^2 / \sigma_p^2$. In this equation, σ_a^2 is the additive genetic variation and σ_p^2 is the phenotypic variation, which can be broken down into $\sigma_a^2 + \sigma_e^2$. In these equations, σ_a^2 is the animal variance and σ_e^2 represents the residual variance.

Results

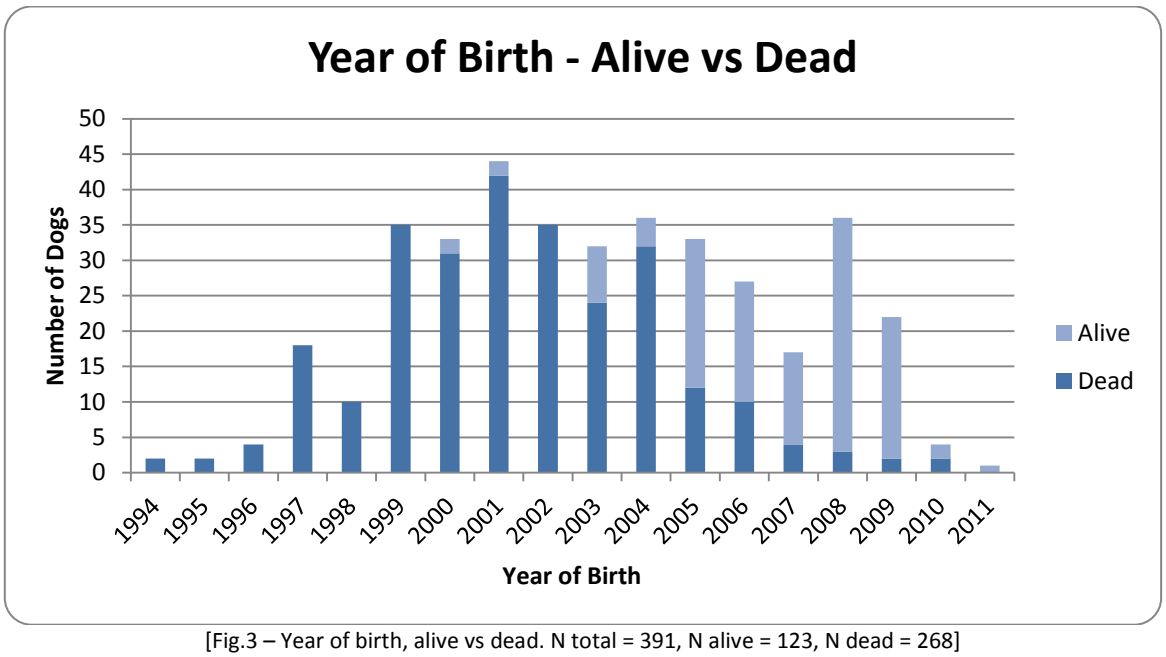
INCIDENCE

Of the total of 532 dogs whose owners were contacted, 123 dogs were still alive (23.1%), 268 dogs had passed away (50.4%), and 141 dogs remained of unknown status (26.5%). The dogs that were still alive were dismissed from the study, as they did not fall into either of the two deciding categories (o - positive for bone cancer, or x - other cause of death) yet. The remaining 268 dogs consisted of 40 dogs positive for bone cancer, though this amount dropped to only 17 dogs when the diagnostic criteria had to be met (at the very least, radiographic imaging should have been used to diagnose a bone tumor). This leaves 228 dogs who suffered another cause of death, and did not suffer from bone cancer in their lifetime.(Fig.2)



[Fig. 2 – Result tree]

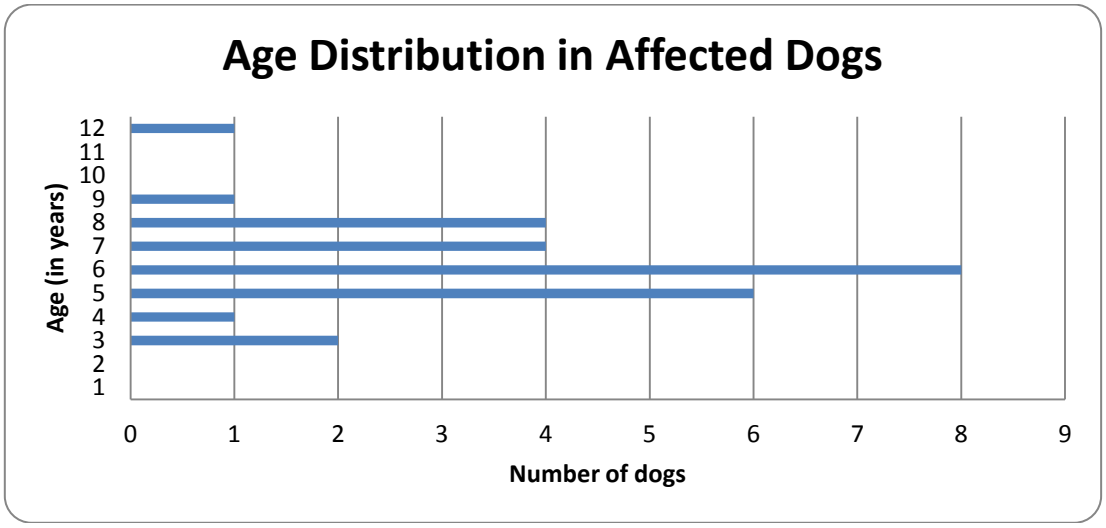
In the figure below (Fig. 3) the distribution between the dogs that are still alive and the dogs that have passed away compared to their year of birth is shown.



