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Peste des petits ruminants Control Programme and Strategies in India: Current Scenario

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ABSTRACT

Peste des petits ruminants (PPR) is an acute, highly contagious, OIE notifiable and economically important transboundary viral disease of sheep and goats. PPR is enzootic in India as a greater number of outbreaks have occurred in the past and now occurring regularly round the year and throughout the country. The PPR outbreaks in sheep and goats have declined in some of the Indian states Viz., Andhra Pradesh, Telangana, Karnataka, and Chhattisgarh after implementing the strategic mass vaccination campaigns. The decreased number of outbreaks, as well as changes in the disease severity patterns and distribution, might be due to the effectiveness of the vaccine, timely vaccination, and most importantly effective implementation of the vaccination strategic plan. This review is focused upon the overall understanding of the National PPR Control Programme (PPR -CP) and its strategies vaccination implantation in India for the control and eradication of PPR.

Key Words: PPR, Sheep and Goats, Control program, Strategies, Vaccination, India

INTRODUCTION

Peste des petits ruminants (PPR) is one of the highly contagious and economically important viral diseases of small ruminants, especially goats and sheep, with morbidity and mortality rates as high as 100% and 90%, respectively. The disease is manifested by severe pyrexia, discharges from eyes and nasal orifices, necrotizing and erosive stomatitis, enteritis and bronchopneumonia (Balamurugan *et al.*, 2014a). The causative

agent of the disease is small ruminant morbillivirus (SRMV), formerly known as PPR virus, which belongs to the genus *Morbillivirus* of family *Paramyxoviridae*. India has a considerable sheep and goat population of around 200 million, (2012 Census, DAHD and GOI). In India, PPR was first reported from Arasur, Villupuram district (Tamil Nadu State) during 1987 (Shaila *et al.*, 1989). The disease is restricted to southern India till 1994 and after that, it took enzootic in many northern states of India. Now, PPR is enzootic in India as outbreaks occur in small ruminants regularly throughout the country, and is a major constraint in small ruminant

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production incurring great economic losses in terms of morbidity, mortality, productivity losses, and trade restriction (Balamurugan *et al.*, 2014a). Epidemics of PPR may have enormous consequences in terms of the dramatic effects of this disease on livestock productivity and high costs of control or eradication (Govindaraj *et al.*, 2016). Epidemics affect not only individual farmers but also the livestock sector as a whole and as a consequence, the national economy. PPR is present in countries, which are either developing or under-developed thereby adding to the economic woes.

For effective control of the disease, the development of both suitable vaccines as well as rapid, specific and sensitive methods for the diagnosis is highly imperative. To control PPR, there is a need for baseline epidemiological data on the disease prevalence, strong support of diagnostic methods, and proper timely vaccination of the susceptible population. Monoclonal antibody-based competitive-ELISA(c-ELISA) and Sandwich ELISA (s-ELISA) for PPR antibody detection and antigen detection, respectively developed at Indian Veterinary Research Institute (IVRI) are the currently employed test for sero-surveillance and sero-monitoring PPR (Singh *et al.*, 2004a; Singh *et al.*, 2004b). Further, live attenuated Vero cell-based vaccine (Sungri-96 strain) developed at IVRI is being used for vaccination of susceptible populations throughout the country. Despite strict control measures including statutory regulations along with the availability of scores of vaccines and diagnostics, this infection still remains a constant threat to sheep and goats (Singh *et al.*, 2009). Vaccination is a recommended

tool to support eradication efforts and limit economic losses due to PPR. The only way to control PPR is by the use of a homologous vaccine against the disease. The present review is focused upon the overall understanding of the National PPR Control Programme (PPR-CP) and its strategies vaccination implantation in India for the control and eradication of PPR.

Control strategies

Control strategies in developing or under-developed countries the choices are limited. Social acceptance, public and regulatory support is essential for the success of any disease control and eradication program. In India stamping out policy is not feasible because of economic and socio-cultural reasons (Singh *et al.*, 2009). However, society will readily accept the vaccination program without much hindrance. Hence, vaccination is a recommended tool to support control and eradication efforts. For the proper control of PPR, there is a need for strong support of diagnostic methods and proper, timely vaccination of the susceptible population upon understanding the epidemiology of the disease is imperative (Balamurugan *et al.*, 2016). Hence, the availability of attenuated cell culture vaccines and various diagnostic techniques or kits for the diagnostic of PPR, public and regulatory concern along with control measure and strategies favours for a strong recommendation of National control programme (NCP) for PPR in India in order to alleviate the poverty in the country initially and continent later. As no specific therapy is available for PPR infection, this way, at least the immediate loss could be prevented and the small and

marginal farmers rearing sheep and goats will be benefited. Therefore, PPR control and eradication depends mainly on rapid and accurate diagnosis or surveillance or monitoring and implementation of the prompt vaccination program.

Studying prevalence of SRMV antibodies in sheep and goats from different geographical areas with varying agro-climatic conditions may be helpful in devising effective appropriate disease control strategies, as the presence of SRMV specific antibodies indicated in situations where either the subclinical or in-apparent or non-lethal infection was suspected (Balamurugan *et al.*, 2014b) or in sheep or goats are exposed to virus naturally (Balamurugan *et al.*, 2012) and recovered in unvaccinated areas or immune response in vaccinated region.

Control Programme

Recent success with the rinderpest eradication program (NPRE) in the country has provided the confidence that is required to launch a similar program with PPR too initially on the lines of NPRE. All the elements (potent vaccine, kits for disease diagnosis & sero-surveillance and the tested infrastructure, etc.) required for a control program are available in the country. They have further been recommended for a collaborative nationwide program implemented by state Animal Husbandry Departments under the direction of the Department of Animal Husbandry and Dairying (DAHD, GOI) and the cooperation of the local public under the guidance from the policymaker at the center.

