Int.J.Curr.Microbiol.App.Sci (2020) *9*(9): 3260-3266

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 9 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.909.404

Scholarly View of Canine Distemper Cases in Mizoram

Tasso Yama[1], J. B. Rajesh[1*], Hridayesh Prasad[1], T. K. Rajkhowa[2], Kalyan Sarma[1], Parimal Roychoudhury[3], Devajani Deka[4] and Suvendu Kumar Behera[1]

[1]Department of Veterinary Medicine, [2]Department of Veterinary Pathology, [3]Department of Veterinary Microbiology, [4]Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences and Animal Husbandry (Central Agricultural University), Selesih, Aizawl, Mizoram, India

**Corresponding author*

Keywords

Antigen, Canine Distemper, Dog, Incidence, Mizoram

Article Info

Accepted:
24 August 2020
Available Online:
10 September 2020

A B S T R A C T

The objective of this study is to determine the incidence of Canine Distemper and its effects on hematology in the canine population in Mizoram. The animals were selected on the basis of history and suspected clinical signs of Canine Distemper (CD). The Antigen Rapid CD Virus Ag Test Kit (BioNote Inc, 2009) used for the qualitative detection of CDV in conjunctiva, urine, serum or plasma. Ten CD positive and ten healthy and CD negative animals were selected. Blood was collected and haematology (Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte count (TEC), Total Leukocyte count (TLC), Differential Leukocyte count (DLC) and thrombocyte) was performed using Automated Blood Cell Counter. Statistical analysis (T test) was employed and interpreted. Out of 900 animals, 40 were suspected for CD on the basis of clinical signs and 10 cases were confirmed with antigen detection test. The incidence rate of canine distemper was observed as1.11% (10/900). 80% (8/10) positive cases were observed in age group of two to six months. Hb, HCt., TEC, TLC, Lymphocyte and Monocyte values of CD infected dogs were significantly ($p<0.01$) decreased than healthy ones. T test was used. CD is prevalent in the canine population of Mizoram. It can be detected by clinical signs combined with Antigen detection. It causes considerable haematological changes. Immunization can prevent the incidences of CD.

Introduction

Canine distemper is a highly contagious, systemic, serious viral illness seen worldwide that affects several organ systems in dogs. Most cases of Canine distemper are fatal. Canine distemper (CD) is the most important worldwide infectious disease of domestic dogs (*Canis familiaris*), and its fatality rate is second only to that of rabies (Degene *et al.*, 2019). CD is caused by canine distemper virus (CDV), first isolated by Carre´ in 1905 (Ogbu *et al.*, 2017). CDV is a Morbillivirus under Paramyxo viridae family (Swati *et al.*, 2016). CDV has been suggested to have mutability and a zoonotic potential that require few amino acid changes (Kennedy *et al.*, 2019). Immunization of pups with

multivalent vaccine which protect against canine distemper virus, canine parvo virus, canine adenovirus type 2, canine parainfluenza virus, and leptospirosis is between six to eight weeks of their age (Vasu *et al.,* 2019).

The causative agent, CDV, is an enveloped, negative-sense, single stranded RNA virus. The virus contains six structural proteins termed as nucleocapsid (N), phospho (P), large (L), matrix (M), haemagglutinin (H) and fusion (F) protein, and two accessory non-structural proteins (C and V) that were found as extra transcriptional units within the p gene as in other paramyxoviruses (Ruiz-Saenz *et al.,* 2019). CDV is a pantropic virus that shows a broad cell tropism.

Accordingly, CDV can be found in cells of the respiratory, gastrointestinal and urinary tract, as well as in lymphoid tissues, endocrine organs and the 2 central nervous system (CNS) (Pratakpiriya *et al.,* 2017). Except for one report (Lalrinkima *et al.,* 2019) there are no detailed literatures on the incidence of CD in Mizoram. This study was conducted with the objective of finding the incidence of CD in Mizoram and to report the haematological parameters of the affected dogs.

Materials and Methods

This work is having approval of Institutional Animal Ethics Committee.

Selection of animals

The animals were selected on the basis detailed history and suspected clinical signs of CD. Animals with symptoms of diarrhoea, vomition, ocular and nasal discharge, hyper-keratinization of nasal and foot pad and nervous signs were selected. Recording of vital signs (temperature, pulse rate and respiration rate) and physical examination (mucous membrane, capillary refill time (CRT) and skin tenting test (STT)) were done. Blood (5 mL) was collected in K3 EDTA vial from the selected dogs and from apparently healthy dogs for haematological evaluation. The haematological examination was done using Automated Blood Cell Counter by MeletSchloesing Laboratories (MS4e, Netherlands). The haematological parameters that were recorded were hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC) and thrombocyte count.

