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Research Article

Identification of Risk Factors for Canine Transmissible Venereal Tumour (CTVT) in Owned Dogs in Pakistan

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Abstract

A matched case-control study was conducted to investigate the risk factors for canine transmissible venereal tumour (CTVT) in owned dogs of Lahore District, Pakistan. Ninety six laboratory-confirmed CTVT positive dogs were matched on the date of clinic visited with 96 control dogs. The univariate analysis was employed to assess the relationship between different population characteristics and the presence or absence of CTVT. Significant factors associated with CTVT that were obtained using univariate analysis were further included in multivariable logistic regression analysis.. Important identified risk factors were breeding strategies (OR=6.36), dog keeping purpose (OR=4.11), stray dog population (OR =3.35), dogs in neighborhood (OR=2.87), history of skin/genital diseases (OR=2.86) and high density population area (OR=2.54). Protective factors included chain/restrain the dog, use of separate cage, use of proper fencing around the kennel and knowing the difference between normal red discharge and clotted blood. With rigorous protective measures and controlling these risk factors, would decrease the spread of CTVT among the canine population in Pakistan. This is the first study performed regarding the quantification of risk factors for CTVT in Pakistan.

Keywords: *Canine, Pakistan, Risk factors, Case control, Transmissible venereal tumour*

Pakistan'da Sahipli Köpeklerde Köpek Bulaşıcı Veneral Tümör (CTVT) İçin Risk Faktörlerinin Belirlenmesi

Özet

Pakistan'ın Lahor Eyaletinde sahipli köpekler arasında köpek bulaşıcı veneral tümör (CTVT) hastalığının risk faktörlerini araştırmak amacıyla eşleştiriliş vaka kontrol çalışması yürütüldü. Doksan altı adet laboratuarda CTVT pozitif olduğu tespit edilen köpek kliniğe getirilen 96 adet kontrol köpek ile karşılaştırıldı. Değişik popülasyon özellikleri ile CTVT'nin bulunup bulunmadığı durumu arasındaki ilişkiliyi belirlemek amacıyla tek değişkenli analiz yöntemi uygulandı. Tek değişkenli analiz yöntemi kullanılarak CTVT ile ilgili olduğu saptanan anlamlı faktörler çok değişkenli lojistik regresyon analizine dahil edildi. Belirlenen önemli risk faktörleri olarak çiftleştirme stratejileri (OR=6.36), köpeği bulundurma sebebi (OR=4.11), sokak köpeği popülasyonu (OR =3.35), yakın çevredeki köpekler (OR=2.87), deri/genital hastalık geçmişi (OR=2.86) ve yüksek yoğunluk popülasyon alanı (OR=2.54) belirlendi. Koruyucu faktörler köpeği bağlama/kısıtlamayı, ayrı kafesin kullanılmasını, kulübe etrafında uygun çitin kullanılması ile normal kırmızı akıntı ile pıhtılı kan arasındaki farkın bilinmesini içermekteydi. Yoğun olarak koruyucu önlemlerin alınması ve risk faktörlerinin kontrol edilmesi Pakistan'da köpek popülasyonu içerisinde CTVT'nin yayılmasını azaltabilir. Bu çalışma Pakistan'da CTVT için risk oluşturan faktörlerinin belirlenmesinde yapılmış ilk çalışmadır.

Anahtar sözcükler: *Canine, Pakistan, Risk faktörleri, Vaka kontrol, Bulaşıcı veneral tümör*

INTRODUCTION

Canine Transmissible Venereal Tumour (CTVT) is one of the unique neoplasms that can be physically transmitted during coitus by direct contact such as licking, scraping, and biting [1,2]. Thus it represents the cells with high number of mutations that are developed into pathogen. Tumorous cells can be transmitted only across abraded

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mucosa with broken epithelium. Acting like an infectious pathogen, cells propagate and establish in the organism. These cells can also transmit from mother to the offspring during social interactions such as grooming and other maternal behaviour. It is a well-known tumour of domestic dogs [3,4]. Based on these, CTVT is seen as a major threat to the reproductive efficiency of canines [5].

CTVT is much prevalent in countries with tropical and sub-tropical settings due to loose import laws [6]. Due to similar settings and loose canine import laws, it is possibly a prevalent disease in Pakistan. Although considerable work regarding diagnosis and therapeutic management has been reported in Pakistan [7,8]. However, as compared to other countries, there is little work has been done in Pakistan regarding the risk factors associated with CTVT [6,9,10].

