The global distribution and epidemiological features of

Canine Transmissible Venereal Tumor

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1 Summary/Abstract

Canine Transmissible Venereal Tumour (CTVT) is a unique type of cancer which has been transmitted between unrelated hosts as a clone, by physical transfer of cancer cells during coitus, for thousands of years. Despite many studies investigating its immunology and molecular genetics, the global prevalence and epidemiologic features of CTVT are still debated. In this study I have carried out a worldwide survey by means of a questionnaire in order to characterise CTVT worldwide distribution, epidemiology and pathogenesis. 515 responses to the questionnaire provided data on the prevalence of CTVT in 106 countries. A clear correlation was identified between CTVT prevalence and the presence of free roaming dogs as well as a weak negative correlation between CTVT prevalence and Gross Domestic Product and distance from the equator. Responses indicated that there is no gender bias for CTVT infection or any evidence for frequent concurrent disease in CTVT-affected dogs. Vincristine treatment was confirmed as the most successful therapy; however, resistant cases were also reported. Furthermore, cases of spontaneous regression were recorded in various countries, providing the first clear account of this phenomenon in naturally occurring CTVT cases. The results of the study are an important step towards informing more people about CTVT’s existence and may help veterinarians and epidemiology workers to develop future strategies minimizing CTVT spread.

Keywords: cancer, transmissible, canine, veterinary, epidemiology

2 Introduction

Canine Transmissible Venereal Tumour (CTVT, Figure 1), also known as Sticker’s sarcoma, Infective Venereal Tumour or Canine Transmissible Venereal Sarcoma (CTVS), is an infectious disease affecting dogs (Stimmelmayr, 2010). It is one of only two known naturally occurring transmissible cancers, along with the Devil Facial Tumour Disease (DFTD) (Pearse
and Swift, 2006; reviewed by Murchison, 2009). The process of development of a cancerous cell from a normal cell occurs when cancer cells acquire advantageous somatic mutations which promote the survival and, later on, dissemination of the best adapted lineage (Nowell, 1976), which ultimately often leads to the death of the host, as well as the death of the cancer clone itself. However, transmissible cancers are unique in acquiring the additional property of being able to transfer between hosts allowing them to live long after the death of the individual they have arisen from (Mukaratirwa and Gruys, 2004; Murchison, 2009).

The transmissible nature of CTVT was first documented in 1876 by the Russian scientist Nowinsky (Nowinsky, 1876), who performed serial passage of CTVT cells by transplantation between unrelated puppies of different breeds. This was further confirmed by extensive transplantation studies performed by Anton Sticker (Sticker, 1906). Further experimental transplantation studies (Stubbs and Furth, 1934; Karlson and Mann, 1952; Kudo, 1974) indicated that the living tumour cells are the true infectious agents and can therefore be thought of as a ‘new parasitic dog species’ on its own (Frank, 2007). Cytogenetic studies of tumours from all over the world confirmed a chromosome number of 57-59, compared to constitutive number of 78 in dogs, highlighting the common origin as well as the remarkable stability of CTVT despite its aneuploidy (Murchison, 2009). Clonal transmission of CTVT has further been confirmed by molecular studies, which found that the pattern of microsatellite and major histocompatibility complex (MHC) polymorphisms is highly conserved in globally distributed set of CTVT tumours, indicating a monophyletic origin (Murgia et al, 2006; Rebbeck et al, 2009).

CTVT affects a wide variety of genetically distinct dog breeds and is able to overcome the immunological barrier to transplantation imposed by the MHC. The ways by which it is able to escape immune rejection and allow tumour progression are thought to be by down regulating MHC, suppressing NK cells, killing B cells and preventing the maturation of dendritic cells (reviewed by Murchison, 2009).
CTVT is primarily a sexually transmitted disease (Smith and Washbourn, 1898; Karlson and Mann, 1952; Cohen, 1985), facilitated by the long-lasting ‘mating tie’ during the coitus of dogs, during which the muscles of the vagina lock firmly around the engorged penis and the mates cannot separate. The nature of mating can cause injuries to the delicate vaginal and penile mucosa, which enables efficient transplantation of the tumour cells between individuals (Feldman, 1929; Rust, 1949; Cohen, 1985). However, other routes of transmission have also been described, including licking, sniffing, scratching and biting of affected areas (Feldman, 1929; Higgins, 1966; Brown et al, 1980). These various modes of transmission probably explain why the majority of tumours are associated with external genitalia, whereas others affect extra-genital sites, reports of which include eyes, mouth, skin and nose either with or without genital involvement (Feldman, 1929; Ajello, 1980; Batamuzi and Bittegeko, 1991; Stimmelmayr, 2010). CTVT metastasis has mostly been described in immunosuppressed dogs and dogs in poor condition (Higgins, 1966) and secondary sites include the inguinal or iliac lymph nodes, and occasionally the skin, spleen, kidney, liver, brain, tonsils, pituitary gland, ovary, uterus, lungs, oro-nasal cavity and eyes (see Das and Das, 2000 for manuscripts published before 2000, see Stimmelmayr, 2010 for manuscripts published before 2010). The frequency of metastasis in otherwise healthy dogs is unknown.

Experimental transplantation studies have led to the description of three distinct stages of CTVT tumour growth - progressive, stable and regressive (Wade, 1908; Karlson and Mann, 1952; Cohen, 1985; Chu et al, 2001a). After experimental transplantation of CTVT cancer cells, the tumours enter a period of progressive growth followed by a stable phase, following which up to 80% of the experimental tumours enter a regressive phase, shrink and eventually disappear completely (Wade, 1908; Cohen, 1985; Chu et al, 2001a). This is in contrast to naturally occurring CTVT tumours, in which spontaneous regression has been poorly documented (Stubbs and Furth, 1934; Rust, 1949; Higgins, 1966; Brown et al, 1980; Mukaratiwa et al, 2006). It is possible that either spontaneous regression does not
occur in naturally occurring tumours or that naturally occurring cases of CTVT are usually treated and so spontaneous regression has been difficult to observe.

Despite the fact that no cases of naturally occurring CTVT have been reported in other members of the Canidae family, the tumour has been successfully experimentally transplanted into fox, coyote, jackal (Cohen, 1985) and wolf (Dungern, 1912). The question whether CTVT has the potential to naturally infect wild canids is still to be answered; it is possible that natural cases of CTVT do occur in wild canids but that this has not yet been reported in the literature.

