RESEARCH ARTICLE

Therapeutic Management of Transmissible Venereal Tumour in Dogs

Naiya P. Parikh[1]*, Mahesh T. Panchal[1], Jignesh J. Parmar[2], Dinesh J. Ghodasara[3]

Abstract

A total of 18 dogs affected with transmissible venereal tumour **(**TVT) were studied for therapeutic management under three groups each of 6 animals using vincristine alone (group 1), bacillus Calmette-Guerin alone (BCG) (group 2), and a combination of BCG and vincristine (group 3). Dogs having TVT were subjected to the confirmatory diagnosis using fine needle aspiration cytology (FNAC), impression smear and histopathological examinations prior to treatment. Treated dogs were monitored for their response to therapy with regards to physical regression of tumour masses along with histological changes. Complete regression of tumour masses was observed in the dogs treated with vincristine alone and combination of BCG and vincristine therapies. No such regression of tumour masses was observed in the dogs treated with BCG alone. Histologically the response of the tumour mass to the treatment was evinced by the appearance of macrophages and increased numbers of tumour infiltrating lymphocytes (TILs) followed by tumour cell apoptosis and necrosis with reduction in the mitotic index in group 1 and 3. The study indicated that combined therapy comprising BCG and vincristine was more effective than vincristine alone in treating TVT, however, BCG alone was not effective.

Keywords: Dog, FNAC, Histology, Impression smear, Therapeutic management, Transmissible venereal tumour.

Ind J Vet Sci and Biotech (2023): 10.48165/ijvsbt.19.4.16

Introduction

The cause of cancer is uncontrolled proliferation of cells. The main purpose of cancer therapy is to promote death of those abnormal (tumour) cells, without too much damage of normal ones. Before introduction of vincristine chemotherapy, canine transmissible venereal tumour (CTVT) was menace till the 1990s because surgery, immunotherapy, radiation therapy and gene therapy were not able to treat it effectively. Vincristine sulfate is an alkaloid obtained from *Vinca rosea* that blocks mitosis by arresting cells in the metaphase (Said *et al*., 2009). Immuno stimulants, including bacillus Calmette Guerin (BCG) (Patard *et al*., 1998) and a mycobacterial cell wall extract from *Mycobacterium phlei* (Morales *et al*., 2001) have been assessed in preclinical and clinical studies as therapy or adjuvant therapy for several tumours. Local immunotherapy with BCG has been utilized to treat several tumours (Knottenbelt and Kelly, 2000). The anti-tumour effect of BCG is well established, and is the choice of treatment in human recurrent bladder carcinomas (Han and Pan, 2006), and equine sarcoid (Knottenbelt and Kelly, 2000). Immunochemotherapy (BCG + Vincristine) treatment of CTVT is more efficient than vincristine alone. Vincristine caused tumour cell apoptosis, while BCG stimulated the local host immune system resulting in an increase in macrophages and tumour infiltrating lymphocytes (TILs) that induce tumour cell necrosis and apoptosis (Mukaratirwa *et al*., 2009). The objective of this study was to compare the therapeutic efficacy of BCG immunotherapy, vincristine chemotherapy and BCG + vincristine combination therapy for CTVT.

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How to cite this article: Parikh, N.P., Panchal, M.T., Parmar, J.J., & Ghodasara, D.J. (2023). Therapeutic Management of Transmissible Venereal Tumour in Dogs. Ind J Vet Sci and Biotech. 19(4), 78-83.

Source of support: Nil

Conflict of interest: None

Submitted: 20/05/2023 **Accepted:** 10/06/2023 **Published:** 10/07/2023

Materials and Methods

The study was conducted on a total of 18 dogs of either sex that were presented at the Veterinary Clinical Complex (VCC), College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand (India); Jivdaya Charitable Trust, Shree Danev Foundation, Ahmedabad; and Vadodara Centre for Animal Rescue and Emergency (VCARE) from November, 2020 to November, 2021 with the complaints of continuous vaginal or preputial bleeding and/or cauliflower like mass present on the genitals. The confirmatory diagnosis

