Genealogy research of the Groenendael and the Tervueren Belgian Shepherd diagnosed with gastric carcinoma.

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Summary

OBJECTIVE: *The incidence of gastric carcinomas is remarkably higher in Belgian Shepherds than in any other breed of dogs. Results from earlier scientific research suggested a genetic predisposition. The objective of this study is to perform a pedigree analysis of a small cohort of Belgian Shepherds diagnosed with gastric carcinoma and to obtain a database of clinical signs from the known affected animals.*

RESULTS: *The overall pedigree contains a total of 385 dogs: 85 Groenendael (11 affected), 195 Tervueren (33 affected) and 105 unspecified Belgian Shepherds. In addition to this, 222 litter mates were included as well. A questionnaire held among the owners of the affected dogs showed that the patients suffered from a range of clinical symptoms, including vomiting of mucus, bile, food and blood, decreased appetite or anorexia, lethargy, and weight loss.*

CONCLUSION: *The presence of a large number of dogs with a single connection within the pedigree possibly suggests some genetic diversity which may be needed to decrease the incidence of the disease. The focus of further research is to unravel the genetic background. If such a genetic marker can be found, this would enable breeders to exclude carrier dogs from breeding in order to decrease the incidence of gastric carcinoma in future generations.*

Introduction

In companion animals, tumours of the gastrointestinal tract are relatively rare: according to scientific literature they comprise less than 1 percent of all the neoplasia occurring in cats and dogs[1-5]. In dogs, the types of gastric tumours that are found most frequently are adenocarcinomas, malignant lymphomas, leiomyo(sarco)mas and fibro-sarcomas[6-8]. Occasionally, polyps occur in the stomach as well. Benign gastric tumours are found more often in dogs than in cats; however, most gastric neoplasia are malignant in nature. With the exception of malignant lymphoma, which may also occur in young dogs, gastric carcinomas occur mostly in older dogs. They usually develop when dogs are at average between six and ten years of age[1]. Males appear to be predisposed, being affected 2,5 times more often than females[8].

In the dog, adenocarcinomas are the main occurring type of gastric tumour, accounting for 42 to 80 percent of all cases described in scientific literature[5]. Adenocarcinomas are usually located at the minor curvature or the pyloric antrum of the stomach[5]. The tumours have an aggressive, infiltrative growth pattern and they often metastasise, especially to regional lymph nodes, the liver, and the lungs[1, 8]. Clinical symptoms, of which progressive vomiting, anorexia, weight loss and haematemesis are the mainly occurring examples[8], often only become evident at an advanced stage of development and up to 95 percent of the tumours has metastasised at the time of diagnosis[1].

Gastric tumours are preferably diagnosed by means of endoscopy, during which multiple biopsies are obtained. Almost all gastric tumours and other mucosal diseases can be determined in this way, but histology is

necessary to confirm the diagnosis. The presence of inflammation, ulcerations, and necrosis may complicate histological examination (Figure 1)[1,4,8]. Momentarily, surgical removal of the tumour is the only possibly remedial treatment available[4]. Chemotherapy appears to be ineffective, and radiation therapy is not well tolerated by the adjacent organs[4]. As clinical symptoms manifest at an advanced stage, surgical therapy becomes extremely difficult or even impossible. In addition to this, excision is often complicated by the fact that most tumours are located at the minor curvature of the stomach. At this point, the main blood supply enters the stomach and (partial) removal of this part often results in a strong reduction of gastric motility. Therefore, the prognosis of gastric tumours is generally fatal[9, 10].