In the past, CTVT had been reported from countries on all six continents. A high CTVT prevalence was reported from Central and South America, specifically noting Puerto Rico (Rust, 1949) and the Bahamas (Higgins, 1966) In the twentieth century, many cases were reported from the United States (Brown et al, 1980) However, although the number of cases was relatively high at the beginning of the 20th century, it kept gradually decreasing (Karlson and Mann, 1952), which could possibly be attributed to changes in dog management, such as breeding control or leash laws. CTVT has been reported to be prevalent in New York and Philadelphia and cases were occasionally seen in Ohio, Kansas, Michigan, Minnesota, California and Chicago (Moulton, 1990).

In parts of Africa, reports have also indicated a high CTVT prevalence (Kimeto and Mugera, 1974). Additionally, parts of Asia, including China, Japan, the Far East and Middle East also reported common CTVT cases (Rust, 1949; Higgins, 1966). Furthermore, CTVT has also been seen in Australia (Locke, 1975) and Papua New Guinea (Rust, 1949; Hamir, 1985). In Europe cases have been reported in the south-east regions in particular, while Great Britain seemed to have occasional rare cases and France had more frequent occurrence of CTVT in the 20th century, compared to Sweden and Denmark which were free of CTVT (reviewed in Das and Das, 2000). Interestingly, prevalence of CTVT has clearly been decreasing in the UK, as no reports of cases have been published since 1970. This could be
contributed once again to changes in leash laws and dog breeding management as well as reinforcement of dog import quarantine.

Precise prevalence data have been provided from some countries, as in one report 1% of dogs admitted to University Hospital in Kabete, Kenya had CTVT (Rottcher and Frank, 1972) but in another report the prevalence was given as high as 11% in Kenya (Kimeto and Mugera, 1974). Furthermore, 32% of dogs were infected in Sri Lanka (Wettimuny and De, 1975), 10% in the former USSR (Osipov and Golubeva, 1974). A very high prevalence of CTVT, over 20%, was seen in India (Singh et al, 1996). Recently in 2007, CTVT prevalence in bitches in Merida, Yucatan, Mexico was reported to be 15.3%, as found during a study into reproductive patterns of stray bitches (Ortega-Pacheco et al, 2007). This was in contrast to prevalence of 2.64% in 2003 carried out also in Merida (Ortega-Pacheco et al, 2003). In Mexico, the prevalence of CTVT can rise up to 37% when the density of stray dogs is very high (Ortega-Pacheco et al, 2007). Another similar study carried out on street dogs in 2007 in Mexico City reported the prevalence to be 17.48% (Cruz, 2010). In Lima, Peru the prevalence has been reported as 8.4% between years 1998-2004 and no gender bias was found (Mendoza, 2010).

Although CTVT has been reported in many countries worldwide, there has been no study regarding the global distribution of the disease. Only two reviews describe rough distribution of the transmissible tumour: one published in 2000 (Das and Das, 2000) and another review, which includes a summary of the current reported worldwide distribution of CTVT, was published in 2010 (Stimmelmayr, 2010). The latter indicates that CTVT has been reported in 33 countries worldwide and also includes the only world map published showing countries where CTVT is endemic. However, this review did not systematically compare CTVT prevalence within each country. Other studies have claimed that CTVT is more prevalent in tropical and semi-tropical areas (Higgins, 1966) and that it is inversely correlated to geographic latitude and positively correlated to higher mean annual temperature and increased rainfall (Hayes et al, 1983). CTVT has also been linked to number of stray dogs
(Cohen, 1985). However, no previous studies have performed a global survey to test the association between CTVT prevalence, number of street dogs and climate.

Records of CTVT worldwide prevalence are sparse, incomplete and out of date. Furthermore, little is understood about CTVT risk factors, epidemiological features and disease course. Therefore, I have collected worldwide epidemiological data on CTVT and additionally specifically investigated the link between street dog density, climate, gross domestic product (GDP) and CTVT prevalence. I also collected and analysed information about CTVT pathogenesis, spontaneous regression and response to therapy around the world.

3 Materials and methods

3.1 Questionnaire design and piloting

In order to collect epidemiological information, the first step was to design the questionnaire, which can be referred to in Appendix 1. After consultation with epidemiology experts, vets working in rural communities (in Kenya and South Africa), pathology specialists and experts in the field of cancer research, I have designed a questionnaire with 20 mostly multiple choice questions. The draft version has gone through extensive piloting phase, in which various people from different backgrounds (veterinarians, representatives of welfare organization, epidemiologists and researchers) were involved.

The piloting phase resulted in a final version of the questionnaire, which was to be sent out to potential participants all over the world. The number of questions was cut down to 17, the wording was simplified to enable participation of veterinarians in non-English speaking countries and photos illustrating typical CTVT cases were included. In addition, the questionnaire contained optional extended answer sections to enable respondents to contribute additional information. The final version of the questionnaire has gained the approval of the Department of Veterinary Medicine Ethics and Welfare Committee (CR105). In order to reach the wide target population, the questionnaire was translated into Spanish, Portuguese, Chinese, Russian and French.
Similarly, in order to increase the circle of participants, I created both an online interactive (site must not be revealed) and a Microsoft Word document version of the questionnaire (Appendix 1). I designed the online version, using the text editor ConTEXT, with the support of the IT officers at xxxx College. The online version is hosted on the website of xxxx (must not be revealed), with the kind help from the IT officers, and all the responses are directed to my email address. The Word document version, which could be sent out in any of the 6 languages listed, was aimed at people with limited Internet access and it also allowed the distribution of printed copies.

The main emphasis of the questionnaire was to determine the approximate prevalence of CTVT in each country, using a range of percentages as categorical variables. I included an optional answer box to allow further information to be provided if available. The respondents were asked to base their estimates for prevalence on percentages of number of CTVT cases seen per number of the total of all cases seen in a year.

In addition to obtaining prevalence data, the questionnaire was designed to provide information about the type of area and type of organisation. Respondents were required to fill in the country they come from, but additional contact details (name, email address) were optional. Other categorical value multiple choice questions gave further information about the number of dogs in the area, sterilization status of the majority of dogs, population size of free roaming dogs in the area, urban/rural location within the country, percentage of cases with metastasis, observation of spontaneous regression, treatment used and response to this treatment, gender and general health status of majority of CTVT affected dogs.