Therefore, the launching of the programme appears technically feasible, economically viable and a practically attainable proposition. Hence, in the way to FMD Control Programme (FMD-CP) (DAHD), it was decided by DAHD, Government of India to undertake a national control programme on PPR (NCP-PPR) in the 11th five-year plan (2007-12) with an aim to control and eradicate this disease from India in a time-bound manner on the lines of rinderpest eradication (<http://dahd.nic.in>) following the eradication pathway of OIE (Balamurugan *et al.*, 2016). Accordingly, this proposed program has been initiated during the year 2010-11 with a sum of INR 432.5 million in the first phase for undertaking various activities of the program. The DAHD, GOI launched NCP for PPR would be run in three phases during India's 11th (2007–12) and 12th (2012–17) 5-year plan and 2017-2020 plan periods. Moreover, vaccination against PPR has been practiced in some states of India since 2002 to control the disease. The activities of the program include identification of animals, procurement of cold-chain equipment and vaccine, assessment of the randomly collected samples of vaccines for their quality, mass vaccination, virus typing in case of outbreaks, recording/regulation of animal movement and sero-surveillance or monitoring of animal population on a random basis (www.dahd.nic.in).

During the first phase, the states and UTs in Southern peninsular India viz. Karnataka, undivided Andhra Pradesh, Tamil Nadu, Kerala, Maharashtra, Goa and Lakshadweep, Daman & Diu, Dadra & Nagar Haveli, Puducherry, and Andaman & Nicobar Island were included in the

vaccination program (www.dahd.nic.in) (Balamurugan *et al.*, 2016). However, the remaining states and UTs of India were also included in the second phase of PPR-CP during 2014-2015 (Balamurugan *et al.*, 2016). Due to PPR-CP, the disease has been brought under control in some Indian states and PPR threat reported declined progressively and substantially in areas under continuous vaccination (Balamurugan *et al.*, 2016) and benefits outweighs the cost of a vaccination program (Govindaraj *et al.*, 2019). In some states, where focused vaccination is adopted, disease outbreaks are being reported sporadically. However, neither a surveillance plan nor a systematic sero-monitoring was initiated to assess the effectiveness of the vaccination program. In the past five years, vaccination and sero-monitoring were carried out extensively in the PPR -CP in some states of India especially Andhra Pradesh, Telangana, Chhattisgarh, and Karnataka.

The government of India also providing funding / grand in-aid under this PPR-CP to research institutions for assisting and undertaking surveillance and monitoring of PPR during the surveillance stage with states / UTs animal husbandry department. Professional commitment on the part of veterinarians and associated personnel involved in a mass immunization program is crucial to succeeding in the vaccination program.

Recent prevalence study in Andaman Nicobar (AN) Islands of India (Balamurugan *et al.*, 2019a) showed that the overall 1.39 % true prevalence of PPRV antibodies in goats, which implies that the goat population in the villages (epidemiological units) were

having less than 30 % seroprevalence or free from PPRV antibodies, as there were neither PPR outbreaks reported nor PPR vaccination strategies practiced in goats in the AN Islands. This necessitates the comprehensive active intensive surveillance program and imperative for monitoring of the occurrence of sporadic outbreaks in different clinical forms of the diseases in the islands to make disease-free Islands by implementing effective disease control measures /strategies for PPR (Balamurugan *et al.*, 2019a). Further, serosurvey in the control programme implemented states in Southern Peninsular India (Balamurugan *et al.*, 2019b), showed that the immune protection in sheep and goats were greater in regularly vaccination practiced states (Andhra Pradesh, Telangana, and Karnataka), when compared with irregularly- or focused- or non-vaccinated states / UT (Puducherry, Kerala and Tamil Nadu), where the disease is endemic and outbreaks are being reported. Further, the seroprevalence study of the PPR in sheep and goats carried out in different states in the Central and Western regions of India revealed that the small ruminants in most of the epi-units (n=190) had < 70 % seroprevalence (unpublished data). This necessitates the active intensive continuous mass vaccination program for a few more years to achieve the desired protection level and surveillance programs to make these regions free from PPR. Therefore, zoning the PPR risk regions and initiating vaccination program at a specified period with complete vaccination coverage of all the risk population in the identified zone is of paramount importance along with monitoring and surveillance.

Vaccination strategies

In India, sheep and goats are an important productive asset of settlers, landless, marginal, and small landholder farmers and it generates a flow of income and employment throughout the year. A number of PPR outbreaks have occurred in the past and now being occurring regularly, round the year and throughout India, as the disease is endemic in nature (Balamurugan *et al.*, 2014b). India practiced focused PPR vaccination in outbreak places in some states since 2002 (Singh *et al.*, 2009) and in program mode in some states since 2011 even before the global framework was planned (Balamurugan *et al.*, 2016). Experimental vaccination against PPR after field testing has been practiced in 15 states of India since 2002 to control the disease during outbreaks (Singh *et al.*, 2009). Ongoing vaccination strategies for the control of PPRV would be slightly different from vaccination programs for rinderpest. A mass vaccination campaign to cover 80% flock immunity would be needed to account for the population dynamics of sheep and goats, disparities in sheep and goats husbandry practices and the agro-climatic conditions affecting the pattern of disease. The slaughtering of male goats at an early age combined with the high fecundity of the caprine species results in the replacement of population (~30-40% naïve population appears) every year.

Though vaccination has been successfully implemented in some states, still its implementation is largely elusive in many states. Hence, the status of PPR vaccination and the impact of the vaccination especially in the successfully

implemented states is necessary to generate evidence for extending the control program. The above points highlight the necessity of sero-monitoring or surveillance in the vaccinated population as well as the impact of the vaccination in different states of India. Further, we need to generate baseline information on the antibody titers across species, geographical location and under different rearing, environments to generate evidence if the country plans to eradicate the disease from the country by 2030. It is also necessary to generate information on the impact of the vaccination including strategies or constraints for effective implementation of the control programs in the country.

In India, currently, three live attenuated PPR vaccines (Sungri 96, Arasur 87 and Coimbatore 97 stains) are available, of which, Sungri 96, developed by Indian Veterinary Research Institute (IVRI), Mukteswar has undergone extensive field trial. PPR virus is one serotype, so any vaccine lineage can protect against all other field viruses. These vaccines may be sufficient to protect against the circulating field isolates or strains of PPRV in India and provide long-term (more than 6 years) protective immunity. These vaccines can be used for the control and eradication of the disease not only from India but also from other Asian and some African countries following the example of the global RP eradication program (GREP), as now Lineage IV virus expand its geographical locations. Seroconversion and protection have been observed in vaccinates by the PPR vaccine (Singh *et al.*, 2009). With a field dose of 10^3 TCID₅₀, protective immunity is ensured for >6 years (Saravanan *et al.*, 2010)

without a booster, the vaccine is well suited for mass immunization (Balamurugan *et al.*, 2016). The vaccine production and quality control technology generated in IVRI, Mukteswar has been transferred into different multinational companies (MNCs) viz. M/s Indian Immunologicals Ltd. Hyderabad, India; M/s MSD Animal health, Intervet India Pvt. Ltd. Pune India; M/s Hester Bioscience Ltd, Ahmadabad, India; and M/s Bio-Med Private Ltd. Ghaziabad, India, apart from IVRI, Uttar Pradesh and Veterinary Biological Production Units (VBPU) or Institute of Animal Health and Veterinary Biologicals of Telangana, Karnataka, Madhya Pradesh, Punjab, Tamil Nadu, Haryana and Kerala states for commercialization.