Confirmation of CD by Anigen Rapid CDV Ag Test Kit

The Anigen Rapid CDV Ag Test Kit (BioNote Inc, 2009) is a chromatographic immunoassay for the qualitative detection of Canine Distemper virus antigen in conjunctiva, urine, serum or plasma. After collecting the specimen using swab, the specimen was immediately extracted and tested. The specimens that were not immediately tested were refrigerated at 2~8℃ for storage not less than 48 hours and the specimen was freezed at -20℃ or below. The test was performed as per the standard protocol (BioNote Inc, 2009). The test reads as follows:

Negative: The presence of only one band within the result window indicated a negative result.

Positive: The presence of two colour bands indicates a positive result

Invalid: If the color band was not visible within the result window

Statistical analysis was employed (Snedecor and Cochran 1994) and interpreted.

Results and Discussion

Incidence of canine distemper

During a two-year trial, 900 dogs were presented at OPD, Teaching Veterinary Clinical Complex, College of Veterinary Sciences and Animal Husbandry, Selesih, Aizawl and State Veterinary Hospital, Khatla.

In these, 40 cases were suspected for CD on the basis of clinical signs and 10 cases were positive with antigen detection test. The incidence rate of canine distemper was observed as 1.11% (10/900). 80% of the positive cases were observed in age group of two to six months and 20% (2/10) were in adult group.

Haematological parameters

Alterations of haematological parameters in CD infected dogs are shown in Table 1.

Discussion

Diagnosis by CDV antigen test kit

In this study CDV antigen was detected in serum, plasma, eye and nasal discharge by the use of CDV antigen test kit. Ten out of 40 dogs suspected for CD, tested positive to CDV. This study showed that rapid CDV antigen test kit is highly sensitive and specific. Kit can detect CD antigen qualitatively in samples like eye and nasal discharges, serum and plasma. Out of 10 cases, only one positive dog was vaccinated. It showed that the dogs harbor CDV antigen despite vaccination. This could be attributed to vaccination failure (Anis *et al.,* 2018). Previously, studies have shown that one of the causes of vaccine failure could include result of maternally derived antibody (MDA) or passively acquired antibodies (PAA) at the time of vaccination (Babalola *et al.,* 2015). Under developed immune system, nonresponsiveness to vaccine antigens, poor vaccines and immunosuppression are causes of vaccine failure (Eghafona *et al.,* 2007).

Incidence of canine distemper in and around Aizawl district of Mizoram

An incidence (1.11%) of CD in dogs reported in this study. Its s observed that the incidence of CD is more in higher host density area where the contact rates and exposure were high. Non vaccinated pups between eight weeks to six months are more vulnerable to infection and also the roaming behavior adds to infection as there is higher chance of exposure to pathogen (Curi *et al.,* 2016). Human assisted movement facilitates transmission between rural and urban areas (Ng *et al.,* 2019). The virus secretion can continue 60 to 90 days following infection. In some studies reports, CDV seropositive healthy animals were observed which denotes virus circulation, infection and recovery (Martinez-Gutierrez and Ruiz-Saenz 2016). CDV infection can result in subclinical infection or clinical infection but majority of the infections (75%) occurs as subclinical infections (Gizaw *et al.,* 2016).

The percentage of CD in different research works in different areas varies. This variation in CDV prevalence may be related to the degree of specificity of evaluation method, phase of CD present and the immunological status of dogs. The CDV affect unvaccinated dogs or dogs with imperfect immunization. Such dogs need further immunization to reduce mortality, to limit clinical cases and to control spread (Nova *et al.,* 2018). Hospital-based surveys may not reveal the real prevalence of canine distemper in urban dog populations when compared with field surveillance studies (Headley and Graça, 2000).

Table.1 Alterations in haematological parameters

No	Parametres	Infected dogs (n=10)	Healthy dogs (n=10)	t-value	Sig
1	Hb (g/dL)	11.49+0.5b	14.75+0.89	-3.066	0.007**
2	HCt	34.89+1.70b	47.73+3.01	-3.708	0.002**
3	TEC (M/mm^3)	5.91+0.29b	7.20+0.45	-2.368	0.029**
4	TLC (M/mm^3)	11.55+1.75b	17.53+1.66	-2.478	0.023*
5	Lymphocyte (%)	9.85+1.47	13.69+1.48	-1.834	0.083*
6	Monocyte (%)	1.84+0.13b	2.92+0.27	-3.548	.002**
7	Granulocyte (%)	88.03+1.55b	83.46+1.58	2.055	.055*
8	Thrombocyte (x $10^3/\mu L$)	246.0+53.71	174.70+25.04	1.203	0.245^{NS}

Table.2 Clinical signs in CD cases

No	Clinical sign	CD Positive cases (n=10)	Percentage (%)
1	Fever	7	70
2	Vomition	6	60
3	Diarrhoea	8	80
4	Inappetance	7	70
5	Nasal discharge	1	10
6	Respiratory distress	0	0
7	Alopecia	1	10
8	Scabes	1	10
9	Pustules	3	30
10	Chorea	2	20
11	Sezures	2	20
12	Discharge from eyes	2	20
13	Hyperkeratosis	1	10
14	Champing of jaws	1	10

In age wise incidence study pups between 4 weeks and 6 months of age were 80 % more likely to contract the virus than adult dog. This observation was in agreement with earlier studies too and this can be attributed to poor maternal immunity and poor immunity at younger age (Buragohain *et al.*, 2018) whereas the presence of CDV infection in adult dogs might be due to the lack of routine vaccination and poor development of vaccine antibodies because of the maternal immunity interference during primary immunization or faulty storage and handling of 50 vaccine or immune status of the animal which results in quick depletion of antibodies (Day *et al.*, 2016).