was made using impression smears and histopathological examinations of fine needle aspirates of the lesions prior to treatment. Fine needle aspiration cytology collection (FNAC) involves a thin, hollow needle to remove samples of cells from tissue or fluid from the mass for making thin smears for microscopic examination. When the lesions were solid and exposed and/or ulcerated, the samples were directly taken from the site, using an impression smear (touch imprint). Biopsy tissue samples were collected in 10% neutral buffered formalin and subsequently processed for histopathology. The dogs were subjected to the three clinical management protocols involving 6 animals in each group. Dogs in group 1 (Vincristine alone) were treated with vincristine sulphate @ 0.025 mg/kg body weight, intravenous (IV) once a week for 3-5 weeks. In group 2 (BCG alone), bacillus Calmette-Guerin) 2-8×10^6 CFUs dissolved in 5 mL normal saline was used intratumorally as a local spray once a week for 3 to 5 weeks, and in group 3, a combination of Vincristine and BCG was used with above said regimens. Biopsy samples of TVTs were taken from the dogs (2-4 per group) of cooperative owners and according to the feasibility for histopathological examinations prior to treatment as well as after 2nd - 3rd and 4th week post-treatment for assessment of histological recovery.

Results and Discussion

FNAC of the cutaneous nodules and ulcerated nodules revealed large, individual, round or ovoid cells which contained discrete spherical hyperchromatic moderately pleomorphic nuclei with coarse chromatin granules and prominent single nucleolus and moderate rim of lightly basophilic cytoplasm with discrete vacuolation. Mitotic figures were evident in multiple fields (Fig. 1). The presence of inflammatory cells was characterized by neutrophils and lymphocytes.

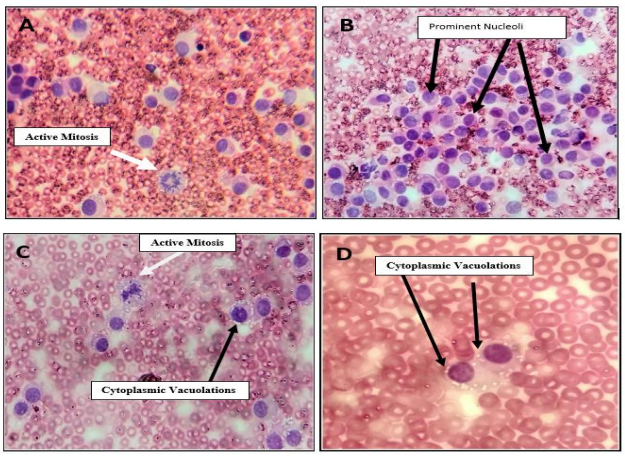

Fig. 1: Cytological features of nodular masses of TVT by FNAC in canines: A-active mitosis, B- prominent nucleoli, C- active mitosis as well as cytoplasmic vacuolations, D- cytoplasmic vacuolations) (40 X, Haematoxylin & Eosin stain)

The impression smears revealed distinct round to polyhedral cells arranged in sheets or individually. There was coarse nuclear chromatin pattern. The characteristic feature was presence of distinct vacuolation in the cytoplasm in majority of the cells. There was prominent anisocytosis and anisokaryosis. After staining, the confirmatory structure was visible, *i.e.,* highly vacuolated cytoplasm (Fig. 2).

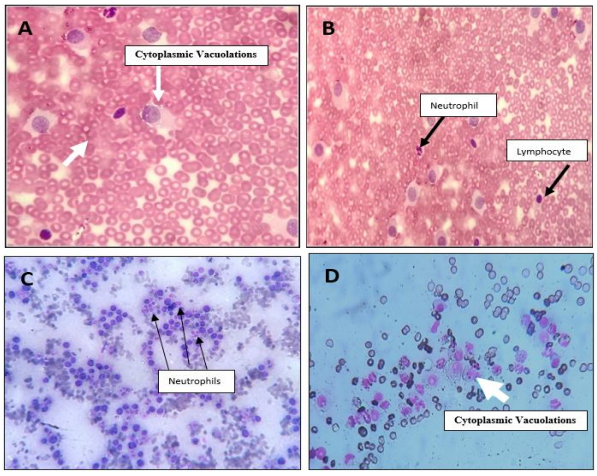

Fig. 2: Cytological features of impression smear of TVT in canines: A- cytoplasmic vacuoles, B- neutrophil and lymphocyte infiltration, C-neutrophils, D- cytoplasmic vacuolations. (40X, Giemsa stain)