Vaccinated animals, infected and recovered animals are protected from re-infection for the remainder of their lives. Hence, in this direction, the strategies were proposed in the PPR_CP involving intensive vaccination of all susceptible sheep and goats and their three subsequent generations (approx. 30%) with 100% fund from central assistance. The basis for selection of some states in the first phase for control program may be due to high prevalence of disease in the region or dense population of small ruminants, availability of facilities and personnel to cover the vaccination in a stipulated time periods, etc. or to make disease-free zone in case of UTs where less population of sheep and goats.

At present, the disease occurrence, severity of the clinical disease and number of outbreaks have progressively and substantially declined in areas under regular vaccination mostly under National Control

Programme on PPR (NCP-PPR) and partly under ASCAD (Assistance to States for Control of Animal Diseases) of the Government of India. The situation in India is improving as a result of progressive mass vaccination. The disease incidence has been in decline over the past 5 years. In India, decreased numbers of outbreaks, as well as changes in the severity of disease patterns recently observed, might be due to the effectiveness of live attenuated vaccines, timely vaccination of sheep and goats, and circulation of a single Asian lineage IV PPRV, since the disease was first reported in India. Currently, vaccination programs are being implemented in some states of India which will alter PPR epidemiology, particularly the distribution of the disease and pattern of disease.

The second alternative strategy may be focusing vaccinations initially on high-risk group animals, namely young animals (6 months to 1 year aged) and goat population rather than sheep and migratory flocks (Singh, 2011) in a suitable period preferably during lean periods. The third strategy might be intensive vaccinations based on populations to make disease-free areas (zone) by identifying the hotspots and implementing vaccinations followed by screening, testing, and overall revaccination, if required, in those areas as reported earlier (Balamurugan *et al.*, 2014b; Singh, 2011). However, keeping in mind the current approach and achievements, as a novel alternative strategy suggested by Cameron (2019), that a trench warfare approach, where the eradication strategy should be modeled on guerrilla tactics: use exceptionally good, locally relevant and timely intelligence; strike rapidly

and effectively in small areas with high vaccination coverage; achieve the goals, and keep moving. The author also points out that to achieve this developing powerful, effective and sustainable surveillance systems is essential (Cameron, 2019). This strategy may be followed if required for a national control program.

Overall, fixed strategies may not work for all the states or regions or countries. However, in the mass vaccination in pulse polio model covering entire population initially, followed by biannual vaccination in a pre-designated stipulated period, covering the naïve young population of sheep and goats at least four to five years will have a tremendous impact on the control of PPR outbreaks in sheep and goats. Thus, after three to four rounds of vaccination, the population in the state may be immune to the disease, but the threat persists from ingress of disease from other bordering states, hence vaccination on the migratory population at the check post or border regions of the states or inter-state border or in the place of entry or place of trade market of animal through transport from other states are to be targeted for mass vaccination as and when required. Finally, it is hoped that PPR in the direction of RP will be eradicated in India within a decade or few more years.

National PPR Control and Eradication Strategy

Recently DAHD department, Ministry of Animal husbandry and Fisheries, Govt. of India prepared the strategic planning for National PPR Control and Eradication Strategy (NPCES) by 2025 with a hope

that PPR in the direction of RP will be eradicated in India within a decade or even earlier. The silent features of this strategy include intensive vaccination with 100 % coverage of sheep and goat populations till 2022, with attaining targeted herd immunity and stoppage of virus circulation through clinical surveillance by 2023/24 and freedom from PPRV infection by 2025. The mass vaccination will be in pulse polio model in the designated time period with two to three cycles of vaccination to reach 70-80 % immunity level, with each cycle of covering entire population of sheep and goats initially, subsequently bi-annual vaccination covering the 30% naïve young population in each of the states with traceability of the vaccinated animals. Moreover, at the time of declaring India is provisionally free from PPR, surveillance of PPR in different states /UT also needs to be carried out as per GCEP guidelines to support the demonstration of freedom from disease in unvaccinated populations.

Prospective

At present, the disease has been brought under control in goats and sheep by available effective and safe live attenuated cell culture PPR vaccine. In overall, the present scenario of PPR in India warrants the studies to be undertaken with the objective to know the effect of agro-climatic changes on the occurrence of PPR in small ruminants in different agro-climatic zones and to analyze the relationship of disease occurrence and risk factors to formulate modules for forecasting and forewarning. The epidemiology of PPR is likely to change due to vaccination as the disease occurs more severely in the naïve

population. This warrants the study to be undertaken to know the changing pattern of the disease and its severity in vaccinated and unvaccinated regions. Andhra Pradesh, Telangana, Karnataka, and Chhattisgarh states have shown a declining trend with more than 90% reduction in the number of reported PPR outbreaks during the preceding five years due to implementation of strategic mass vaccination plan. Implementation of vaccination and control strategies adopted for PPR in sheep and goats by India may motivate other countries for similar initiatives leading to progressive mass vaccination and control of PPR.

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CONFLICT OF INTEREST

No conflict of interest exists.

AUTHORS' CONTRIBUTION

V. Balamurugan planned and wrote the draft of the manuscript, K. VinodKumar and G. Govindaraj provided inputs, support, and edited & formatted the manuscript and Parmal Roy provided guidance and support.