[Fig.3 – Year of birth, alive vs dead. N total = 391, N alive = 123, N dead = 268]

Osteosarcoma

Of the 40 dogs diagnosed with bone cancer, of only 27 of those dogs there is additional information (such as age at time of tumor occurrence, location of the tumor and the diagnostic approach) available. The sex distribution is uneven, with 7 males and 20 females. The average age of the dog at the time of occurrence of the tumor is 6.35 years, with the majority of the dogs in the age range between 5 - 7 years of age (18 dogs)(Fig.4).



[Fig.4 – Age distribution in affected dogs at time of tumor occurrence (N = 27 dogs with bone cancer)]

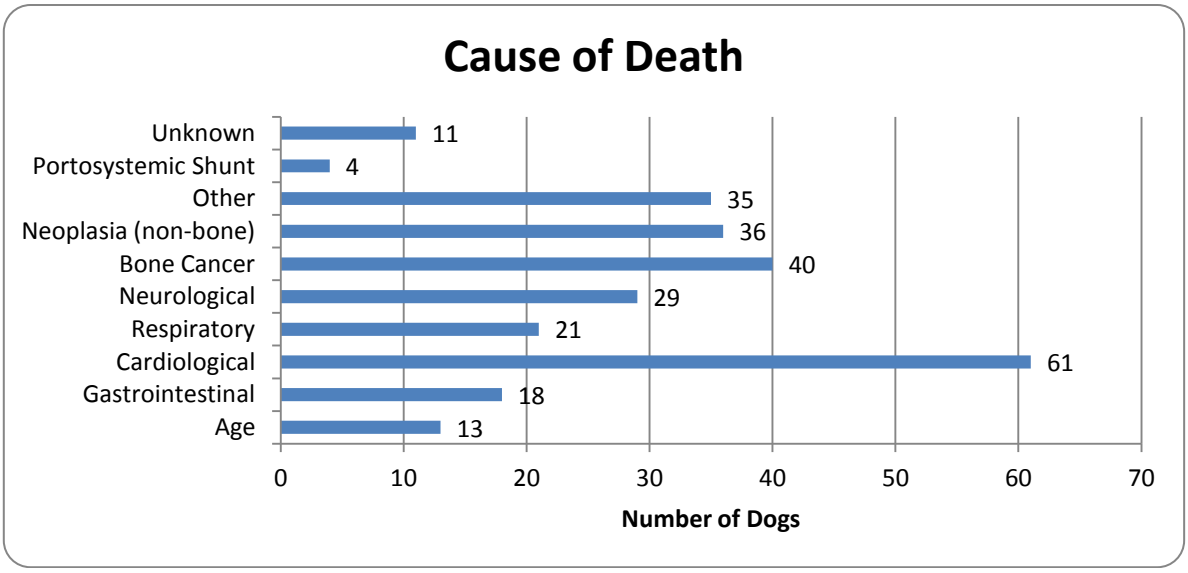
The majority of the tumors were located on the front leg (19 front leg vs. 5 hind leg) and most of them occurred in the carpal region (14 tumors). Tumor locations were distributed as follows: front leg (2 cases), shoulder (2 cases), humerus (1 case), carpus (14 cases), tibia/knee (2 cases), tarsus (3 cases), digit (1 case) and unknown (1 case).

The level of diagnostics performed in each cases differ greatly, from clinical evaluation (5 cases), radiographic imaging (16 cases) to CT followed by biopsy (1 case). In 5 of the cases the method of diagnostics was unknown.

Cause of death

In the group of dogs that died from another cause than bone cancer - and had never encountered it in their lifetime - the sex distribution is surprisingly even - 113 males and 113 females (2 unknown). Their ages range from 1 month of age to almost 12 years of age.

The distribution of other causes of death is detailed in the graph below (Fig.5). For comparison, the number of dogs that died from bone cancer have also been added.