Previous studies have revealed that introduction and transmission of CTVT is associated with age, breed, sex, purpose of dog keeping [3,10], contact with stray dogs and high human population density areas [9]. These mentioned risk factors are important in identification of high-risk areas and kennels, which could be planned for interventions like treatment. Furthermore removal of these identified risk factors could be important part of disease control policies.

Based on such importance of breeding inefficiency and epidemiology of CTVT, the current study was planned. The main objective of this study was to investigate the risk factors associated with CTVT in owned dogs under local geographical conditions of Pakistan. These findings may also provide baseline for studying the spread of CTVT in similar geographical conditions.

MATERIAL and METHODS

A case control study was conducted in the Lahore District, Punjab, Pakistan;included the clinical cases between January 2014 and January 2015. Data related to cases and controls were collected from a total of 24 clinical practices in Lahore. From this data, 96 cases of CTVT and 96 controls (n=192) were required to achieve the study power of 80% for detection of 2.0 having odds ratio with 95% confidence interval [11]. A team of researchers visited the homes/kennels to collect the data related to risk factors. A structured questionnaire was used to get information from the dog owners.

Cases were included from the records of those pet dogs that exhibited the clinical signs (cauliflower like lesions on external genitalia, serosenguinus clotted bloody discharge, lethargy) of CTVT and were confirmed by (impression smear and aspiration needle) cytology with Leishman-Giemsa (LG) staining techniques followed by the microscopic examination [2]. In control, dogs visiting the clinics on the same day for other problems like vaccinations, routine check-ups, fever of unknown origin, ectoparasites, dyspnoea, wounds, and other infectious diseases were included. These dogs were considered as negative if they did not show any clinical signs [2,12,13]. Only those dogs were considered as cases which are kept as owned dogs and are not neutered. Those dogs were excluded from the study which were neutered and not owned.

A pre-designed questionnaire was used for the collection of data. The questionnaire contained several questions representing the different risk factors on the baseline parameters of the dogs including sex, age, type of dog (Purpose of dog keeping), breed and conditions related to management and breeding practices. Stray dog population was defined as the presence of free-roaming dogs with no owners in 1 km of the distance of the area. Proper fencing was considered as the presence of boundary walls around the house/kennel.Dogs in neighbourhood were defined as the presence of pet dogs in ten houses around the house/kennel. Usually bloody discharge during the proestrus phase and vaginal cytology are used to confirm the approaching oestrus. But considering only bloody discharge as the sole indicator heat can be easily confused with the clotted blood that is released during CTVT. That is why the knowledge related to breeding practices involving bloody discharge was also included as risk factors. These selected risk factors were the result of extensive review of previous studies and observations of local canine keeping practices [3,8-10]. The questionnaire and the variable boundaries were explained to the owners by the interviewer so that owner could answer those questions easily.

The location of all the cases was recorded with Global Positioning System (GPS, Garmin, Olathe, KS, USA) in WGS-84 datum. Chloropleth map was generated in Q-GIS 2.6.1 (Quantum-GIS Group). Geographical data of Lahore District consisting of boundaries and administrative town divisions were downloaded from the internet (http://www.diva-gis.org/datadownand http://lwmc.com.pk/company_profile.php).

To manage the data, a database was created in Microsoft Excel 2010. Data was analysed by using SPSS® (Version 16.0, Chicago, USA). Univariate analysis for dichotomous variables was applied to observe the association among different variables divided into 2 levels encoding Yes/High/Guard/Own = 1 and No/Low/Common/Pet = 2. Odds ratio (OR) was calculated for the assessment of risk factors. Logistic model regression was also applied to analyse the complete relation between the variables. Variables with P value <0.2 were selected in multivariable logistic regression model with forward method. Similar method was repeated until all remaining variables showed the P value <0.05 [14]. The two tailed P value < 0.05 was considered as statistically significant. The Hosmer and Lemeshow goodness-of-fit test was also employed in order to analyse the fit of the model to the data.