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Full Length Article

Establishment of Embryonic Stem Cell like Cell Colonies from *In-vitro* Produced Buffalo Compact Morulae

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ABSTRACT

The study was aimed at establishing the embryonic stem cells (ESC) like cell colonies from *in-vitro* produced buffalo (*Bubalus bubalis*) compact morula (CM). A total of 33 CM were subjected for zonalysis using pronase and embryonic cells were derived by three different methods viz., **T 1**: Intact CM; **T 2**: Disaggregation of CM by gentle pipetting and **T 3**: Slicing of CM using Bard Parker blade. The efficiency of establishment of ESC-like cell colonies in the three groups were 66.7, 83.6 and 6.7 per cent respectively. There were no significant differences on the day of attachment and initiation of primary colony formation between T1 and T2 groups. However, the primary colonies established significantly earlier (Day 3.8) in T2 group than in T1 group (Day 4.8). In both the groups, the primary colonies spread out vigorously exhibiting rapid proliferation rate. The ESC-like cell colonies of T1 and T2 groups survived upto four passages. Immunostaining of CM, primary and passaged ESC-like cell colonies revealed distribution of Oct-4 protein. The study demonstrated successful establishment of ESC-like cell colonies from pipette dissociated blastomeres of *in-vitro* produced CM.

Key Words: Buffalo, Compact morula, Embryonic stem cell colonies

INTRODUCTION

Embryonic stem cell (ESC) is a powerful tool to understand the mechanisms

that control early developmental processes and substantial attention is being given for their potential in regenerative medicine. The stage of embryonic development at which ESCs are derived may be crucial to their subsequent establishment into colonies and cell lines (Ito et al., 1996). Successful attempts have been made in mouse, bovine and human to produce pluripotent ESC lines after the culture of blastomeres obtained from morulae (Eistetter, 1989; Strelchenko, 1996; Strelchenko and Verlinsky, 2004). However, such attempts to produce ESC-

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like cells from morulae in buffaloes remained unsuccessful (Verma *et al.*, 2007 and Huang *et al.*, 2010).

We hypothesized that compact morula would be a more appropriate stage to derive ESC like cells, when blastomeres are not committed for differentiation, but has more lineage towards undifferentiated ICM than morula. An analogous cell aggregation occurs in the morula stage embryos prior to formation of blastocyst, which could be visually identified as compact morula (CM). Hence, the present investigation is attempted to derive ESCs from blastomeres of *in-vitro* produced buffalo CM.

MATERIALS AND METHODS

All the chemicals used for *in vitro* production of embryos and culture were procured from Sigma Aldrich (USA) unless otherwise mentioned specifically.

***In-vitro* production of compact morulae**

Ovaries from sexually mature buffaloes (*Bubalus bubalis*) were collected irrespective of their age, breed and stage of oestrous cycle from Chennai Corporation abattoir and utilized for the study. In the laboratory, the extra-ovarian tissues were trimmed off and the ovaries were washed in running tap water and normal saline. All the visible follicles (>2 mm diameter) were aspirated using a sterile 18 or 20G hypodermic needle attached to a 10 ml disposable syringe. The collected follicular fluid was transferred to 90mm gridded petridish and screened for oocytes. All the cultureable quality oocytes i.e., oocytes enclosed in a compact cumulus mass of ≥ 3 layers with evenly granulated

cytoplasm were selected and subjected for *in-vitro* production of embryos. The *in-vitro* maturation, *in-vitro* fertilization and *in-vitro* embryo co-culture were performed as described by Satheshkumar *et al.* (2017). Embryo development was observed for nine days post-insemination and embryos that attained the CM stage were utilized for the study.

Preparation and inactivation of buffalo fetal fibroblast feeder layer

Buffalo fetuses (around 60 days old) were collected from slaughtered animals and used for preparing buffalo fetal fibroblast feeder layer (BFF) as described by Verma *et al.* (2007). The feeder layer was inactivated with mitomycin C (10 μ g/ml) for two hours (Munoz *et al.*, 2008), after which the feeder layer was washed off and replaced with ESC media.

Derivation of embryonic stem cell like cells

CM were zonally incubated by incubating in 0.5 per cent pronase and were subjected to three protocols for the derivation of embryonic cells viz., **T1**: The zona free CM were seeded intact (n= 12 intact CM); **T2**: The blastomere cells of the zona free CM were dissociated by repeated pipetting with a narrow bore pipette (n= 44 cell clusters) and **T3**: The zona free CM were placed individually in 5 μ l PBS media and sliced mechanically using No.11 Bard Parker blade (n= 30 sliced cells). The intact CM / cell clusters/ slices (Figure - 1) were washed in ESC medium and seeded over the mitomycin inactivated feeder layer. A total of 18 trials were conducted with six trials in each group.

Seeding and culturing of embryonic cells

The intact CM/cell clusters/slices of CM seeded onto the inactivated feeder layer (Day 0) were cultured at 38.5°C in a humidified atmosphere of 5% CO₂ in air. The cells were monitored regularly at 24 h interval. Once the cells were found attached to the feeder layer the ESC medium was changed every two days. The cell clusters were observed for attachment, formation and establishment of primary colonies as described by Tesar (2005). When got established, the primary colonies were dissociated mechanically as described by Verma et al. (2007) and reseeded to another culture plate of mitomycin C treated feeder layer. Further passages were performed in the same procedure, every four to five days until the ESC like cell colonies lose their characteristic attachment and establishment.

Characterization of Embryonic stem cell colonies

Morphological characterization

The ESC-like cell colonies were identified and monitored based on their morphological characteristics as described by Chen *et al.* (1999) and Li-Wang *et al.* (2005). The colonies which have flat, polygonal/round shaped cells with clear border and faster proliferation rate were considered as typical ESC-like cell colonies.

Fluorescence Antibody Technique

One representative primary and passaged colony in each trial was retrieved mechanically along with some accompanying layer of BFF and then subjected for Fluorescence antibody technique (FAT) against *Oct-4* protein to

confirm the property of stemness by the method described by Peura *et al.* (2007). The derived ESC like cell colonies were fixed with 4% Paraformaldehyde (Merck UN 2213) in a glass slide and incubated in primary antibody (Anti Oct-3/4 (N-19)-Goat IgG; Santacruz Biotechnology-SC-8268) and secondary antibody rhodamine conjugate (Anti-Goat IgG, Donkey-Rhodamine Conjugate; Millipore AP 18-0R). The cell colonies were observed under fluorescence microscope at a wave length of 520-560 nm after adding DAPI /antifade (Millipore S1173). The fluorescence of reddish yellow colour was considered positive for stemness characteristics.