Furthermore, questions with extended answers were seeking information on observation/absence of CTVT from other regions, unusual cases of CTVT, natural infection of CTVT in other species (such as fox, coyote, jackal) and also provided space for further comments. In addition, the questionnaire asked for photos of CTVT cases (with the person’s approval) and offered collaboration on a separate genetic project, which would include collection of CTVT samples in RNAlater.
3.2 Identification of participants and distribution

I decided to use a country-based division of the world, with further division of some larger countries (including Russia, China, Australia, USA and Brazil) into smaller regions, while discarding uninhabited regions. The first step was to use the Internet to identify potential participants. The questionnaire was targeted at veterinarians in private practice, veterinary schools, veterinary hospitals, welfare or veterinary charitable organizations and non-governmental organizations (NGOs) in each country/region and I was aiming to obtain more than one response from each country in the world. I created a database of the first contacts and sent out an e-mail asking for contribution in the project by filling in the questionnaire. Furthermore, I contacted international veterinary organizations in order to circulate the questionnaire via their mailing lists. Local veterinary organizations were contacted with the request to publish an advertisement in their local newspaper or periodical. Additionally, personal contacts were used to disseminate the questionnaire all around the world and e-mail recipients were kindly asked to forward the questionnaire on to a more appropriate person if unable to answer themselves. I sent out a total number of 901 emails to potential participants, and received 515 completed questionnaires (response rate of 57.2%).

The questionnaire was sent around many international mailing lists, including the Veterinary Society of Surgical Oncology (VSSO), Veterinary Cancer Society, World Vets, World Small Animal Veterinary Association (WSAVA), International Veterinary Students’ Association (IVSA), Israel Veterinary Medical Association, Italian Veterinary Oncology Society, American College of Veterinary Internal Medicine (ACVIM), Rural Vets South Africa, Kenya-Kabete 1229 Vets Group, Uganda 600 Vets Forum, Veterinary Students Association (Kenya), Veterinary Students Association Kabete. Furthermore, an advertisement for the questionnaire was published in the Ukrainian magazine МИР ВЕТЕРИНАРИИ (World Veterinary Medicine) and in the Russian veterinary periodical VetPharma.

Additionally, the project was publicized at international conferences, which also involved distribution of printed questionnaires, including the Nigerian Veterinary Medical

### 3.3 Data analysis

To enable a uniform data analysis, the categories for prevalence values (none, less than 0.5%, 0.5%-1%, 1-3%, 3-5%, 5-10%, 10-20% and more than 20%) were assigned a number from 0 (for ‘none’) up to 7 (for ‘more than 20%’), which allowed me to add up the point value of each response from one country and calculate the average, using all of the responses for each country obtained from the survey. The point value was then converted back into the categorical prevalence value, giving an average prevalence for each country.

In order to create the choropleth map showing CTVT distribution, I used RStudio and a set of packages including knitr, xlsx, rgdal, ggplot2, maptools, mapproj, ggmap, countrycode and doBy. The names of countries were converted into ISO 3166-1 alpha-2 codes published by the International Organization for Standardization (ISO). Furthermore, the locations from which responses were recorded were converted into GPS coordinates, to enable processing by R and subsequent display on the map. Additionally, a map with finer resolution of prevalence in each country was created using Adobe Illustrator.

The data concerning the risk factors associated with CTVT prevalence were partly obtained from the responses to the questionnaire by asking the respondents whether majority of dogs in their area are free roaming. The number of ‘yes’ and ‘no’ responses was counted and the more numerous answer displayed on the map. If respondents specifically stated that there are only some free roaming dogs in their area, this was displayed on the map as well. Other data, including GDP values and climate data, have been obtained from The World Bank database and analysed using graphs and statistical test (Pearson’s Correlation coefficient and statistical significance two tailed test). Furthermore, countries were divided into low income, low middle income, upper middle income and high income economies (The
World Bank “Country and lending groups”, 2012) in order to further analyse effects of socio-economic status on CTVT prevalence.

The questions included in the second part of the questionnaire were analyzed by various means including a bar graph to show which organizations the responses came from, a pie chart showing gender predisposition, the health condition of CTVT affected dogs and the treatment used. Additionally, the response to treatment was analyzed worldwide by displaying the data graphically. Furthermore, the average percentage value for metastatic spread has been calculated based on the responses given. Since the response was a categorical variable, each category was assigned a mean value (for example category 0-5%, mean value 2.5%), which was multiplied by the number of responses for this category and the results for all categories were added up to give a total value, which was divided by the total number of responses to give the estimated average percentage of cases with metastasis.

4 Results

4.1 The worldwide distribution of CTVT

During the project, I collected a total of 515 responses to the survey from 106 countries from all inhabited continents.

The responses were received from a variety of organizations (Figure 2), including private veterinary clinics and specialist referral practices (60.3%), veterinary schools (26.4%), animal welfare organizations (for example rescue organizations, dog shelters and sterilization clinics) (7.1%), pathology laboratories (2.3%), academic research institutes (1.4%) and governmental offices (2.5%). The majority of responses were obtained from the online version (386 responses), while other respondents chose to fill in the Microsoft Word Document version, mostly in foreign languages (128 responses). Answers to the survey were received in all the languages in which the questionnaire was available.
The choropleth map (Figure 3) displays CTVT worldwide distribution according to countries, as obtained from the survey. The countries are shaded in different colours corresponding to their estimated average prevalence of CTVT.

According to the responses, the highest prevalence was recorded as being more than 20% in Lesotho and Morocco, while the Gambia and Ivory Coast were reported as having prevalence of CTVT 10-20%. However, these data are based on only a single response from each of these countries, so we are aware of possible bias. The prevalence was found to be also very high (5-10%) in American Samoa, Belize, Botswana, Cambodia, Ecuador, Ghana, Grenada, Honduras, Laos, Mauritius, Mexico, Nicaragua, Romania, Samoa, Sri Lanka, Thailand, Turkey, Venezuela and Zambia. A high percentage of dogs infected with CTVT (3-5%) was seen in Argentina, Chile, Costa Rica, Cuba, Dominica, Fiji, Guyana, Kenya, Malawi, Malaysia, Micronesia, Mozambique, Paraguay, Peru and Timor-Leste.