American veterinary databases show that the incidence of gastric carcinoma in the dog is 0.1 percent[11]. Certain breeds appear to be predisposed to developing gastric tumours, the Belgian Shepherd (Tervueren, Groenendael and Malinois) being one of them[2-4,11]. Other predisposed breeds are the long haired Scottish Shepherd, Staffordshires, Chow Chows, and Shar Peis[4,11]. Previous American and Italian and recent Norwegian research suggests the influence of a hereditary component in the development of gastric tumours in Belgian Shepherds[5,11,12]. A retrospective study in The Netherlands showed an incidence of 1.18% and a heritability of 0.09 among Dutch Tervueren Shepherds[13]. This incidence is remarkably higher than the American average of 0.1 percent. Subsequent to this study, the aim of this research is to perform a pedigree analysis of a small cohort of Belgian Shepherds previously diagnosed with gastric carcinoma.

Materials and methods

Participating patients and control dogs

The study started in 2000 and is still ongoing. All dogs were examined by the primary study observer (PJJM), who was responsible for recruiting and enrolling all participants and their respective dogs. The dogs that suffered from clinical signs of gastrointestinal disease were referred to the Veterinary Specialist Centre *De Wagenrenk* to be assessed. The examination included a routine physical and a gastroduodenal workup during which survey abdominal radiography and ultrasonography and an upper gastrointestinal endoscopy were performed. The workup procedure is described in detail in an earlier article from the primary study observer[14]. From all dogs that were examined, biopsies were taken for histological examination and EDTA blood samples for DNA-extraction. The owners were requested to provided their dog's pedigree and to submit a questionnaire. The questionnaire concerned questions about the dog's general information (name, breed, gender, neutered/spayed yes or no, age, etc.), living environment, food, medical history (reproduction included for female dogs), and clinical signs.

Figure 1 *- Stomach from a dog with adenocarcinoma. A large ulcer and thickening of the wall is visible at the gastric antrum (black arrows)*[8]*.*

Pedigree analysis

The Dutch cynology organisation *Raad van Beheer* provided data of all the Tervueren and Groenendael litters born between 1996 and 2011 and registered by the organisation. The human genealogy computer programme *Family Tree* was used to digitalise all the pedigrees and then an overall pedigree was drawn by hand to provide an overview of the breed's interrelations.

Data handling and statistics

Statistical analyses were performed using IBM SPSS Statistics 22.0 and descriptive statistics were used to report baseline data (average ± standard deviation and range, or mode).

Results

Patients' questionnaire - descriptive statistics

Age at death

Sixty-nine Belgian Shepherds were diagnosed with a gastric carcinoma. Of 54 dogs, the exact age at death was registered. The average age at death was 9.11 years old, with a standard deviation of 2.18 and a range from 5.23 to 14.06 years of age (Figure 2).

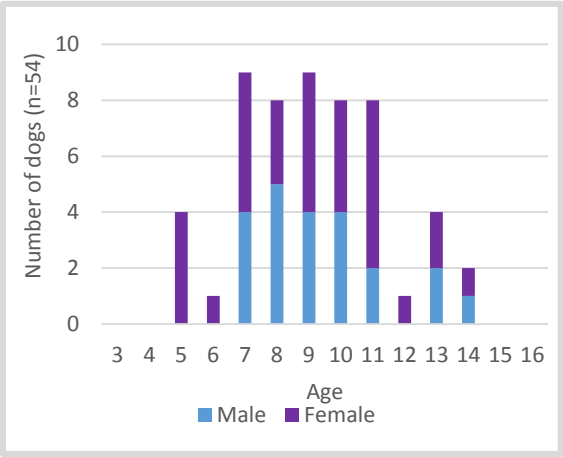

Figure 2 *- Average age at death of male and female Belgian Shepherds (n=54).*

Ratio male/female

The 69 dogs comprised 28 male dogs (40.6%) and 41 female dogs (59.4%). Based on the data provided by the Dutch cynology organisation *Raad van Beheer*, the male/female ratio of Tervueren and Groenendael Shepherds in The Netherlands is 1:1. There is no significant difference between this ratio and the ratio of the study cohort ($P < 0.05$).

Castration and sterilisation

Twenty-nine dogs (42.0%) were neutered or spayed.

Completed questionnaires

Fifty-three out of the 69 owners returned a completed questionnaire.