The dogs covered under treatment group 1 (Vincristine) had complete regression of tumour growth within 4 cycles (Fig. 3a), *i.e.,* with a total therapeutic dosage of vincristine ranging from 0.45 to 0.75 mL per cycle. These dogs evinced the side effects, *viz.,* dullness, depression, inappetence, lethargy, and weight loss, which were managed with therapeutic support. Gastrointestinal disturbances like vomiting, diarrhoea, nausea and anorexia were also noticed. The dogs treated with BCG (group 2; Fig. 4a) evinced no regression in tumour mass grossly, however did not show further growth of the tumour and the masses had a marginal reduction in firmness during the course of treatment. Dogs treated with combination of vincristine and BCG (group 3; Fig. 5a) had complete regression of tumour growth within 4 cycles, *i.e.,* with a total therapeutic dosage of vincristine ranging from 0.40 to 0.60 mL and BCG 2-8×10^6 CFUs administered uniformly. These dogs evinced a comparatively rapid regression in the tumour mass and its firmness with cessation of oozing of fluid from the end of 1st cycle itself, and by the end of 3rd cycle most of the mass had regressed, however 4th cycle was required for complete remission of the tumour mass.

Four weekly regimen of intravenous administration of vincristine sulphate at 0.025 mg/kg body weight alone in dogs under group 1 was very effective in the complete remission of TVT in dogs (Fig. 3b). The similar observations were reported by Tella *et al.* (2004), Saibaba *et al.* (2015) and Hiblu *et al.* (2019) in dogs. It inhibits mitosis and bonded with tubulin by preventing the formation of mitotic spindles. Das *et al.* (1991) also found Vincristine sulphate @ 0.025 mg/kg body weight intravenously at weekly interval for 3 to 4 times very effective in treating the TVTs.

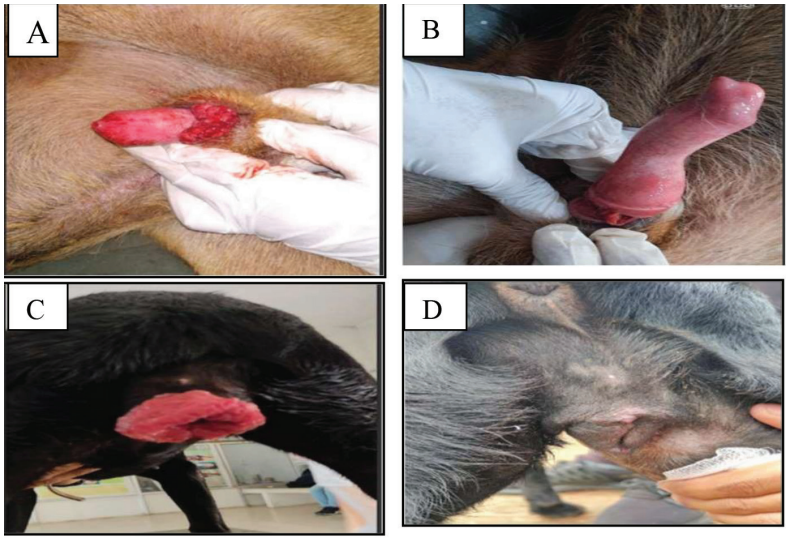

Fig. 3a: Gross images of dogs affected by TVT before (A, C) and after the therapeutic management with the complete remission (male-B, female-D) under group 1 (Vincristine)