In contrast, the majority of countries of northern, western and central Europe were found to be free of CTVT, except for cases imported from abroad. These countries included Austria, Belgium, Czech Republic, Denmark, Finland, Germany, Hungary, Malta, Netherlands, Slovenia, Sweden, Switzerland and the United Kingdom. Some cases of CTVT were reported from southern regions of France, south-west of Spain and Portugal. In the countries of southern and eastern Europe, CTVT was found to be endemic with less than 0.5% prevalence in Belarus, Estonia and 0.5-1% prevalence in Bulgaria, Greece, southern Italy including Sicily, and Ukraine. A much higher prevalence (5-10%) was reported in Romania and Macedonia.

To show the fine resolution of prevalence data and variation within one country, which is especially relevant for larger countries, the responses have all been displayed on a map using a colour-code (Figure 4A, B). Each dot on the map represents a single response from the particular site where it is located. Grey dots were used to indicate areas or countries where CTVT was seen but where a definitive prevalence value was not provided.
4.2 Risk factors influencing CTVT prevalence

I investigated whether CTVT prevalence was associated with density of free roaming dogs, socio-economic status, climate, difference between urban and rural areas and other factors.

**Free roaming dogs.** Number of free roaming dogs was found to be associated with CTVT and a clear linkage between prevalence of CTVT (Figure 3) and presence of free roaming dogs in the country (Figure 5) was observed. However, CTVT was not found to be correlated to the previously reported (Hughes and MacDonald, 2013) human:dog ratio in the country or region (Figure 6).

Lack of knowledge and education about CTVT as well as its nature and route of transmission can also contribute to increasing transmission of CTVT from street dogs to owned, but free-roaming dogs. One questionnaire response from Quito, Ecuador commented that many owners are unaware that CTVT can be transmitted sexually and therefore they let their dogs roam freely and breed, even though they seem to have a bloody discharge or visible tumours on their genitalia.

**Socio-economic development.** The development status of each country was determined on the basis of GDP per capita value, and this was found to be weakly negatively correlated with CTVT prevalence (Figure 7). Additionally, Figure 8 shows the difference in responses received from various countries, divided into groups based on their income economies.

**Climate and distance from equator.** Despite previous publications (Hayes, 1983) indicating a positive correlation between increased rainfall and CTVT prevalence, no such correlation has been found in this study (Figure 9). On the other hand, an inverse correlation with the distance from the equator was observed (Figure 10), as was suggested by previous reports correlating CTVT prevalence and geographic latitude (Hayes, 1983). Although claims about climate and latitude association have previously been made, this study was the first one to investigate the relationship on a global scale.
**Urban/rural areas.** The extended answers provided by the respondents suggested that CTVT is more common in rural areas in comparison to urban areas. However, the analysis of difference in prevalence of CTVT between rural, semirural and urban areas of country did not show an association between urban/rural status and CTVT prevalence (Figure 11).

**Other CTVT risk factors.** No gender predisposition for CTVT was found from the questionnaire (Figure 12). 120 out of the 339 responses to this question (35.4%) answered saying that CTVT occurs equally in females and males. 102 respondents (30.1%) claimed that CTVT is more common in females, while 117 people (34.5%) have noticed CTVT more commonly in males (difference between sexes is not statistically significant as shown by the $\chi^2$ test, $\chi^2=1.027$, so $p=0.31$, $0.31>0.05$). Therefore it seems that, in contrast to previous studies which suggested a gender bias (Karlson and Mann, 1952; Ajello, 1980; Boscos, 2004), there is no gender predisposition for CTVT infection.

Whether age is associated with CTVT status is unclear. This study has shown that CTVT occurs in dogs within a wide age range from 6 months to 13 years. Therefore it might seem that age is not the predisposing factor by itself, but other variables, such as sexual activity of the dogs might be more important.

Dogs with CTVT have been reported to be in varying health conditions (Figure 13), from completely healthy (apart from the tumour) (54%), to thin, emaciated (16%) and infested with internal as well as external parasites or otherwise diseased (26%). A small percentage of the 351 answers to this question (4%) have noticed other injuries and bite marks, which are likely to be facilitating the spread of the tumour. However, no definite associated infection or parasite has been found.

Although the questionnaire did not include a specific question about breeds of dogs which are more susceptible to CTVT infection, the extended answers provided information to suggest that most of the CTVT cases are in mixed breed dogs and no particular breed has been shown to be associated with CTVT infection. However, this does not mean that some
breeds are not more susceptible than others, as a response from Ghana claimed exotic breeds, such as Boar bulls, German shepherds, Rottweilers and to a much lesser extent Doberman to be most frequently infected with CTVT.

Additionally, respondents were asked to report if they had observed CTVT occurring in wild canids. The majority of respondents did not observe any such cases, but there was a case of CTVT in a fox reported from a pathology lab in Chile. However, no further information about this case is available. Furthermore, one case of naturally occurring CTVT in a wolf was recorded in Minnesota, but since the case was over 15 years ago, the details are, again, unclear. In contrast, no cases have been seen in the population of the African Wild Dog nor the Canadian wild canid population or the jackal population in South Africa, despite the fact that I have received responses from people who worked extensively with these wild canids and so probably would have had a chance to observe CTVT if it were there.

Investigation of CTVT risk factors has shown a link between density of free roaming dogs, socio-economic development of the country, distance from the equator and CTVT prevalence. On the other hand, there is no evidence that CTVT prevalence is associated with rainfall, gender, breed, age or health status of the dogs.

4.3 CTVT pathogenesis and treatment

In addition to obtaining data on CTVT prevalence and associated risk factors, I have collected information concerning spontaneous regression, metastasis and treatment of CTVT. The results provide evidence on the prevalence of natural spontaneous regression, the frequency of metastasis and the type of therapies that are frequently used as well as the effectiveness of these therapies for treating CTVT.

