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Occurrence of canine distemper virus infection in guwahati (Assam)

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Abstract
In this study, a total of 167 clinical samples were screened by cytological examination, where 58 cases (34.73%) were found positive. The most common clinical signs recorded in the positive cases were pyrexia (102.8-105°F), mucopurrulent ocular and nasal discharges, hyperkeratosis of the digital pads, vesiculo-pustular dermatitis and nervous disorders. The variable neurological signs were chorea, paddling and cycling movement, muscle tremor, epileptic seizure with facial twitching. Occurrence of Canine Distemper (CD) was recorded highest in young dogs 0-6 months (55.17%) of age group. Season-wise more occurrence rate was recorded in winter (48.81%) months. Highest occurrence was also recorded in local non-descript (43.10%), unvaccinated (72.41%) and male (58.62%) pups. Canine distemper infection based on age, season, breed, sex and vaccination status were found significant ($P<0.05$) in statistical analysis. The present study suggests that regular annual vaccination against CD followed by booster dose can protect the dogs from infection.

Keywords: Canine distemper, Dogs, Occurrence, Vaccination

1. Introduction
Canine distemper (CD) is a highly contagious and fatal viral disease of canines, which was recognized as one of the leading cause of dog mortality [1]. Canine distemper virus (CDV) infects the susceptible animals by inhalation and the virus shed primarily in the ocular and nasal secretions [1, 2]. Infection caused by CDV might lead to subclinical diseases, which involves respiratory problems, rhinitis, fever and often linked with CNS disorders [1, 3]. CDV most commonly infect the puppies, when there are low maternally derived antibodies [4]. The disease is highly prevalent in winter season [5]. A variety of clinical parameters and different types of assays have been suggested to use for the definitive antemortem diagnosis of distemper. However, due to the unpredictable and variable course of the disease, final diagnosis for most of the animals remains uncertain. Various samples including conjunctival and nasal swabs, blood smears and cerebrospinal fluid have been used for the diagnosis of the disease [6, 7]. The virus produces inclusions of variable shape and size in different cells and can be used for diagnosis of the disease [8, 9].
CDV vaccines are commercially available and given in combination with other vaccines such as leptospirosis, hepatitis, Para influenza and parvovirus vaccines (DHPPi+L). Now a days regular vaccination against CD is almost customary to prevent the occurrence of the disease. However, the disease remains as a major problem in dogs even after regular vaccination against the disease [10]. In recent years, the occurrence of CD has also been recorded frequently in Guwahati city of Assam. Therefore present study was carried out to determine the occurrence of CD in Guwahati (Assam) in relation to age, sex, season, breed and vaccination status of the affected dogs.

2. Materials and Methods
2.1 Sample collection
One hundred and sixty seven blood samples, conjunctival and nasal swabs were collected from the suspected dogs brought to Teaching Veterinary Clinical Complex, College of Veterinary Science, Assam Agricultural University and other private clinics within Guwahati city after getting ethical approval from Institutional Animal Ethics Committee. The blood samples were collected by venupuncturing cephalic or sephanus vein and collected in an EDTA vacutainer, while conjunctival and nasal swabs were collected at room temperature with sterilized cotton swabs. Dogs showing clinical signs of oculao-nasal discharge, gastroenteritis, high rise of

temperature (102.8-105°F), pustular dermatitis, hyperkeratosis of digital pads and central nervous system disorders were suspected for the clinical cases of canine distemper. The samples were collected during the period from March, 2016 to February, 2017. Age, breed, sex and history of vaccination of the clinically suspected dogs were recorded before collection of samples.

2.2 Cytological examination
The disease was diagnosed based on demonstration of viral inclusion body in the cytological examination of ocular and nasal epithelial cells as well as in blood cells. Immediately after collection of conjuctival and nasal samples, smears were prepared on clean grease free glass slides. Similarly a thin blood smear was also prepared from the blood collected from peripheral veins. Smears were fixed with methanol and stained with Giemsa stain [11] for microscopic identification of viral inclusions in ocular and nasal epithelial cells [12] with different circulatory blood cells [13, 14, 15, 16].

2.3 Occurrence study
To study the age-wise occurrence it was divided into four different age groups viz. 0-6 months, 6-12 months, 12-24 months and above 24 months. To study the effect of seasons on occurrence of CD, the whole year was divided into four seasons viz. (i) Pre-monsoon (March-May), (ii) Monsoon (June-September), (iii) Post-monsoon (October-November) and (iv) Winter (December-February) according to the Meteorological Department, Govt. of Assam, Borjhar, Kamrup. Susceptibility of different breeds to CD was analysed by dividing into three categories viz. Local non descriptive, cross bred and pure breeds. Occurrence was also studied in both the sexes. The occurrence of the disease was also studied in vaccinated, irregularly vaccinated and unvaccinated dogs.

3. Ethical consideration
The ethical approval was granted by the Institutional Animal Ethics Committee.

4. Statistical analysis
Results are reported as percentage and the differences between groups were compared with Chi-square analysis. All analyses were performed with standard software (IBM SPSS Statistics 20 software); values of $p<0.05$ were considered significant.

5. Results and Discussions
Out of total 167 clinically suspected samples, screened by cytological examination, 58 cases were found positive for CDV infection, thus indicating 34.73 percent positivity. Canine distemper infection was also reported by earlier workers in Assam, which was slightly lower than the present study [5]. Their study was purely restricted on neurological signs and post-mortem pathology and that might be reason for lower prevalence. However, prevalence study made by different workers from different areas showed variable prevalence percentage, which might be due to managemental and different geo-climatic conditions [10, 17, 18]. As the CD virus is pantropic in nature, has affinity towards different cells including epithelial and circulatory blood cells. The virus replicates within these cells so, viral inclusions were found in the different cells [19]. Previous workers examined the cytology of different cells and considered as an easy diagnostic method for CD diagnosis. The size, shape and characteristics of inclusions in blood and exfoliated epithelial cells were well described in earlier workers and agreed with the present findings [12].