RESULTS

A total of 96 exposed dogs and 96 non-exposed dogs were visited and interviewed.Locations of exposed dogs were recorded and marked on the map in *Fig. 1*. Map depicts that cases were most frequentin urban areas/ towns of Lahore district compared to rural areas/towns.

Univariate analysis screened a total of 15 variables from which 12 variables (dog keeping purpose (guard/pet), dog in neighborhood, high density human population area, history of skin/genital diseases, mating partner, number of dogs per house, stray dog population, red vulvular discharge as only heat indicator, chain/restrain the dog, use of separate cage, use of proper fencing around the kennel and knowing the difference between normal red discharge and clotted blood were found associated with either case or control *(Table 1)*. The 3 intrinsic factors of dogs i.e. age, sex and breed were found having no association with CTVT. Of these 12 variables, 8 were found as potential risk factors i.e. associated with increasing the odds of exposure in cases as compared to controls. Among these, the type of breeding dog/mating partner is the most affecting variable with 170% increase followed by history of skin/genital diseases and dogs in neighbourhood affecting 110% increase in disease occurrence. But the Remaining 4 factors were determined as protective factors i.e. associated with decreasing the odds of exposure in cases to controls. These factors included chain/restrain the dog, use of separate cage, use of proper fencing around the kennel and knowing the difference between normal red discharge and clotted blood.

In first multivariable, all the variables except red blood discharge as sole indication of breeding and number of dogs per householdhad P-value <0.05 *(Table 2)*. Therefore a new multivariable model was produced without these two variables *(Table 3)*. No interactions were found in the final multivariable model under all the parameters with interaction coefficients found statistically non-significant. Confounding was not found among variables with difference between ORs lower than 0.012 The P-value for Hosmer and Lemeshow goodness-of-fit test was 0.6, which shows a good fit of theoretical model.

DISCUSSION

In Pakistan, limited information is available regarding canine diseases especially CTVT [6]. This study is the first document that explains the epidemiological aspects associated with the spread of this disease in Pakistan.

CTVT can be diagnosed with different methods but the commonly used methods are histopathology and cytology. During the study, cytology based approach was less costly and rapid as compared to 2-3 day long costly histopathology [2,12,13].

Current study implicates that there is no significant difference between genders, age groups with various breeds either imported or mongrels that can influence the disease burden. These results are similar to previous studies [9,10]. The possible cause of variable frequency among the groups is might be due to their key importance in dog breeding business and notion of obtaining higher sperms and ova quality [15,16].

This study entails that factors allowing contact between dogsare the main reasons that can spread this disease. This study involves several factors that directly relate to biosecurity of the kennel/homestead (i.e. chain/restrain the dog, use of separate cage, use of proper fencing around

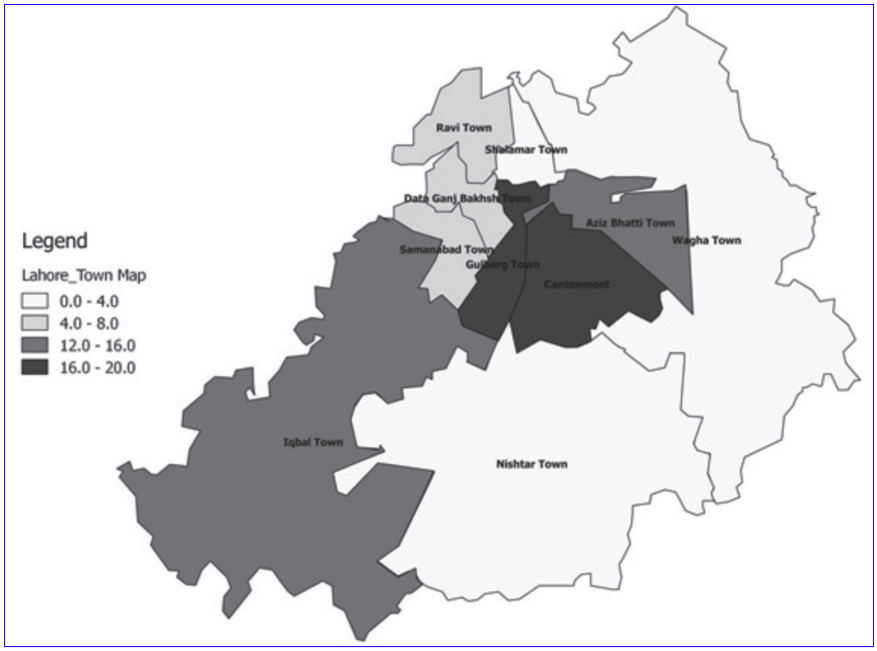

Fig 1. Range of CTVT cases in different towns of Lahore District

(Urban Towns = Ravi Town, Shalamar Town, Data GanBakhsh Town, Samanabad Town, Gulberg Town, Aziz Bhatti Town; Rural Towns = Wagha Town, Nishtar Town, Iqbal Town)