Statistical analysis

The data on various parameters of establishment of ESC-like cell colonies were compared and analysed between different treatment groups by student t-test (Snedecor and Cochran, 1994)

RESULTS AND DISCUSSION

A total of 1420 oocytes were retrieved from 865 buffalo ovaries with a mean recovery rate of 1.7 ± 0.1 oocytes per ovary. Out of the 562 (39.6%) cultureable quality oocytes, 33 (5.9 ± 0.8 %) CM were produced.

The characteristics of primary ESC like cell colonies established in all the three treatment groups (T1, T2 and T3) were presented in Table 1 and Fig.1 (a, b and c). When zona free intact CM (T1) were seeded, two variable modes of establishment of primary colonies were observed. In the first mode, the intact embryos initially got dissociated and then

attached to the BFF layer by Day 5 and the resultant atypical ESC colony got detached by Day 7. These colonies did not establish further on passaging. In the second mode, primary ESC-like cell colonies were established and the data from these colonies were considered for further statistical analysis. The overall establishment rate

of 66.67 per cent in the present study was much higher than 36.4 per cent reported by Zhang et al. (2006) while intact CM were cultured for establishing human ESC colonies. Eistetter (1989) suggested that the variability in establishment of ESC colonies when cultured intact might be due to over exposure of cells to the differentiation stimulating influences.

Table – 1. Characteristics of Primary Embryonic Stem Cell like cell colonies in different groups

S.No	Treatment Groups	No. of Cells/ Clusters/ Slices seeded	No. of Primary Colonies formed	Day of initial attachment (Mean ± SE)	Day of initiation of colony formation (Mean ± SE)	Day of establishment of primary colony (Mean ± SE)
1.	T 1	12	8 (66.7 ± 21.2) ^a	1.0 ± 0.0 ^a	2.3 ± 0.3 ^a	4.8 ± 0.3 ^b
2.	T 2	44	36 (83.6 ± 7.6) ^b	1.0 ± 0.0 ^a	2.2 ± 0.2 ^a	3.8 ± 0.3 ^a
3.	T 3	30	2 (6.7 ± 0.0) [@]	1.0 ± 0.0	2.0 ± 0.0	--
Significance			**	NS	NS	*

Percentage in parenthesis (Mean ± SE)

Values within columns with different superscripts differ significantly

*: Significant ($P \leq 0.05$) **: Significant ($P \leq 0.01$)






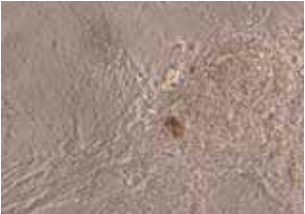



NS : Not significant ($P > 0.05$) @: Statistically not comparable

When pipette dissociated embryonic cells (T2) were seeded onto the BFF, their efficiency of attachment and establishment of primary ESC like cell colonies was 81.8 per cent. The present finding was higher than that reported (61.0 %) by Strelchenko and Stice (1994), who disaggregated bovine morulae using trypsin (0.25%) or pronase (0.5%). The reduced response reported by these researchers might be due to the damage caused by the enzymes to the zonolysed embryos as suggested by Ito et al. (1996). The characteristics of formation, establishment and proliferation rate of primary colonies corroborated with the

reports of Eistetter (1989), who utilised the pipette dissociated blastomeres of mouse morulae.

None of the sliced cells (T3) established as colonies in concurrence with the findings of Huang et al. (2010). They also disaggregated the buffalo morulae into several pieces using pin head, similar to the method followed in the present study and found that none of the isolated cells formed colonies. Verma et al. (2007) suggested that the irreparable damage caused to the blastomeres during slicing might be the cause for non-establishment of colonies.

Figure -1. Characteristics of ESC-like cell colonies derived by different methods

T1			
	Day 0- Zonalysed CM (200x)	Day 1- Attachment of intact CM (400x)	Day 5 – Establishment of ESC-like cell colony (200x)
T2			
	Day 0 - Pipette dissociated CM (200x)	Day 1 – Attachment of cell cluster (400x)	Day 3 – Establishment of ESC-like cell colony (200x)
T3			
	Day 0- CM slices (200x)	Day 1 – Attachment of cells (400x)	Day 3 – Detachment of colony (400x)

Thus it was concluded that establishment of colonies was better ensured when pipette dissociated blastomeres of CM were utilized and there was a high inconsistency when seeded intact or sliced. Similarly First et al. (1994) also expressed that morula derived cell lines have not usually established without disaggregating the cells.

ESC-like cell colonies were found to be flat, polygonal shaped cells with clear border and easily distinguishable from the feeder layer. The typical ESC-like cell colonies were also found to spread out vigorously in a faster rate. In both the T1

and T2 groups, the ESC-like cell colonies successfully survived upto four passages. Verma et al. (2007) and Huang et al. (2010) who utilized *in-vitro* produced CM also reported failure in passaging the colonies.

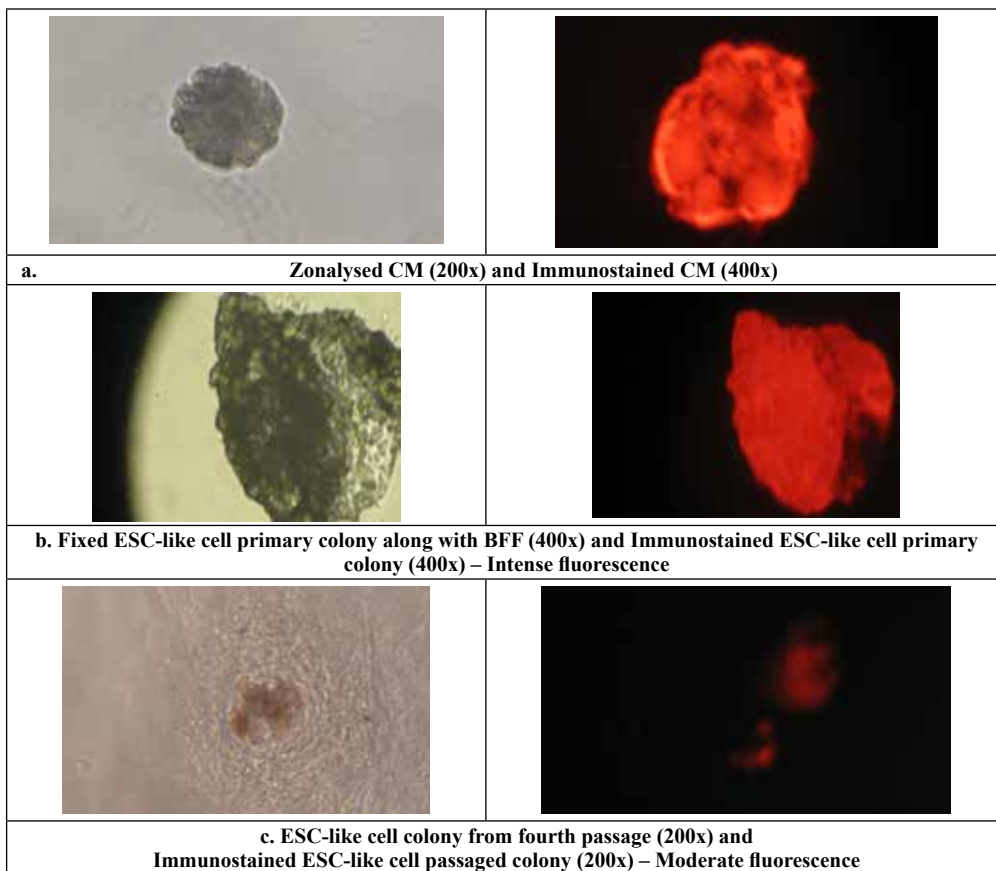
The pluripotency of ESCs was reported to be maintained by transcription factor, *Oct-4*, a product of the *Pou5f1* gene and has been widely accepted as a pluripotent cell marker. Mitalipov et al. (2003) stated that embryonic expression of *Oct-4* initiated at the 4- to 8-cell stage with strong nuclear localization of the protein in all blastomeres during the morula stage. In the present study, immunostaining of CM (Fig. 2a) revealed