[Fig.5 – Causes of death (N=268)]

Incidence

Because of the very different levels of reliability in the data collected, the incidence has been calculated multiple times using different sets of data, to see whether there were any significant results.

The incidence calculated using every positive bone cancer case, regardless of what the source was or if the diagnostic requirements were met, resulted in an incidence of 14.93% (40 dogs of a total of 268 dogs). If, since we are not holding this group to any requirements, add in the extra group of dogs collected because of the heritability estimation, it results in an incidence of 16.54% (132 dogs of a total of 798 – 532 dogs with blood samples, 266 dogs without). If we look only at the dogs for which additional information was available, the incidence drops to 10.07% (27 dogs of a total of 268 dogs). If the diagnostic requirements (i.e. radiographic imaging) had to be met, the incidence lowered again to 6.34% (17 dogs of a total of 268 dogs).

HERITABILITY

The estimated heritability was 0.15 (± 0.09) on the liability scale (the logistic model), and 0.33 (± 0.12) on the observed scale (the linear model). The heritability calculated on the liability scale did not differ significantly from zero, the heritability on the observed scale did ($P \leq 0.05$). Age was found not to be a source of variation in both models, and as such was eliminated from the model. Sex did significantly affect the outcome in both models, with female dogs having 2.33 times more risk of encountering bone cancer in their life time than their male counterparts ($P \leq 0.05$).

Discussion

Incidence

If we look at the most reliable calculation of the incidence – considering only the small group of dogs where the diagnostic requirements have been met – it is only 6.34%. If all the requirements and extra information is ignored, the largest incidence calculated is 16.54%. Since there has been no earlier record of an incidence (in percentages) of bone cancer in the Irish Wolfhound population, it is difficult to compare this result to any reliable source. As stated previously, in two earlier studies the increased risk of the Irish Wolfhound has been determined (Dorn et al, 2012; Ru et al, 1998), but these results are difficult to compare with a calculated percentage. Given these risks, however, it was expected that the incidence rate would be higher.

A reason for the lower than expected incidence could be a case of censored data. With the Irish Wolfhound being susceptible to a variety of diseases (such as dilated cardiomyopathy, gastric dilation and volvolus, porto-systemic shunting, etc.) it is not uncommon for dogs to die young – young enough that they did not yet reach the age at which bone cancer commonly occurs (±6.35 yrs). Looking at the data collected, the youngest dog to acquire bone cancer was 3 years old. Dogs that died before 3 years of age of another cause accumulate to only 12 dogs, but if we assume the majority range (5-7 years of age) that would give us an additional 36 dogs that might have developed bone cancer if they had lived longer and had not suffered another illness.

Then there is the case of the large group of right-censored data, which, while not affecting the incidence directly since they were not counted (only the total of dogs deceased were used), they might still develop bone cancer in the future. Of the 532 dogs, 123 dogs are still alive at this point in time, with the oldest being 12 years old, the youngest only a year old. While 37 of the dogs are past the age at which bone cancer usually occurs, for now they cannot be appointed to either evaluated group.

The aspects considered above are only of any concern assuming that the result calculated in this study is correct. The data collected was very variable with very little diagnostics performed, which could affect the reliability. Owners often needed time and effort to recollect the appropriate answers to the questions put before them, as a lot of the dogs in this study died at least a couple of years ago and without knowing that the circumstances surrounding their dog's death could one day be important for research, the owners could hardly be blamed for being forgetful. The contact information available for the dogs was also extremely limited (for some dogs only a name and phone number) and oftentimes no longer correct, and while every effort was made to find the owners, this still resulted in a fairly large group of dogs of unknown status. The information provided by the breeders of the dogs was also very variable. Some breeders are more involved in the lives of their dogs after they leave the litter than others. This means that while multiple breeders gave information on the dogs known to them, the level of accuracy of this information is hard to evaluate.

Heritability

The estimated heritability is significant on the observed scale is 0.33 (±0.12) However, given the binary nature of the trait, fitting a logistic model is more appropriate. This model showed an estimated h^2 of 0.15 (±0.09), which did not differ significantly from zero.

Using this data set the liability for bone cancer is a moderately heritable trait. Since there have been no previously published heritability results for bone cancer in the Irish Wolfhound, there are no means of comparing this result.

However, the same objections put forward in the discussion of the incidence calculation apply to this estimation. The data used came from a variety of sources and for this estimation even a separate group of dogs was included of whom no other information was known other than that it had been reported to the sampler that they had died of bone cancer or some other cause. Since there is no way of knowing whether or not this diagnosis was correct, it could be that the status assigned to each dog was not in all cases the right one.