Table 1. *Univariate analysis of the factors included in the study*

S. No.	Risk Factors	Levels	Case	Controls	P Value	OR	CI 95%	
1	Age	< 5 Years	60	54	0.35	1.33	0.72	2.45
		> 5 Years	36	42				
2	Gender	Male	46	45	0.89	1.03	0.611	1.75
		Female	50	51				
3	Chain/Restrain the dog	Yes	38	55	0.019	0.51	0.291	0.9
		No	58	41				
4	Cage Type	Separate	35	53	0.0079	0.43	0.233	0.819
		Common	61	43				
5	Knowing the difference between normal discharge and clotted blood	Yes	33	47	0.04	0.56	0.31	1
		No	63	49				
6	Dog breed	Imported	47	45	0.87	1.08	0.5	1.8
		Mongrels	49	51				
7	Proper fence	Yes	28	41	0.032	0.48	0.24	0.95
		No	68	55				
8	Purpose of dog keeping	Guard	59	41	0.014	2.01	1.13	3.5
		Pet	37	55				
9	Dogs in neighbourhood	Yes	68	41	0.00015	3.25	1.7	6.2
		No	28	55				
10	Density of human population in area	High	68	38	0.00001	4.2	2.1	8.37
		Low	28	60				
11	History of skin/genital diseases	Yes	58	26	0.00001	4.2	2.1	8.37
		No	38	70				
12	Type of breeding dog	Common	69	30	0.0000013	5.4	2.6	11.08
		Own	27	66				
13	No of dogs per house	< 2 Dogs	58	36	0.0011	2.83	1.46	5.47
		> 2 Dogs	39	60				
14	Stray dog population in the area	Yes	71	49	0.0009	3.01	1.51	5.93
		No	25	47				
15	Red bloody discharge as sole indicator of heat	Yes	66	51	0.03	2.05	1.076	3.71
		No	30	45				

the kennel).These factors were found protective (OR <1.0) in this study and in previous study [17].

With compromised biosecurity, certain factors (i.e. stray dog population in the area, dogs in neighborhood, high density human population area and number of dogs per household) favour the introduction of infected dog and consequently the disease spread rapidly. All these factors were included in the multivariable models and they revealed high association with the occurrence of CTVT (OR >2.0). This multivariable model explained the guard dogs with common mating partner/practices and stray dog population in the area have more risk of getting the disease. Among all the variables, mating practices is the most important risk factor that can increase the risk of getting disease 3 times more. Although other variables, excluding above discussed variables, have less higher OR values but still they can pose a risk.

Stray population is considered as reservoir of CTVT infection [3]. In the final multivariable model, Stray population of the area showed the higher association with risk of contracting CTVT (OR =3.35, P<0.001). As mentioned in previous studies, failing the biosecurity measures introduces the infected stray dogs into kennels/ households that mightlead to spreading the infection [9]. Apart from the stray populations, infected dog in the kennels and nearest vicinity can be remained undiagnosed especially females due to less obvious clinical signs. Final multivariable modelrevealed that presence of dogs in neighborhood wasfound associated with the CTVT (OR=2.87). High density human population areas are

Table 2. *Mutivariable regression model including all the variables with P-value <0.25*