expression of *Oct-4* protein confirming their pluripotent nature, and thus supporting our view for utilizing the CM stage embryo for development of ESC like cell colonies. The primary and passaged ESC-like cell colonies specifically expressed *Oct-4*, where as the BFF feeder layer cells around the colony served as negative controls and did not expressed the marker signal (Fig. 2b and 2c). It was observed that primary colonies expressed intense fluorescence, while the colonies of fourth passage exhibited moderate fluorescence indicating the probable loss of pluripotent nature. These expression patterns ascertained the status of

pluripotency in the cultured ESC-like cells derived from CM. Similarly, Strelchenko and Verlinsky (2004) opined that morula derived human ESC lines have expressed the ESC specific markers, including *Oct-4* same as that of the ESCs derived from blastocysts.

Based on the findings, it was concluded that buffalo ESC-like cell colonies could be successfully established from CM and the derivation of embryonic cells from pipette dissociated CM was found to be highly efficient and consistent in establishing colonies.

Figure – 2. Immunostaining against *Oct-4* of CM and ESC-like cell colonies



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Selective Red Cell Variables in Chippiparai Hound Breeds of Tamil Nadu- a Pilot Study in 30 Dogs

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ABSTRACT

Globally, many clinico-pathologic differences between hounds and other breeds have been investigated and most of the research has been focused on differences in the hematologic values between the breeds and hematologic reference intervals for the Greyhounds, which have been recently published. However, our native hound, Chippiparai, closely resembles the morphological traits of the hounds and yet there are no published reports on the hematological parameters of the Chippiparai breed from Tamil Nadu. Hence, this study was carried out to investigate the red cell variables like Hemoglobin, Packed Cell Volume and RBC counts which were compared with that of non-hound breeds. Chippiparai hound dogs (n=30) and non-hound dogs (n=30) were selected amongst the dogs presented at Madras Veterinary College Teaching Hospital and various native hamlets. The Hb, PCV and RBC level in the Chippiparai breeds were found to be higher which may probably be attributed to the need for appropriate oxygen supplementation under extreme climatic conditions, for running and hunting and probably as an inheritance from the other sight hounds. Also, these breeds can be recommended as good blood donors since a lesser volume of their transfused blood (with greater PCV) can increase the hematological parameters of the anemic recipient dog as compared to an equal volume of blood from the non-hound blood donors.

Key Words: Chippiparai, hounds, hemoglobin, RBC, blood donor

INTRODUCTION

Chippiparai dogs are native breeds of Tamil Nadu and are believed to be linked with the ancient Egyptian Saluki breeds and the Grey hounds. The morphology, appearance and characteristics resemble the Saluki's to a greater extent as compared to the Grey hounds. Recent awareness

regarding raising our native hounds, amongst pet owners and breeders to prevent their extinction are leading to progressive increase in the population of this breed in Veterinary practice. Interestingly, these hounds were found to have higher values of hematological parameters such as Hemoglobin, Packed Cell Volume, Red Blood Cell, and Mean corpuscular volume when compared with other non-hound breeds during our observation. Hence, a pilot study was carried out to report the unique high normal hematological parameters in healthy Chippiparai dogs. Earlier studies have reported that Greyhounds have higher

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HCT, Hemoglobin (HB) concentration, MCV, MCH and MCHC when compared with values of non-Greyhound dogs. Thus, a need to establish reference intervals specific for *Chippiparai* dogs was studied at the Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University during 2013-14.

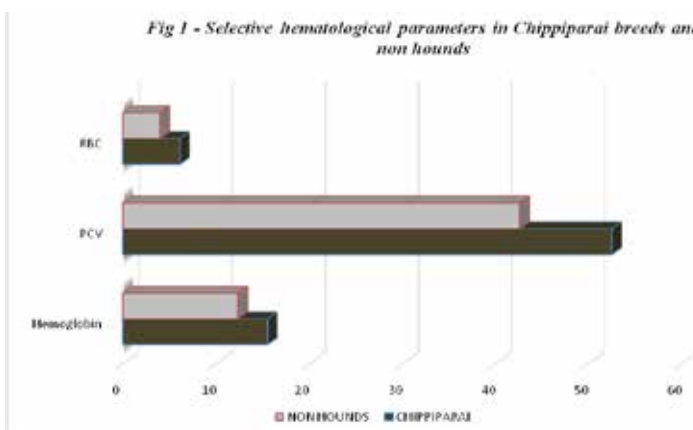
MATERIALS AND METHODS

Chippiparai dogs ($n=30$) between the age group of one to eight years were analyzed for normal hematological parameters wherein utmost care was maintained in morphologically assessing the confirmation of these hounds. Non hound dogs($n=30$) like German Shepherd, Mongrels, Labrador, Boxers, Spitz and the

hound breed Chippiparai brought to the Madras Veterinary College and Teaching Hospital, from kennel clubs/ dog breeders around Chennai, in and around hamlets of Tirunelveli and Madurai were included in this study. Emphasis was given to the Chippiparai hounds based on the previous study of the prevalence of DEA blood groups. Sick dogs and free roaming dogs were excluded from the research. About two ml of whole blood was collected from the cephalic vein, stored at 4°C and tested within 24 hours. Hematology was performed using hematological auto-analyzers (BC Vet auto-analyzer) to estimate Hemoglobin, Packed Cell Volume, Red Blood Cell count and the results were statically analyzed using Independent T test (Table- 1 and Figure - 1).