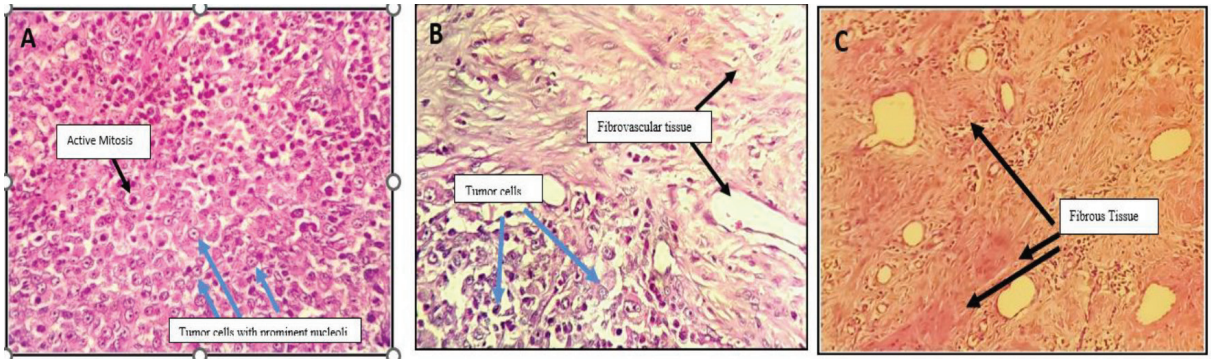

Fig. 3b: Histopathological regression of TVT in canine patients under group 1 (Vincristine): A- pre-treatment tumour cells with prominent nucleoli along with active mitosis, B- tumour cells along with fibrovascular tissue 2-3 weeks post-treatment, C- complete remission by fibrovascular tissue at 4 weeks post-treatment (40 X Haematoxylin & Eosin stain)

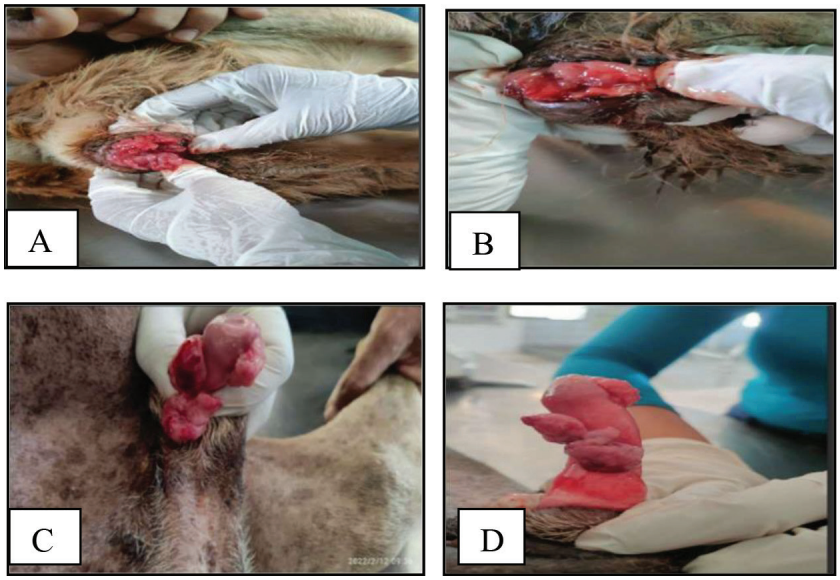

Fig. 4a: Gross view of TVT before (female-A & male-C) and after BCG treatment with no regressive changes, but tumour being flimsy and stagnant with reduced firmness (female-B, male-D)

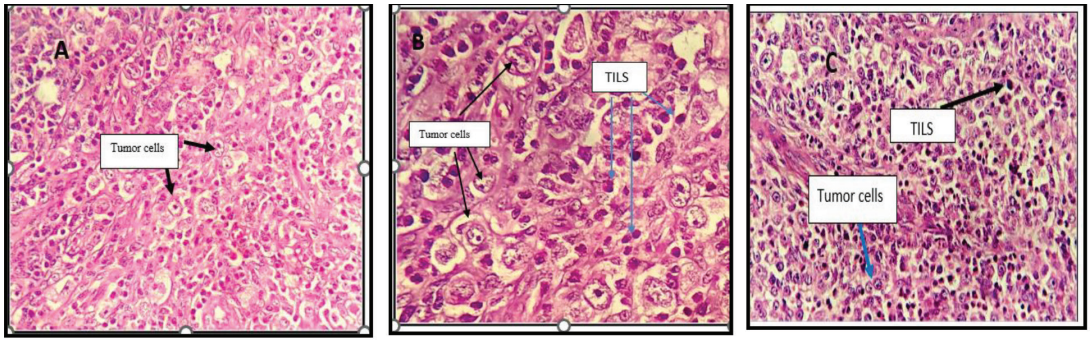

Fig. 4b: Histopathological appearance of TVT in canine patients under group 2 (BCG alone): A-pre-treatment tumour cells with prominent nucleoli along with active mitosis, B- tumour cells along with TILS 2-3 weeks post-treatment, C-tumour cells along with TILS 4 weeks post-treatment (40X, Haematoxylin & Eosin stain)