Potential Risk Factors	Levels	Regression Coefficient	Standard Error	OR	95% C.I.	P value
High density population area (N= 68)	High	0.91	0.385	2.503	1.176 - 5.327	0.017
	Low					
Dogs in neighborhood (N=68)	Yes	1.07	0.388	2.937	1.371 - 6.289	0.005
	No					
Stray dog population (N=71)	Yes	1.28	0.404	3.626	1.640 - 8.014	0.001
	No					
No of dogs per household (N=58)	< 2 Dogs	0.63	0.387	1.896	0.887 - 4.05	0.098
	> 2 Dogs					
Dog keeping type (N=59)	Guard	1.25	0.413	3.520	1.565 - 7.915	0.002
	Pet					
Mating partner (N=69)	Common	1.70	0.413	5.480	2.434 - 12.334	< 0.0000
	Own					
History of diseases (N=58)	Yes	1.04	0.390	2.838	1.319 - 6.105	0.007
	No					
Red bloody discharge as indication of heat (N=66)	Yes	0.67	0.392	1.967	0.911 - 4.25	0.084
	No					

Table 3. *Final multivariable regression model excluding the red discharge as indication of heat and number of dogs per household*

Potential Risk Factors	Levels	Regression Coefficient	Standard Error	OR	95 % C.I.	P value
High density population area	High	0.93	0.38	2.54	1.21-5.32	0.01
	Low					
Dogs in neighborhood	Yes	1.05	0.38	2.87	1.36-6.03	0.01
	No					
Stray dog population	Yes	1.21	0.40	3.35	1.54-7.25	0.00
	No					
Dog keeping type	Guard	1.41	0.40	4.11	1.87-9.05	0.00
	Pet					
Mating partner	Common	1.85	0.40	6.36	2.88-14.01	0.00
	Own					
History of diseases	Yes	1.05	0.38	2.86	1.35-6.05	0.01
	No					

The same no of cases were mentioned as in Table 2

also considered as risk for contracting CTVT (OR=2.54); might be because of more congesting living style and more interaction. Relationship of presence of dogs in neighborhood and high density human population areas with CTVT has been also discussed in previous studies [9,10].

Breeding and general management of kennel is considered to be playing very important role here in the spread of this disease. Factors such as purpose of dog keeping, red vulvar discharge as sole breeding indicator, knowing the difference between normal red discharge and clotted blood, and type of breeding partner were showing significant differences (P<0.001). Purpose of dog keeping was included in the final multivariable model that resulted in higher risk of contracting disease (OR=4.11). Guard dogs have also been considered as prone to risk of getting CTVT in previous studies [9]. Regardless of estrous cycle/breeding management, guard dogs are routinely released in night for security purposes which entails the risk of their mating with infected dog. While such practices are absent in pet dogs.

In canine breeding, red vulvar discharge in female dogs is considered as good indication of approaching oestrus but it should be further confirmed with the vaginal cytology [18]. Red discharges can also be produced in

different pathological conditions therefore should be confirmed [19]. This study implicates that owners considering red vulvar discharge as sole breeding indicator were posing their dogs to contract and spread this disease to other dogs (OR=1.96*)*. While knowing the difference between normal red discharge and clotted blood is considered as protective factor that can prevent the mating with infecting dog (OR=0.5*)*.

Another factor in the canine breeding practices is utilization of one of the two types of breeding dog i.e. common breeding dog and own breeding dog. Common breeding dog for several kennels/household is usually allowed to mate with any other dog on the account of money while own dog is not allowed to mate with any other dog outside the house and kennel. This study has revealed that due to utilization of common dog unchecked, this disease is usually spreading and persisting in Pakistan (OR=6.36*)*. In previous studies specifically related to CTVT [7,8], this factor has not been studied. This factor is commonly studied in all other reproductive disorders that spread through venereal transmission [20]. In addition to this, owners having less number of dogs are more exposed to this disease because they usually introduce the common breeding dog that exposes them to potential risk factor of getting this disease. These risk factors are newly devised to the risk factor studies related to CTVT. This is new addition to knowledge about epidemiological factors of this disease.

The basic objective of the study was the identification and quantification of potential risk factors associated with CTVT among owned dogs in Pakistan. This study has identified some new risk factors like utilizing common mating dogs and poor breeding practices along with similar risk factors that were already documented from previous studies. Guidelines and policies focusing the control of these risk factors could reduce the risk of CTVT infection in owned dogs in Pakistan and in other developing countries oftropics. Good management practices and strict biosecurity together can prevent the unchecked introduction of infected dogs. There should also be improvement in breeding practices and management. There are many examples of quarantines and biosecurity policies that include prohibits introduction of any dog into kennel either stray or owned, clinical examination before mating and confirmation of estrus.

Conflict of Interest Statement

The authors declared that they have no conflict of interest.

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