Table - 1. Red Cell variables in *Chippiparai* breeds ($n=30$) and Non-hound breeds German Shepherd, Labrador and Boxers ($n=30$)

Parameters	<i>Chippiparai</i>		Non-Hound		T Stat	P - Value
	N1	MEAN ± SE	N2	MEAN ± SE		
Hb	30	17.45 ± 2.1381	30	11.61 ± 0.1706	2.72**	0.0086
PCV	30	53.55 ± 0.6165	30	41.56 ± 0.3007	17.48**	0.0000
RBC	30	5.88 ± 0.0868	30	4.22 ± 0.0704	14.88**	0.0000



RESULTS AND DISCUSSION

The results indicated an average Hb of 17.45 ± 2.1 g/dl, PCV of 53.55 ± 0.61 % and RBC count of 5.88 ± 0.08 million/cmm in the current study. The mean values were obtained for the above-mentioned parameters for the two groups under study i.e. Chippiparai dogs and Non-Hound dogs and compared using Independent T test statistics. The following results indicate that the difference in the mean of all the parameters under investigation vary greatly between the two groups under study. The P – value for all the above studied parameters were found to be < 0.001 , which indicates that the mean values of the parameters Hb, PCV and RBC for both the groups under consideration vary significantly. There is a highly significant difference among the means of Hb, PCV and RBC between both the groups under the study, indicating that the two groups differ significantly from each other.

The results thus obtained from the study reveal that the indigenous *Chippiparai* hound breed has a higher value of the red blood cell variables such as Hb, PCV and RBC which is statistically significant.

In western countries, much studies on breed specific reference range for Red Cell variables and other biochemical profiles have been reported for German shepherds and Golden retrievers (Lund *et al.*, 2000). Greyhounds and Saluki hounds for that matter closely resemble our native Chippiparai breed and seem to have an acquired physiological adaptation similar to them. These breeds are well known for their athleticism, superior hunting and racing

performance and widely used for hunting in the mountain areas which probably contribute to their higher total oxygen-carrying capacity (Zaldivar-López *et al.*, 2011). More research has been emphasized in establishing breed specific parameters for greyhounds and have opined a higher than average value of haematological parameters as compared to other non-hound breeds (Campora *et al.*, 2011).

Higher muscle mass and acquired physiological adaptation differentiates these hounds from non-hound breeds. Thus, it is imperative that field veterinarians, academicians and researchers including clinical pathologists need to update and be aware of this unique high hematologic characteristics of the breed which would prove to be vital in making accurate diagnosis for these breeds (Mesa – Sancez *et al.*, 2012). The high concentration of RBC and Hb in the Chippiparai breeds may probably be understood as a need for appropriate oxygen supply under extreme climatic conditions for running and hunting attitudes and probable inheritance from the sight hounds as per literatures (Sullivan *et al.*, 1994).

More over these breeds can be recommended as canine blood donors since a lesser volume of their transfused blood (with greater PCV) can increase the hematological parameters of the anemic recipient dog.

For example, for an anemic recipient dog weighing 10 kg with PCV of 15 % would need approximately 300 ml from a non-hound donor or 200 ml (approx) from a Chippiparai donor dog to achieve a desired PCV of 30 based on the following formula-

$$(\text{Vol of blood required (ml)}) = \text{Bwt of recipient (kg)} \times 80 \times \frac{(\text{Desired PCV} - \text{Recipient PCV})}{\text{Donor PCV}}$$

$$\text{Vol of blood required from a non-hound dog} = 10 \times 80 \times \frac{(30-15)}{40} = 300 \text{ ml}$$

$$\text{Vol of blood required from a Chippiparai dog} = 10 \times 80 \times \frac{(30-15)}{55} = 218 \text{ ml}$$

Further, mean cell volume, potassium, urea, alanine amino transferase, aspartate amino transferase, alkaline phosphatase and cholesterol were also found to be significantly higher in deer hounds as compared to the average dog populations (Sheerer *et al.*, 2013), which needs further investigation in our indigenous *Chippiparai* breeds.

With regards to *Chippiparai*, further investigation into other parameters including, thyroid hormones, cardiac troponin, oxyhemoglobin dissociation curve, RBC 2,3-diphosphoglycerate (2,3-DPG) concentration, the partial pressure of oxygen and other underlying factors that influence the unique high hematological features should be investigated in a larger population.

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Comparison of different pheromone dispensers in luring *Musca domestica* towards delta trap

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ABSTRACT

The house fly sex pheromone (Z)-9-tricosene was impregnated into different pheromone dispensers (septa, plastic and polythene) and their efficacy in luring *Muscadomestica* towards red acrylic delta trap was studied. Out of the 514 house flies trapped, (Z)-9-tricosene when impregnated in circular plastic green lure attracted the maximum number of flies (165), followed by polyvial (132), silicone septa (111) and rubber septa (79) whereas the least number of flies was attracted with rectangular plastic dispenser (27). Among the house flies trapped, 46.49 per cent (239) were males and 53.50 per cent (275) were females. Predominance of male flies was observed with circular plastic green lure, polyvial and silicone septa, whereas in rubber septa and rectangular plastic lure, more number of female house flies were trapped. Observations revealed that circular plastic green lure, polyvial and silicone septa can be used as an effective dispensers to impregnate (Z)-9-tricosene in order of preference to facilitate slow and sustained release of pheromone and thereby trapping the optimum number of house flies towards delta trap.

Key Words: *Muscadomestica*, (Z)-9-tricosene, Pheromone dispenser, Delta trap.