Spontaneous regression, a phenomenon which has been debated in naturally occurring CTVT cases for a long time (Higgins, 1966; Brown et al, 1980; Mukaratirwa and Gruys, 2004) has been reported from 20 places around the world, as is shown by the red dots in Figure 14. Most cases of spontaneous regression were seen in South America and Asia.
This proves that spontaneous regression does happen even in naturally infected dogs, which has not previously been well documented in the literature.

Metastasis was observed on average in 3.38% of cases (see methods for calculation), which confirmed the previous reports published where metastasis develops in less than 5% of cases (Karlson and Mann, 1952) and was reasonably close to other reports indicating metastasis in 2 out of 30 (6.7%) naturally occurring CTVT cases (Brown et al, 1980). Sites of metastasis varied widely and the most common ones included lymph nodes, nasal and oral cavity (nasal mucosa, hard palate, tongue), skin (flank, neck, whole body) and eyes (conjunctiva). Other sites of CTVT metastasis included lungs, liver, spleen, bladder, brain, spinal cord, testicles, bone, mammary glands, paws, nasal turbinates, lips, ovary and uterus. However, in some cases we cannot be sure whether these sites are primary tumours or metastasis, since the extended answers sometimes gave an unclear answer.

The survey has confirmed that Vincristine is now the drug of choice (Figure 15) for treatment of CTVT worldwide, as 74% of respondents using Vincristine reported the response rate (defined as proportion of tumours that go into complete remission) to be 80-100%, proving it to be a very effective treatment. This was also shown by earlier studies (Brown et al, 1980; Singh et al, 1996; Nak et al, 2005), which have looked at the response of CTVT to various treatment regimes. Vincristine was used by the vast majority of respondents (Figure 16; the number of participants who responded to this question was 351) either on its own (36.3%) or in combination with surgery (33.7%). Additionally, Doxorubicine was sometimes used in Vincristine resistant cases (6.5%) and a combination of surgery, Vincristine and Doxorubicine was also selected by some respondents (3.7%). Some participants used Vincristine and surgery in combination with radiotherapy (1.2%). In some cases (1.5%), if Vincristine didn’t work, the dog was euthanized. However, in places where Vincristine was not available or not known, surgery was the only option (10%). A few respondents (3.1%) from Bangladesh, Guyana or some parts of Chile never used any treatment and the dog was always euthanized. A group of other treatments (4%) included hot
metal cauterisation, electrocautery, prednisone and herbal treatment with aloe vera. Occasionally, non-steroidal anti-inflammatory drugs were used, but all the respondents admitted that there was no effect and in South Africa it was only given as a placebo to calm down the owners, who couldn’t afford to pay for Vincristine treatment. Interestingly, co-administration of Ivermectin and Vincristine has been claimed by a Brazilian vet to improve therapeutic response over Vincristine alone.

In some countries where Vincristine is either not readily available (Vietnam or Kyrgyzstan) or not known or not used for some other reason (such as remote parts of Russia and most parts of China) a dramatic decrease in response rate to their therapy of choice was seen. In addition to identifying the most common means of treatment, the survey helped me to map cases of CTVT which are resistant to Vincristine treatment, which is illustrated by green coloured dots in Figure 14. Some places, such as Brazil, Romania and Thailand reported a large number of Vincristine resistant cases, while other respondents from Botswana, Greece, India, Malaysia, Nigeria and Russia only came across one case.

5 Discussion
During the project, I collected a large dataset about previously unknown worldwide CTVT distribution. CTVT had previously been reported from 33 countries worldwide (Stimmelmayr, 2010), which has, after this study, been expanded to 92 countries where CTVT cases have been reported. Additionally, a distinction has been made between countries where CTVT is endemic (83 countries) and where CTVT cases have been reported very rarely or only in particular areas. A further 14 countries were shown to be free of CTVT, unless a case was imported (Figures 3 and 4A, B).

5.1 Factors affecting worldwide CTVT distribution
The CTVT worldwide distribution map (Figure 3), produced as a result of the survey, gives average prevalence for each country, which is additionally expanded in more detail in Figure
4A and 4B, showing distribution of CTVT within each country as obtained by the questionnaire.

The presence of free roaming dogs was identified as one of the major factors associated with CTVT prevalence (Figure 5), in contrast to lack of association between human:dog ratio and CTVT prevalence (Figure 6). This would suggest that CTVT prevalence is not linked to the actual number of dogs but rather to the number of free roaming dogs. Conditions favouring free roaming dogs include uncontrolled breeding, higher number of entire (non-spayed/neutered) dogs and poor living conditions. These conditions would provide a ground for dissemination of CTVT infection, especially and, most likely, since it is a sexually transmitted disease.

Additionally, the extended answers provided from Botswana, China, India, Ivory Coast, Nigeria, South Africa, Suriname, Uganda and Zambia support this correlation by drawing a link between lack of breeding control and increased prevalence of CTVT, which is facilitated by transmission between street and owned dogs. In some countries (China, Israel, Mauritius, Samoa, South Africa, Thailand, Ukraine), education about breeding control and sterilization are proposed by the respondents to be essential to control the disease. This link is further supported by a response from Chan Island near mainland Thailand, where breeding management and neutering campaigns have eradicated CTVT from the island completely since starting the project in 2001. Furthermore, the observation that CTVT is mainly seen in free roaming dogs in late breeding season or after breeding season in Bangladesh provides additional support for the statement that uncontrolled breeding plays a significant role in CTVT prevalence. However, despite a large number of free roaming dogs in Tennessee, this area is claimed to be free of CTVT by one respondent. This is most likely explained by the strict laws on dog control enforced in Tennessee (2007 Pub.Acts, c. 276, § 1).

Socio-economic status, defined by GDP values per capita (Figure 7) and by income economies (Figure 8), has shown a weak inverse correlation with CTVT prevalence, which corresponded to a higher number of countries with lower CTVT prevalence in the high-
income group. However, the correlation is not very strong, suggesting that GDP and income economies are not the main determining factors in CTVT distribution, but they might rather affect some of the other factors which have a greater influence. A lower socio-economic status, along with cultural practices, would in turn lead to differences in number of free roaming dogs. In addition, fewer dog control laws and more relaxed breeding management in lower GDP countries may also indirectly affect the prevalence of CTVT in the country.