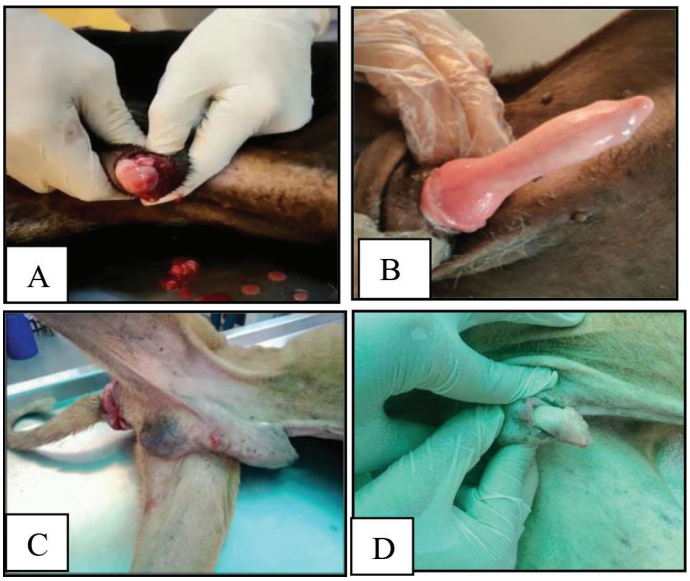

Fig. 5a: Gross images of male dogs affected by TVT before (A, C) and after the therapeutic management with the complete remission (B, D) in group 3 (Vincristine + BCG combination)

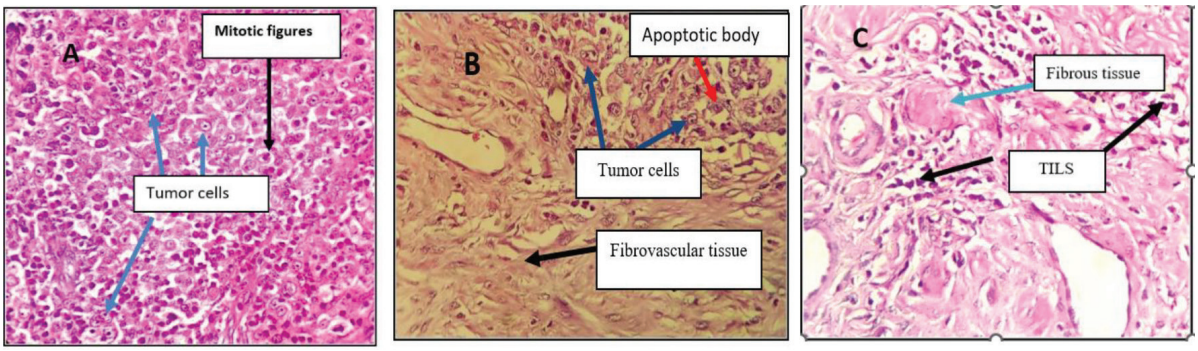

Fig. 5b: Histopathological appearance of TVT in canine patients under group 3 (Vincristine + BCG treatment): A- pre-treatment tumour cells with mitotic figures, B- tumour cells with apoptotic bodies along with fibrovascular tissue 2-3 weeks poast-treatment, C- complete remission by fibrovascular tissue and TILS, 4 weeks post-treatment (40X, Haematoxylin & Eosin stain)

The tumour masses in the dogs under group 2 (BCG) remained grossly stagnant with no further growth (Fig. 4b). However, the present findings are supported by observations of Sylvester (2002), who stated that the BCG had consistently shown to decrease the progression rates of non-muscle invasive bladder cancer (NMIBC) and in preventing progression of NMIBC, the benefit being limited to carcinoma *in situ* only. Another meta-analysis by Bohle and Bock (2004) revealed statistically significant superiority of BCG with maintenance course for reducing the risk of progression in superficial bladder tumours. Hess *et al*. (2006) showed that intralesional BCG therapy of CTVT was effective in causing

tumour regression with a mean regression time of 63 days. Several lines of evidence suggest that BCG attachment to tumour cells and presentation of BCG antigen to T-helper cells are required to trigger effective anti-tumoral activity. *In vitro* studies have provided evidence that cytokines and nonspecific cytolytic mechanisms are involved in the anti-tumoral mechanism of BCG (Hess *et al*., 2006) and also that it decreases the capacity of tumour cells to proliferate (Pryor *et al*., 1995). Contrary to the present findings, Mukaratirwa *et al*. (2009) reported complete regression of TVT in all the dogs treated with BCG, with a medication regimen of five consecutive days intratumourally with a mean regression time to be 44±6 days (40-49 days).