Age at death

Of this cohort, the average age at death was 9.18 years old, with a standard deviation of 2.20 and a range from 5.23 to 14.06 years of age (N=50).

Ratio male/female

There were 20 male dogs (37.7%) in this cohort, and 33 female dogs (62.3%). This male/female ratio was also not significantly different.

Castration and sterilisation

Twenty-four dogs (45.3%) were neutered or spayed. Five of the 33 females (15.2%) had been pregnant with an average of 2.4 litters with a standard deviation of 1.1 and a range from 1 to 4 litters.

The following information was observed from the completed questionnaires:

Food

Most dogs were fed with (combinations of) different kinds of food: 46 dogs received a commercial dry food (68.7%), 15 dogs received fresh meat (22.4%), 5 dogs received wet food (7.5%), and 1 dog took pot luck.
The dogs (N=52) were fed between 1 to 4 times a day, with a mode of 2 meals per day (32 dogs = 61.5%).

Environmental settings

Thirty-three dogs lived in a rural environment or a small village (68,8%) and 14 dogs in a city (29,2%). Forty-five dogs lived inside the house (93.8%), 1 dog lived outside in a kennel, and 1 dog lived both inside the house as well as in a kennel outside.

Twenty-eight households were non-smoking households (52.8%). In 17 households (32.1%), people smoked inside the house daily. In the remaining five households (9.4%), smoking occurred at an average of once a week and in one household, smoking occurred once a month. According to national statistics, 23.3% of the Dutch population smokes. If the 23 households in which smoking occurs are put together, there is a significant difference of 0.0004 with $P < 0.05$. If only the households in which smoking occurs daily are selected, a significant difference of 0.018 remains.

Clinical signs

The patients suffered from a range of clinical symptoms: 28 dogs vomited mucus (53.8%), 40 dogs vomited bile (76.9%), 41 dogs vomited

(remnants of) food (78.8%), 32 dogs had decreased appetite (61.5%), 20 dogs suffered of anorexia (38.5%), and 8 dogs had diarrhoea (15.4%). Other clinical symptoms that were mentioned by 21 of the owners were: haematemesis, nausea, apathy, lethargy, excessive sleeping, expression of pain, having difficulty laying down, panting, fever, and foetor ex ore. Thirty-nine dogs lost weight during their ailment (75.0%). The weight loss was at average 4.76 kilograms with a standard deviation of 2.37 kilograms and a range from one to eleven kilograms (Figure 3).

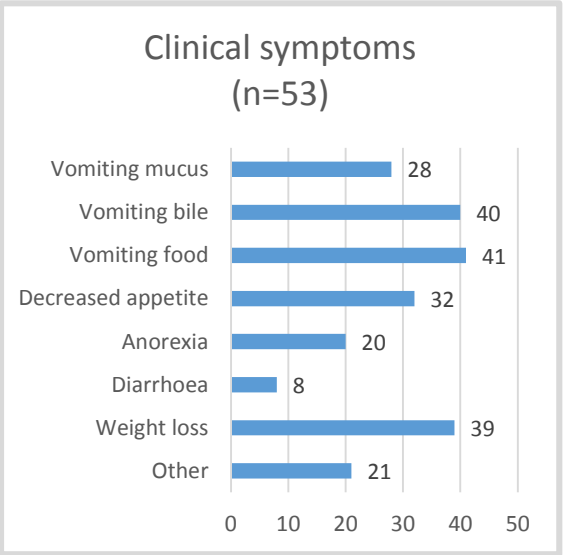

Figure 3 *- Clinical symptoms in 53 dogs diagnosed with gastric carcinoma.*

Pedigree analysis

The overall pedigree contains the combined pedigrees of 44 dogs diagnosed with gastric carcinoma. Out of the 44 affected dogs, 11 dogs belonged to the Groenendael breed and 33 dogs belonged to the Tervueren breed. Besides the affected dogs, there is a total of 341 dogs present in the pedigree: 74 Groenendael, 162 Tervueren, and 25 Belgian Shepherds with more than one connection of which the breed variety was unknown. The remaining 80 dogs had merely one single connection and, in this overall pedigree, their breed variety is not further specified.