A weak correlation of CTVT prevalence with the distance from the equator (Figure 9) has confirmed the earlier reports (Hayes, 1983) and it might suggest an effect of climate on CTVT. However this interpretation should be handled with care due to potential bias, since the link might be more likely caused by the association between socio-economic status and the distance from the equator. The correlation might also be affected by the large number of small countries near the equator (such as in the Caribbean), which have a high prevalence of CTVT and cannot be distinguished on the map (Figure 3). In contrast, no correlation has been found between CTVT prevalence and increased rainfall, which indicates further that CTVT is not likely to be associated with climate.

It has been difficult to be able to convey the varying prevalence between urban and rural areas within each country on a map, as these results have often been obtained as an expanded answer description. No clear difference could be seen between urban and rural areas in Figure 11, however, since these data were obtained from the responses to the questionnaire, potential individual bias should be taken into account. Additionally, reports by the respondents have suggested that rich areas of the cities, where dogs are kept inside houses have a very low or even no prevalence, in comparison to high prevalence of CTVT in suburbs and areas with low-income housing, where owned dogs wonder on streets and interact with the free roaming population. This might have lead to the biased responses to this question, as areas within one city can vary hugely in CTVT prevalence. The extended answers have suggested that rural areas have been claimed by many respondents to have a higher
prevalence of CTVT, once again indicating that living conditions and breeding control, which are likely to vary between urban and rural areas, play an important role in CTVT prevalence.

An interesting phenomenon has been observed in the USA and Australia, where the distribution of CTVT is limited only to Indian reservations and aboriginal communities. Despite the fact that no CTVT cases have been reported in Australia in the literature since 1975 (Locke, 1975), CTVT prevalence was found to be 0.5-1% in the aboriginal communities in Northern Australia (around Darwin and Alice Springs in particular) and northern parts of Queensland and Western Australia, with an occasional case seen in other parts of Western Australia (reported in extended answer questions). Similarly, in the USA, CTVT has often been reported in the Indian reservations, either by volunteers or members of HSVMA-RAVS (Humane Society Veterinary Medical Association - Rural Area Veterinary Services). The higher prevalence of CTVT in these communities could, once again, be attributed to the lower incomes in these areas, which are likely to indirectly affect the number of free roaming dogs, living conditions and breeding management. In contrast, no cases have ever been reported from New Zealand, which could reflect the well enforced quarantine and therefore prevention of import of CTVT cases into the country.

Some countries, Italy being particularly prominent, have a varying geographical prevalence of CTVT between regions within the country. Despite the responses from northern parts of Italy showing prevalence of CTVT on average less than 0.5%, the additional information provided in the extended answers very clearly states that the northern regions are free of endemic CTVT. All responses from the northern parts of Italy describe CTVT presence in these regions about 10-20 years ago, which is also supported by historical reports (Ajello, 1980). On the other hand, in the southern regions, as well as Sicily and Sardinia, CTVT is still quite common with prevalence of 0.5-1% (obtained from responses to the questionnaire) and cases are still being reported in the literature (Albanese, 2006). It is possible that the poorer living conditions and increased number of free roaming dogs in the
south are facilitating CTVT spread more readily; however, a possible difference in breed composition and a susceptibility of particular breeds should also be considered.

Although it has been suggested that CTVT affects more males (Osipov and Golubeva, 1976; Brown et al, 1980), as they are constantly sexually receptive throughout the year, other reports have claimed that naturally found CTVT is more common in females (Ajello, 1980; Singh et al, 1996), as one male dog can spread the disease to 11 of 12 females (Karlson and Mann, 1952). Despite some respondents claiming that CTVT is easier to observe in females and others claiming that it is easier to observe in males, the overall results have shown that there is no gender bias in CTVT infected cases (Figure 12).

Despite the fact that no association has been found between specific parasites or disease and CTVT susceptibility, as all health, diseased, parasite infected or emaciated dogs seem to be affected (Figure 13), a report from the Solomon Islands has drawn a link between neglected, poorly fed, weak and stray dogs and higher incidence of CTVT. The respondent also highlighted that in weak, stray dogs regression with Vincristine treatment is much slower, compared to the healthy, well fed and strong dogs. Furthermore, it has been observed that the progression of CTVT is slower in the healthy population, which once again implies some role of the immune system in controlling progression. Therefore infection or immunosupression might be a predisposing factor, but certainly not the only one, as genetics might play a key role as well. The importance of genetics was suggested in experimental studies which found a correlation between dog MHC and CTVT tumour progression (Bennett, 1975a).

The finding that CTVT was reported in a fox and a wolf, and thus potentially allowing spread of CTVT in other populations of canids, might have further conservation implications if it entered some endangered canid populations, such as the African Wild Dog. However, I did not find evidence of wild canid populations naturally infected with CTVT. Some reasons why the wild canids are not widely infected with CTVT might be because the tumour cannot establish itself in other species due to species-specificity in the tumour's
mechanisms of interaction with its host. Alternatively it may be because there is a lack of opportunity for transmission due to lack of contact between wild canids and domestic dogs.

5.2 Potential biases and problems of self reported data

In order to provide reliable data, I was aiming to obtain several responses from each country which would enable the calculation of an average value and therefore elimination of bias introduced by variation in individual perception of CTVT prevalence in the area. Despite the measures taken to eliminate bias, it is still expected that the values obtained will only be estimates. Other factors, including location and differences between rural/urban areas, were also considered to be potentially influencing responses. Despite the endeavour to map the CTVT prevalence as accurately as possible, I have only obtained one response from 24 countries out of the total of 106 countries. This has to be taken into account when considering the reliability of the data in each country. Figure 4A and B enables judgment of the reliability of the prevalence value in each country. Responses from smaller countries, such as islands in the Caribbean and in the Pacific, can be considered reliable, as local vets receive patients from all regions of the island. On the other hand, in countries including Lesotho, Morocco (prevalence more than 20%), Gambia and Ivory Coast (prevalence 10-20%), caution should be taken when considering these very high values of CTVT prevalence, since only one answer has been obtained for the whole country and so we should be aware of possible bias.