5.1 Age-wise occurrence of CDV infection
The age wise occurrence of CD infection was significantly ($P<0.01$) higher (55.17%, n=32) in dogs of 0-6 months of age followed by 6-12 months of age (22.41%, n=13), 12-24 months of age (17.24%, n=10) and low in dogs (5.17%, n=3) above 24 months of age (Table 1). Previous workers also reported similar findings and opined that lack of maternal immunity and poor immune competency for the acquired immunity at the young age could be incriminated as the cause of high occurrence of CD in young animals [17, 20, 21]. However, some reported that age-wise prevalence of CD was not significant [22, 23]. In contrast to the present findings, many workers reported that dogs above 5 years of age were more susceptible to CD compared to the puppies [24, 25]. However, different workers have reported different age groups in the susceptibility to CD [10, 26, 27].

5.2 Season-wise occurrence of CD
Highest occurrence was observed in winter (70.69%, n=41) compared to post-monsoon, pre-monsoon and summer where occurrence were 20.83 percent (n=5), 7.41 percent (n=2) and 31.25 percent (n=10) respectively (Table 1). The seasonal occurrence of CD was found to be statistically significant ($P<0.01$) in chi-square test. These findings were agreed with earlier workers [5, 17]. As the canine distemper virus is liable and readily inactivated by heat therefore, it cannot thrive in hot environment for prolong period of time [28]. However, the virus is resistant to cold and remains viable in low temperature which enhances the transmission of the disease in winter [26]. This could be the cause of highest occurrence of the disease in winter months. Moreover, droplet infection increases in the winter season due to large amount of dust and dirt particles present in the dry environment which helps to transmit the infectious particles more readily to distant areas. Earlier findings also revealed that stress developed in winter may be one of the predisposing factors for the infection, as stress results decrease immunity in animals [29].

Table 1: Effect of occurrence of CD on season, age, breed, sex and vaccination status

Particulars		Total no. of sample examined	Positive samples	Occurrence (%)	Chi-square value (P)
Season	Pre-monsoon	58	5	8.62	153.63 (0.000)
	Monsoon	58	2	3.45	
	Post-monsoon	58	10	17.24	
	Winter	58	41	70.69	
Age group	0-6 months	58	32	55.17	73.09 (0.000)
	6-12 months	58	13	22.41	
	12-24 months	58	10	17.24	
	> 24 months	58	3	5.17	
Breed	Local	58	25	43.10	14.54 (0.002)

	Cross	58	11	18.97	
	Pure breed	58	22	37.93	
Sex	Male	58	34	58.62	5.95 (0.015)
	Female	58	24	41.38	
Vaccination status	Non-vaccinated	58	42	72.41	105.50 (0.000)
	Vaccinated	58	11	18.97	
	Irregularly vaccinated	58	5	8.62	

5.3 Breed-wise occurrence

Higher prevalence was observed in local dogs (43.1%; n=25) followed by pure breeds (37.93%; n=22) and cross bred (18.97%; n=11) dogs. The study also showed significant differences ($p<0.05$) between breeds. The finding of this investigation was in agreement with other studies [20]. The local non-descript dogs were not vaccinated regularly against CD and their population were higher than cross bred and pure breed dogs, which make them most susceptible to the disease. In contrast to this finding, few workers stated that breed has no relation with occurrence of the disease [22].

5.4 Sex-wise occurrence

In this study, sex-wise occurrence of CD infection revealed highest in male (58.62%; n=34) dogs than their female (41.38%; n=24) counterparts. The study also showed significant differences ($p<0.05$) between sex in chi-square rest. These findings were similar with previous workers [20]. The higher occurrence in male dogs might be due to higher population of male animals in and around the city. Some studies reported that gender has no significant role in the susceptibility of animals to CD. The present findings disagree with the previous study, who reported occurrence of CD has no association with sex [1, 22, 23].

5.5 Vaccination status

In the present study highest occurrence of CD was recorded in non-vaccinated animals (72.41%; n=42) and least in irregularly vaccinated animals (8.62%; n=5). Occurrence of the disease was also reported in vaccinated (18.97%; n=11) group of dogs. The chi-square test revealed significance difference in occurrence of the disease on vaccination status (Table 1). These observations were correlated with previous findings [26]. Occurrences of CD in vaccinated as well as irregularly vaccinated dogs were reported by many earlier workers [10, 26]. Previous workers stated that occurrence of the disease in vaccinated animals might be the result of vaccination failure either due to host factor or vaccine itself [1]. According to the earlier studies, the probable cause of vaccination failure could be the maternally derived antibody or passively acquired antibodies at the time of vaccination. Other factors that have been reported for vaccination failure included delay in the maturation of immune system or organs, genetic inability to respond to certain vaccine antigens, ineffective vaccination, immunosuppression duo to other disease conditions or physiological stress as well as poor vaccine immunogenicity [10] .Improper maintenance of cold chain at the time of transportation of the vaccines may lead to the vaccination failure. Few workers also reported that low dose, multiple and inappropriate route of vaccination were the cause of persistence of the disease in the vaccinated population of animals [30].

6. Conclusions

This study revealed that cytology can be used for diagnosis of the disease where inclusions were recorded in different cells. The most susceptible age for canine distemper infection is 0-6 months. The highest occurrence of the disease was observed in local, male and unvaccinated dogs. However, some percentage of vaccinated animals also revealed CVD infection due to vaccination failure. Therefore, this study suggests that regular vaccination followed by booster dose can protect the animals from the canine distemper infection.

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8. References

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