Sex predisposition

The results showed an increased risk of 2.33 for females of developing osteosarcoma in their life. This result does not correspond with what has been published earlier, but then again those publications differ wildly. Some report similar findings to this study in the Scottish Deerhound with the risk for females 1.6 times higher than males (Phillips et al, 2007), some studies looking at other breeds report the exact opposite, with females having a 29% lower risk than males (Egenvall et al, 2007) and male incidence laying slightly higher (Ru et al, 1998). A study evaluating the lifespan and disease predisposition of the Irish Wolfhound found a higher incidence in males, but only in males that were castrated (Urfer et al, 2007). When comparing the disease in both humans and dogs, one study found a ratio of 1.5:1 for males:females in dogs (though it also reported that this finding is hardly consistent across publications), and in humans there are also more male sufferers (Martano et al, 2011). Finally, there are also studies that report no significant differences between the genders at all in dogs (Anfinsen et al, 2011; Rosenberger et al, 2007).

In the similar study conducted in the Scottish Deerhound population with the same result, it was suggested that a cause of this finding could be a relatively earlier death in males than females due to other causes, such as heart disease, which in that breed mainly affects young males. Those males might have developed osteosarcoma, had they lived to the appropriate age (Phillips et al, 2007). In the Irish Wolfhound, dilative cardiomyopathy is a frequently occurring disease, with a incidence rate of 20%. The estimated age of onset is 4.52 years of age (±2 yrs). Female dogs are less likely to develop the disease, and if they do it is at an older age (Vollmar, 2000). With 61 dogs evaluated in this study dying of heart disease and 35 of those dogs males, this could explain some of the difference.

Another theory is that the sampling group was already uneven in the gender distribution, but in this study the group considered in this part consists of 224 females and 192 males, which is reasonably evenly distributed.

Conclusion

In this study, bone cancer had a lower than expected incidence of 6.34% in the Irish Wolfhound. The estimated heritability in this study was 0.15 (± 0.09) on the liability scale, and 0.33 (± 0.12) on the observed scale. The latter result differed significantly from zero. This indicates that an association study using DNA markers might be fruitful.

If such a study would be repeated, it would be recommended to decide on the test group early on, and to keep the owners of the dogs informed about the existence of such a study and encouraged to contact the research team should bone cancer occur or if the dog dies of another cause. In cases where bone cancer occurs, the accurate level of diagnostics can then be recommended and – should they not wish to follow any treatment – perhaps even a postmortem examination can take place. There is, as stated before, a fairly large group of dogs alive at this time and those owners have recently been contacted. Those dogs could form the beginning of a second group, should there be a desire to test the results found in this study.

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APPENDIX A – Protocol Owner Contact (by telephone) – In Dutch

Goedemorgen/Goedemiddag, spreek ik met (naam eigenaar)?

U spreekt met Lauren de Boer, coassistent van de Universiteitskliniek voor Gezelschapsdieren in Utrecht, bel ik gelegen?

Ik bel over (naam hond), uw Ierse Wolfshond. Er is van (naam hond) een aantal jaar geleden bloed afgenomen voor DNA onderzoek naar verscheidene aandoeningen in het ras. Op dit moment is er een onderzoek gaande bij de Universiteitskliniek naar botkanker bij Ierse Wolfshonden, omdat deze aandoening in dit ras ongebruikelijk vaak voorkomt. We willen graag onderzoeken hoe vaak de aandoening precies voorkomt in de Nederlandse populatie. Daarna hopen we genoeg lijders te hebben geïdentificeerd om DNA onderzoek te kunnen doen, om hopelijk het gen vast te kunnen stellen die bij de Ierse Wolfshond botkanker veroorzaakt.

In het kader van dit onderzoek zou ik u graag wat vragen stellen over de gezondheid van (naam hond).

Indien onbekende status: (de vragen kunnen per individueel dier verschillen)
- Is (naam hond) nog in leven?
    - In leven
        - Hoe is het met de gezondheid van (naam hond)?
        - Eventuele aanvullingen van overige informatie (wisselend per geval)
    - Overleden
        - Wanneer is (naam hond) gestorven?
        - Heeft hij/zij botkanker gekregen?
            - Op welke leeftijd?
            - Welke diagnostiek?
            - Bij welke dierenarts?
            - Wie was de fokker van de hond?
        - Eventuele aanvullingen van overige informatie (wisselend per geval)

Indien al bekend met osteosarcoom: (de vragen kunnen per individueel dier verschillen)
- Is (naam hond) nog in leven?
    - In leven
        - Hoe is het met de gezondheid van (naam hond)?
        - Eventuele aanvullingen van overige informatie (wisselend per geval)
    - Overleden
        - Wanneer is (naam hond) gestorven?
        - Eventuele aanvullingen van overige informatie (wisselend per geval)
    - Osteosarcoom
        - Op welke leeftijd?
        - Welke diagnostiek?
        - Bij welke dierenarts?
        - Wie was de fokker van de hond? (Indien onbekend)