In addition to this, a total of 222 litter mates is included in the pedigree. Of one affected dog the number of litter mates was unknown. Two litters contained more than one affected dog.

Most of the dogs had an average of two to four connections. One male dog had the highest number of interrelations, which was eight (Figure 4).

Discussion

This study assessed the interrelations of Belgian Shepherds diagnosed with gastric carcinoma. Because the collection of affected dogs and control dogs is still ongoing, the analysis of the questionnaires and the overall pedigree is only a representation of a certain moment within the time span during which the research is carried out. The overall pedigree contains the combined pedigrees of 44 affected dogs. Fifty-three out of 68 owners submitted a questionnaire. This means that the results from the questionnaire are limited by the amount of available questionnaires. The results may turn out differently if more completed questionnaires will be returned in the future.

The average age of the dogs was 9.11 ± 2.18 years old with a range of 5.23 to 14.06 years of age. This is in accordance with the average age determined in previous studies, which was about 9.5 years old[3, 4, 13].

Earlier studies showed that the incidence in male dogs was higher than in female dogs[3, 4, 13]. In this study, females were actually more affected than males with a male to female ratio of 1 : 1.19. However, the difference was found not to be significant.

The type of food and number of meals per day appear to be of no significant influence on the development of gastric carcinoma. In order to be able to perform statistical analyses, additional information from healthy control dogs is essential.

The number of households in which smoking occurred did differ significantly from the national figure of 23.3%. The large difference between the two groups that were tested (all the households in which smoking occurred grouped together or only the households in which smoking occurred daily) may suggest