During interpretation of the data, the reliability of the source from which the data have been obtained should also be considered. Some countries, for example Pakistan, Argentina or Canada, only have one or a few responses displayed in the map, but based on the extended information provided it is clear that it is reliable. In the case of Pakistan, the questionnaire has been answered by a whole group of researchers, who also obtained information from other parts of the country and included it in the questionnaire. Similarly in Argentina, the questionnaire was completed by an expert at a university, who has had experience with CTVT in other parts of the country. In Canada, the questionnaire was
completed by a member of a national pathology lab. This issue could potentially be addressed by showing dots of different sizes on Figure 4A and B, correlating in size to reliability. However, this option has been discarded, since the presentation would be unclear.

Distribution of the questionnaire to all countries around the world was inevitably going to be challenging and some difficulties were encountered at this stage, as it proved often difficult to obtain even a single response from particular countries, where internet access is not readily available. In order to overcome this barrier, the questionnaire was available in a Microsoft Word Document printable version as well and personal contacts with people travelling to developing countries were used to disseminate the questionnaire.

5.3 CTVT pathogenesis and response to therapy

The extended questions included in the questionnaire have proved to be very informative about CTVT behaviour in naturally occurring infection. It will be very interesting to correlate the epidemiological information on spontaneous regression, metastatic sites affected and different response to chemotherapy with genetic features of the tumour, in particular the distribution of the two different clades (Murgia et al, 2006) around the world.

In some countries spontaneous regression has been observed (Figure 14), which proves that this phenomenon does occur even in naturally transmitted CTVT. However, 96% of respondents had never observed a single case of spontaneous regression. It is still unclear whether spontaneous regression is just less common in naturally occurring cases compared to experimental CTVT or whether there is a lack of opportunity to observe these cases, since treatment is almost always used.

Furthermore, the study has confirmed that Vincristine is the most successful and widely used agent for treatment of CTVT (Figure 15, Figure 16), as suggested by earlier studies (Nak et al, 2005). Often, however, respondents used surgery to remove the visible tumour mass, which was followed by a course of weekly Vincristine injections. However, using Vincristine or surgery rather than using them in combination is very likely to give
lower response rates, as response to surgery and other treatments was seen to be less effective than response to Vincristine (Figure 15). However, Vincristine might not always be the option because it is not always available or the owner cannot afford to pay for the treatment.

Despite Vincristine being effective in a majority of cases, resistant cases have also been reported all over the world (Figure 14). One explanation could be that due to the extensive Vincristine use and so increasing selective pressure, outbreaks of resistant strains have emerged. It will be interesting to confirm this theory by further genetic studies.

The question related to metastasis, after retrospective consideration, provided slightly unclear answers. Despite this question being related to metastasis in the Word document version, the visual link with metastasis was not very clear in the online version. This resulted in a report of a large number of sites affected; however, in some cases it was unclear whether these were primary sites or metastasis. Despite the ambiguity in the question caused by the layout of the online questionnaire, it was still very informative and has proved that metastasis can occur in a number of naturally occurring cases and therefore CTVT should not be described as ‘benign’, as was the case in some previous publications (Mello Martins et al, 2005). Importantly the survey has shown that metastasis can occur in otherwise healthy dogs and therefore immunosuppression, although being one of the predisposing factors, is certainly not the only one, as was suggested in previous reports (Higgins, 1966).

An interesting relationship to investigate is between CTVT and pregnancy - why are CTVT cases not more common in pregnant females given that it is transmitted during coitus? One explanation could be related to the incubation period of CTVT, which could in most cases be longer than 63 days, which is the duration of pregnancy. Although there is not a lot of information on naturally occurring CTVT and pregnancy, responses from Ecuador, Peru and Suriname have all reported CTVT in pregnant females and so proving that CTVT affected females can still have a normal pregnancy. However, what is not known is whether some pregnancies have been aborted as a result of CTVT in the early stages.
Overall, the study has gathered previously unknown information on CTVT distribution and epidemiology from countries all over the world which has shown that CTVT prevalence is correlated with number of free roaming dogs, socio-economic status and distance from the equator. Furthermore the survey has contributed information about CTVT pathogenesis and response to therapy to the field. In addition to providing important new insights into the global spread of a transmissible cancer, this study may help inform policy-makers and veterinarians to develop measures to more effectively control and prevent CTVT.

6 Reference list


**Figures**

**Figure 1** Canine Transmissible Venereal Tumour (CTVT) affecting (A) a male dog (B) a female dog (C) skin of the whole body (D) the conjunctiva of the eye. Photos provided courtesy of Karina Ferreira de Castro (Brazil), Fanny Gallardo-Arrieta (Venezuela), Natalia Quiros (Universidad de Chile) and Natalia Ignatenko (Ukraine).

**Figure 2** Proportion of different types of organisations involved in the survey. Percentage of the total number (515) of responses is shown. The actual number of responses is shown above each bar.
Figure 3 Choropleth map showing CTVT worldwide distribution by country. The map is based on the responses obtained from the questionnaire (locations from which responses were received are represented by black dots) and an average prevalence value has been calculated for each country.
Figure 4A Map of the whole world showing all of the responses (apart from Europe) received from the questionnaire and the corresponding prevalence for each response. Each dot represents a single response. The arrangement of responses in the boxes is according to timeline of when the answer was received.
Figure 4B Map of Europe showing all of the responses from the questionnaire received from Europe and their corresponding prevalence value. Each dot represents a single response. The arrangement of responses in the boxes is according to timeline of when the answer was received.

Figure 5 Map showing the worldwide distribution of free roaming dogs, which shows a clear association with the countries with high CTVT prevalence (Figure 3). Data on the estimated prevalence of free roaming dogs were obtained from the questionnaire.
Figure 6 Relationship between CTVT prevalence and human:dog ratio. No correlation has been found (two tailed test, DF = 14, when p=0.05, |-0.2732| < 0.497) Data on human:dog ratio in each country/region are obtained from Hughes and MacDonald, 2013. Note that data refers to continents except for in countries where more detailed data on human:dog ratio is known (Argentina, Brazil, Chile, China, Colombia, Mexico, Peru, Venezuela, USA, Canada). Prevalence value displayed represents the top border of each categorical interval.