The dogs treated under group 3 using Vincristine in combination with BCG evinced better treatment response than that in group 1 (Vincristine), with respect to a comparatively rapid regression in the tumour mass and its firmness with cessation of oozing of fluid and milder degree of side effects (Fig. 5b). The present findings are completely in agreement with the observations reported by Mukaratirwa *et al*. (2009), who found complete tumour remission in all the dogs with combined BCG and vincristine therapy being more effective than vincristine alone in treating CTVT. Byrne and Reynoldst (1982) studied recovery of metastatic malignant melanoma with vincristine with and without BCG vaccination and they found trivial advantage in response or survival in BCG treated group over those treated with chemotherapy alone.

The biopsy samples of TVTs collected pre-treatment from all three groups (Fig. 3b, 4b, 5b) for histopathological examination revealed round to polyhedral shaped tumour cells, arranged or grouped in strings and interspersed with delicate stroma. The tumour cells had a high nucleus to cytoplasm ratio with a round nucleus and chromatin ranging from delicate to coarse with prominent nucleoli and cytoplasmic vacuoles. Large number of mitotic figures was present. Minimal proliferation of connective tissues was observed. Inflammatory cells such as lymphocytes, plasma cells, neutrophils were observed. Very similar observations have been reported by many workers (Krithiga *et al*., 2005; Mukaratirwa *et al*., 2009, Saravanan *et al.,* 2015) with large variations in cellular (anisocytosis) and nuclear morphology (anisokaryosis) with prominent anisonucleoliosis in the nucleus of the tumour cells.

In the dogs under groups 1 and 3 (Fig. 3b, 5b), there was a decrease in oedema, appearance of macrophages and an increase in the number of tumour infiltrating lymphocytes (TILs) post-treatment. At the end of the 2nd week and start of 3rd week there was an increase in the apoptosis of the tumour cells, with reduction in the number of tumour cells. Subsequently, by the end of 3rd week, a gradual increase in the number of TILs was noticed followed by tumour cell apoptosis and necrosis, reduction in mitotic figures and gradual replacement of the tumour parenchyma by fibrovascular tissues. At the end of the 4th week, most of the tumour cells were completely replaced by fibrovascular tissues. The histological features found in the dogs treated with vincristine and combination of vincristine and BCG under groups 1 and 3, respectively, corroborated well with the observations made by Mukaratirwa *et al*. (2009), Sethawongsin *et al*. (2018) and Hiblu *et al*. (2019).

In dogs under group 2 (BCG) after the starting of treatment (Fig. 4a), at the end of the 2nd week there was a minimal apoptosis of tumour cells with no reduction in the number of tumour cells. During the subsequent weeks there was a gradual increase in the number of TILs but there was no reduction in tumour cell apoptosis and mitotic figures, with minimal presence of fibrovascular tissues. The present findings, however, did not agree with the observations reported by Mukaratirwa *et al*. (2009).

Conclusions

Based on the present findings it was concluded that vincristine caused tumour cell apoptosis while BCG stimulated the local host immune system resulting in an increase in macrophages and tumour infiltrating lymphocytes that induce tumour cell necrosis and apoptosis. In canines treated with BCG alone the mass did not show regression, however the mass was observed to be suppressed grossly with milder reorganization and capsulation, and no further proliferation. The onset of cessation of bleeding and regression of tumour mass were observed to be comparatively earlier in dogs treated with combination of vincristine and BCG as compared to vincristine alone, however the dogs under both the treatments required 4 treatment cycles for complete regression. The non-curative effect of BCG on TVT in the dogs might be due to variation in the intensity of growth, dosing frequency and the stage of treatment.

Acknowledgements

Authors wish to thank the authorities of Veterinary College, Anand, and Kamdhenu University for the facilities provided. The help extended by Jivdaya Charitable Trust, Shree Danev Foundation, Ahmedabad and Vadodara Centre for Animal Rescue and Emergency (VCARE), Vadodara in providing clinical material is also gratefully acknowledged.

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