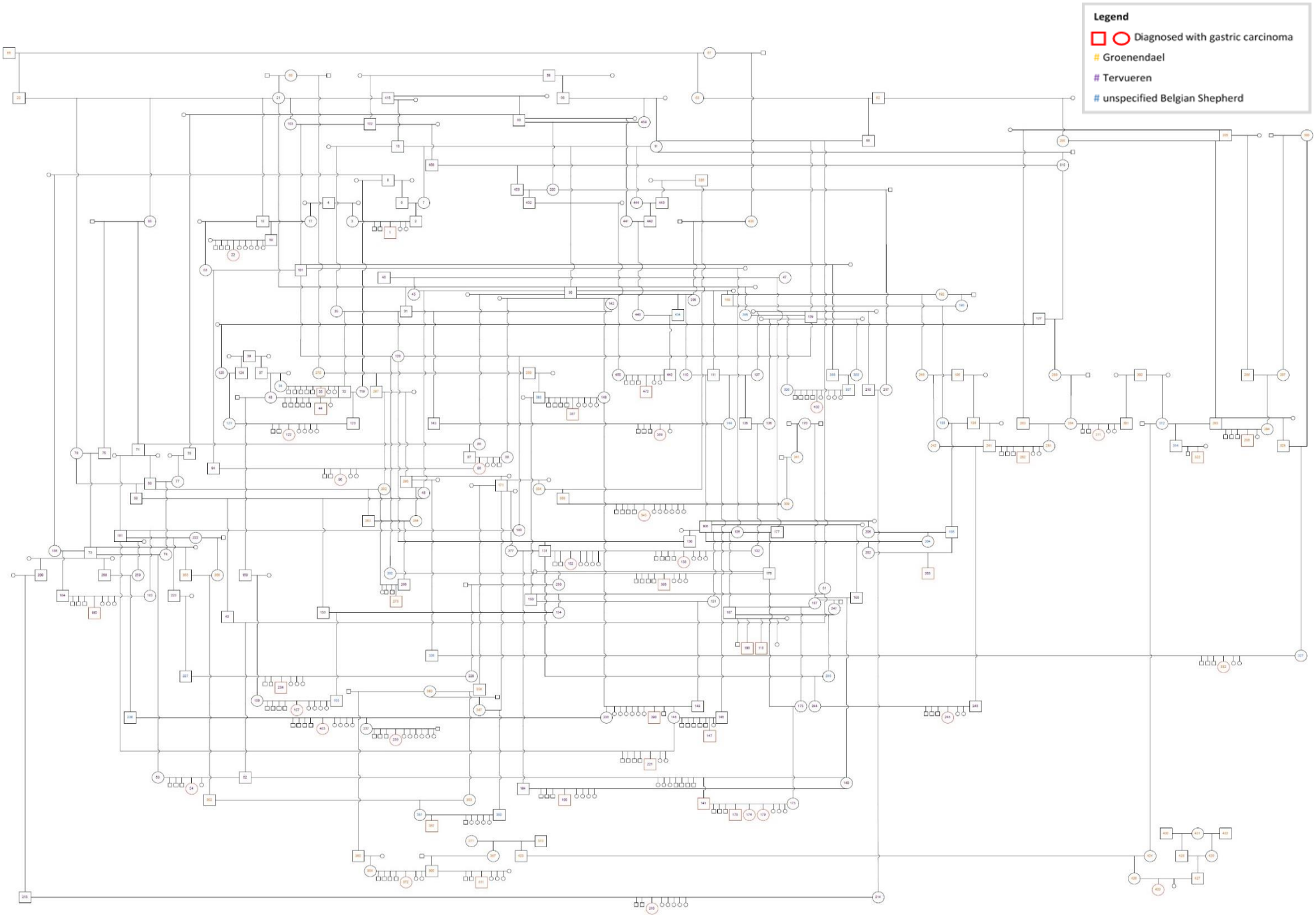

Figure 4 *- Overall pedigree of 11 Groenendael and 33 Tervueren Belgian Shepherds diagnosed with gastric carcinoma.*

that the total size of the groups is too small to provide reliable information. Moreover, the national statistics are based on information about smoking per individual. In the questionnaire, the information provided is on smoking per household. Therefore it may be argued that the two are not to be compared. The significant difference is not easily explained. It may be the case that dog owners smoke more often than people who do not own dogs, or maybe smoking could be an important environmental influence in the development of the disease.

The clinical signs present in the examined cohort are comparable with earlier reported research. Only haematemesis, previously mentioned as one of the most frequent symptoms of gastric carcinoma, was present in merely four of the 48 dogs. This may be due to the fact that haematemesis was not separately available on the list of symptoms in the questionnaire. The four owners that reported vomiting of blood, added the symptom to the additional category "other symptoms".

The overall pedigree is based on the pedigrees provided by the owners of the affected dogs. It may be the case that the dogs are actually more interrelated than the pedigree shows at the moment, due to links that are present but have not become apparent through the available pedigrees. In the overall pedigree, there were two litters of dogs that showed more than one affected dog within the same litter. It may well be the case that there are more litters with multiple affected dogs, but this remains, as of yet, unknown. To provide more insight in the hereditary characteristics of gastric carcinoma it would be worth following up on all the litter mates of the affected dogs to see if there was more than one dog suffering from gastric problems within the litter.

The use of a limited number of stud dogs for breeding may have influenced the incidence of gastric carcinoma in the breed. The presence of a large number of dogs with a single connection within the pedigree possibly suggests a genetic diversity which may be needed to decrease the incidence of the disease.

Conclusion

Scientific research repeatedly suggests that Tervueren and Groendendael Shepherds are genetically predisposed breeds to develop gastric carcinomas. Because the disease develops at a later age, it is likely that dogs may have been used for breeding before clinical symptoms become manifest. Further research is needed in order to identify a causal genetic marker. If such a genetic marker can be found, this would enable breeders to exclude carrier dogs from breeding in order to decrease the incidence of gastric carcinoma in future generations.

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