Figure 7 Graph showing the relationship between CTVT prevalence and GDP per capita values worldwide. GDP per capita values were obtained from the World Development Indicators Database, World Bank. A weak, but statistically significant, negative correlation can be seen (two tailed test, DF = 91, when p=0.01, |-0.4563| > 0.267). Prevalence value displayed represents the top border of each categorical interval and category ‘more than 20%’ is shown by the value of 25% on the graph.
Figure 8 Relationship between CTVT prevalence and socio-economic status, defined as low/lower middle/upper middle or high income economies. The different colours represent division into different socio-economic groups (based on The World Bank “Country and lending groups” (2012)) while the size of the dots represents number of countries with average CTVT prevalence within a given range. Prevalence value displayed represents the top border of each categorical interval and category ‘more than 20%’ is shown by the value of 25% on the graph.
Figure 9 Graph showing the relationship between CTVT prevalence and annual rainfall. No correlation has been found (two tailed test, DF = 91, when p=0.05, |0.037| < 0.205) Rainfall data are based on values from The World Bank. Prevalence value displayed represents the top border of each categorical interval and category ‘more than 20%’ is shown by the value of 25% on the graph.

Figure 10 Relationship between CTVT prevalence and distance from the equator (shown in degrees of latitude). A weak, but statistically significant, inverse correlation can be seen (two tailed test, DF = 181, when p=0.01, |-0.3172| > 0.254). Data on distance from the equator, measured for the capital city in each country, were obtained from News Track India. Prevalence value displayed represents the top border of each categorical interval and category ‘more than 20%’ is shown by the value of 25% on the graph.
Figure 11 Difference in prevalence of CTVT between rural, semirural and urban areas. The different colours show varying levels of urbanisation, while the size of the dots is proportional to the number of respondents.

Figure 12 Gender predisposition of dogs infected with CTVT. Results are based on observation and personal experience of the respondents, who were asked whether they observe CTVT more commonly in males or females. The actual number of responses received for each answer is shown in the boxes.
Figure 13 Health condition of CTVT affected dogs. Results are based on observation and personal experience of the respondents, who were asked about the health condition of majority of CTVT infected dogs. The actual number of responses received for each answer is shown in the boxes. The total number of responses was 351.

Figure 14 Map showing cases of spontaneous regression (in red) and Vincristine resistant cases (green) reported from all over the world.
Figure 15 Differences in response to various treatment options. The colours represent four types of treatment, while the size of dots is proportional to the number of respondents who use this type of treatment (total number of respondents for this question was 331). Each categorical option for responsiveness to treatment (defined as proportion of tumours that go into complete remission) is displayed by its middle value on the graph. Vincristine + surgery + other category might give fewer good outcomes, since respondents might be using the separate treatments on their own, rather than using all in combination.
Figure 16 Treatment options used by respondents to the questionnaire for treatment of CTVT. Results are based on answers from 351 participants. When there is a combination of treatment methods used, it is unclear whether they are used together or separately by the respondent.
Appendix 1

PROJECT ON CANINE TRANSMISSIBLE VENEREAL TUMOUR WORLDWIDE DISTRIBUTION

CTVT (also known as Sticker’s Sarcoma, Infective Venereal Tumour, TVT, Venereal Sarcoma) is a common disease of dogs and is found worldwide. Primary tumours occur on the external genitalia of both sexes and the disease is usually transmitted during mating.

The aim of this research project is to collect data on worldwide prevalence of CTVT, which will help us to identify CTVT risk factors and therefore new possibilities for CTVT prevention. The collection of samples will be used for a genetics study of transmissible cancers and the evolutionary processes which have permitted their emergence and spread worldwide.

All the data will be kept confidential. Your contribution to this project is much appreciated!

YOUR INFORMATION

Name: 

Email address (to keep you updated with project): 

Country: 

Town/village: 

Type of organisation:
- □ Private veterinary clinic
- □ Veterinary school
- □ Pathology laboratory
- □ Animal welfare organisation
- □ Other, please specify: 

Number of dogs in your area:
- □ Fewer dogs than humans
- □ One dog per human
- □ 2-5 dogs per human
- □ > 5 dogs per human

Most dogs in your area are:
- □ Spayed/Neutered
- □ Entire

If you have extra information, please, comment:
Is there a noticeable population of free roaming dogs in your area?

☐ Yes
☐ No

If you have extra information, please, comment:

CTVT QUESTIONNAIRE

1) Have you ever seen a case of CTVT?

☐ Yes
☐ No

2) What is your expert opinion on prevalence (% of affected dogs) of CTVT in your region?

☐ None
☐ Less than 0.5%
☐ 0.5-1%
☐ 1 - 3%
☐ 3 - 5%
☐ 5 - 10%
☐ 10 - 20%
☐ More than 20%

If you have more precise data, please comment:

3) How many CTVT case per year do you see?

4) Most of my CTVT cases are from

☐ Urban area
☐ Rural area
☐ Semirural area
☐ Unknown

5) Have you observed CTVT in other regions of your country or the world?

6) Is it noticeably absent from any region in your country?
7) How often does metastasis (lesions in more than one place) occur in the cases you have observed?

- 0-5%
- 5-10%
- 10-15%
- 15-20%
- More than 20%

What other parts of the body are affected (e.g. skin, lymph nodes,...)?

8) Have you ever observed spontaneous regression of tumours (without treatment)?

- Yes
- No

If yes, please provide details:

9) Treatment used (tick all that apply)

- Vincristine
- Doxorubicine
- Radiotherapy
- Surgery
- No treatment
- Other

If other, please specify:

10) What proportion of tumours goes into complete remission after using the selected treatment?

- 0-20%
- 20-40%
- 40-60%
- 60-80%
- 80-100%

11) Gender of the majority of affected dogs

- Male
- Female
- Equally represented

Comment on percentage of predominant sex:

12) Most of dogs with CTVT are:

- Otherwise healthy
- Diseased/carrying parasites

If diseased, what is the most frequent disease/parasite in dogs with CTVT?

- Injured
- Thin, emaciated

What is the most common disease/parasite in your practice?

13) Comment on any unusual cases of CTVT that you have seen:
14) Have you ever come across CTVT in other species (e.g. fox, wolf, coyote, jackal)?

15) Any other comments:

16) Have you got any PHOTOGRAPHS you could send me?

17) Is there a national veterinary organisation in your country?
   □  Yes
   □  No

If yes, what is its name/contact?

18) WOULD YOU LIKE TO TAKE PART IN RESEARCH COLLABORATION? (All materials required for sampling as well as postage are paid for by us!)
   □  No
   □  Yes