Clinical signs observation in canine distemper infected dogs

In our study, diarrhoea was the predominant signs in CD *i.e.*, in 8 cases (80%) either alone or combined with other systemic signs (Table 2). CDV mainly affects three types of host cells viz. epithelial, lymphoid, and neurological cells. Dogs presented with neurological signs like cerebellar or vestibular signs may progress to tetra paresis or plegia. Such dogs can have distemper encephalomyelitis (Amude *et al.*, 2018). Demyelinating leukoencephalitis occurs as a result of direct virus-mediated damage and the invasion of CD8+ cytotoxic T cells, that

causes over expression of pro-inflammatory cytokines and CD4 + mediated delayed type hypersensitivity and cytotoxic CD8+ T cells contribute to myelin loss in the chronic phase (Ruiz-Saenz *et al.,* 2019). The extra neuronal signs in our study are well correlated with previous reports. Gastrointestinal and/or respiratory signs (systemic signs), frequently with central nervous system involvement, characterize the classical clinical presentation of distemper. The common systemic signs are purulent nasal discharge, coughing, dyspnoea, pneumonia, diarrhoea, vomiting and dermal pustules. Vomiting is a common finding in the early stages of infection. Diarrhoea develops and may be mild to severe. The oculo-nasal discharge was the prominent systemic sign in a study on neurologic distemper (Rendon-Marin *et al.,* 2019; Willi *et al.,* 2015 and Wyllie *et al.,* 2016).

Alteration of haematological parameters

The study revealed that Hb, Hct and TEC values of CD infected dogs were significantly ($p<0.01$) decreased than control group which indicated that CD infection was responsible for causing anaemia due to effect on hematopoietic system. The mean total RBC count, PCV and coagulation time were significantly lower in the canine distemper infected dogs than in the controls ($P<0.05$). Dogs affected with CD have lower TEC, Hb, PCV, and MCV and these changes are due to erythroid hypoplasia resulting from persistence of the virus in the bone marrow (Buragohain *et al.,* 2017). Thrombocytopenia is a result of bone marrow depletion. Hemogram of dogs with CD may indicate iron deficiency with microcytosis, hypochromasia, poikilocytosis, keratocytes and schistocytes (Bohn, 2013). CDV can cause bone marrow suppression and thus affect hematopoietic precursors leading to decreased production (Carter 2018).

There was significant decrease in the value of WBC in dogs affected with CD than healthy dogs. One important finding of CD infection is severe transient immunosuppression that may last for a few weeks to months after clinical cure. Apart from profound leukopenia, CDV induce anergy-like state in immune cells that can result in loss of delayed-type hypersensitivity activity. This makes patients susceptible to secondary infections like gastroenteritis and even pneumonia contributing to morbidity and mortality (da Fontoura Budaszewski and Von Messling, 2016).

The monocytes count of infected dogs showed significant reduction in comparison to healthy ones in the present study. Monocytes and macrophages are the first target cells propagating CDV (Beineke, 2015). There was increase in neutrophils in diseased dogs than normal dogs. The increase in granulocytes might be due to the response of immune system to bacterial infection and inflammatory process (Berghoff and Steiner, 2011).

In conclusion the CD is prevalent in the canine population of Mizoram. The incidence rate of canine distemper was observed as1.11% (10/900). It can be detected by clinical signs combined with Antigen detection. The Anigen Rapid CDV Ag Test Kit can be used for the detection of Canine Distemper virus antigen in different samples. CD causes considerable haematological changes. Hb, HCt. TEC, TLC, Lymphocyte and Monocyte values of CD infected dogs were significantly ($p<0.01$) decreased. Immunization can prevent the incidences of CD.

Acknowledgements

This research article is from the MVSc research work of the first author. The authors

are thankful to the Dean, College of Veterinary Sciences and Animal Husbandry, Selesih, Aizawl, Mizoram for all the help and facilities given.

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How to cite this article:

Tasso Yama, J. B. Rajesh, Hridayesh Prasad, T. K. Rajkhowa, Kalyan Sarma, Parimal Roychoudhury, Devajani Deka and Suvendu Kumar Behera. 2020. Scholarly View of Canine Distemper Cases in Mizoram. *Int.J.Curr.Microbiol.App.Sci.* 9(09): 3260-3266.
doi: https://doi.org/10.20546/ijcmas.2020.909.404