INTRODUCTION

Pheromones are chemicals produced by insects which send signals to other members of the same species. The best known are sex pheromones emitted usually by the females of many insect species in order to indicate their location and willingness to

mate. Sex pheromones of *Musca domestica* have been studied in detail by researchers, amongst which only one pheromone, the (Z)-9-tricosene, produced by female house flies to attract males has been successfully employed for field use (Carlson *et al.*, 1971; Chapman *et al.*, 1998a, 1998b, 1999; Noorman and Den Otter, 2001; Butler, 2007 and Butler *et al.*, 2007). One of the major problems associated with the sustainable field use of (Z)-9-tricosene is its volatility and biodegradability. (Z)-9-tricosene based pheromone baits used to attract house flies do not last long unless they are impregnated into sustained release or slow release

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dispensers (Chapman *et al.*, 1998a). Even though control of flies using pheromones is a viable pest management approach, suitable pheromone dispensers however, are lagging behind the general availability of pheromones (Hummel *et al.*, 2013). Identification of the suitable pheromone dispenser is thus vital for successful impregnation of pheromones. The present *in vitro* study was therefore undertaken to study the comparative efficacy of different pheromone dispensers impregnated with (Z)-9-tricosene in luring *M. domestica* towards delta trap.

MATERIALS AND METHODS

Pheromone

The house fly sex attractant pheromone (Z)-9-tricosene - 97% (Sigma Aldrich, Germany).

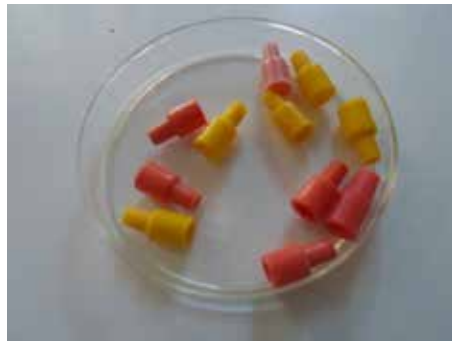
Pheromone dispensers

The pheromone dispensers included septa, both rubber and silicone, plastic dispensers, circular plastic green lure and rectangular plastic dispenser and polyvial, a polythene dispenser (Sun lure, Ambattur, Chennai) (Fig. 1).

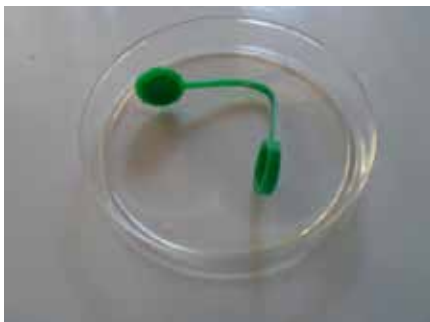
Figure – 1. (Z)-9-tricosene pheromone dispensers evaluated. a: Rubber septa dispenser, b: Silicone septa dispenser, c: Circular plastic green dispenser, d: Rectangular plastic dispenser, e: Circular plastic dispenser, f: Polyvial



a



b



c



d



e
Impregnation of pheromone into dispensers

All the dispensers were impregnated with 20 μ l of 0.22 per cent (Z)-9-tricosene dissolved in hexane.

Delta trap bioassay in insectory for all dispensers

A red acrylic delta trap (fabricated at Technocrats, Chennai) was used for the study (Fig 2). The dimension of the delta trap was 22 cm L, 18.5 cm B, 8.5 cm H and



f
sides of 11 cm with a 20.5 x 16 cm insert at the base where a transparent polythene sheet of 17 x 13.5 cm was placed and glue was applied uniformly on it. The pheromone lure was suspended in the middle of the trap using a thread. The trap was kept in the floor of the insectory for 1 h. At the end of 1 h, trapped flies were counted and sexed under stereo zoom microscope (Leica M-29-5, Switzerland). After each experiment, the delta trap was thoroughly washed in hexane thrice and then used.

Figure – 2. Red acrylic delta trap used to evaluate the efficacy of (Z)-9-tricosene pheromone dispensers a: front view, b: top view



RESULTS AND DISCUSSION

Out of different (Z)-9-tricosene dispensers tested, the circular plastic green lure attracted the maximum number of flies, 32.10 per cent (165 flies) (Fig. 3) followed by polyvial which attracted 25.68 per cent (132 flies). The silicone septa attracted 21.59 per cent (111 flies) and rubber septa attracted 15.36 per cent (79 flies). The least number of house flies was attracted by the rectangular plastic lure, 5.25 per cent (27 flies). Overall, out of the 514 house flies trapped using different pheromone dispensers, 46.49 per cent (239 flies) were males and 53.50 per cent (275 flies) were females. In the circular plastic green lure, out of 165 flies lured, 40 per cent (66 flies) were males and 60 per cent (99 flies) were females. In polyvial, out of 132 flies attracted, 45.45 per cent (60 flies) were males and 54.54 per cent (72 flies) were females. In silicone septa, out of 111 flies attracted, 47.74 per cent (53 flies) were males while 52.25 per cent (58 flies) were females. With rubber septa, out of 79 flies lured, 58.22 per cent (46 flies) were males and 41.77 per cent (33 flies) were females. The rectangular plastic lure impregnated with (Z)-9-tricosene lured 27 flies of which 51.85 per cent (14 flies) were males and 48.14 per cent (13 flies) were females. The house fly sex pheromone (Z)-9-tricosene has been used to attract house flies (Carlson *et al.*, 1971; Chapman *et al.*, 1998b, 1999; Noorman and Den Otter, 2001; Butler, 2007 and Butler *et al.*, 2007). (Z)-9-tricosene indicates the male house fly that the female fly it had encountered is a *M. domestica* and not some other species. (Z)-9-tricosene acts as an attractant or aggregation pheromone. It is a highly volatile compound hence can

only influence the behavior of house flies at short ranges (Noorman and Den Otter, 2001 and Kelling *et al.*, 2003). In order to facilitate slow and steady release of pheromone, it needs to be impregnated successfully in a suitable dispenser. In the present study (Z)-9-tricosene was impregnated into different dispensers and evaluated for their efficacy in attracting house flies in the insectory using delta trap. Among plastic dispensers, circular plastic green lure was better than the rectangular plastic lure. With respect to septa dispensers, the silicone septa lured more flies compared to rubber septa. Polyvial was found superior to septa and rectangular plastic lure. In the present study, although the same concentration of (Z)-9-tricosene was impregnated in all the dispensers, the low efficacy observed in septa dispensers compared to plastic and polyvial dispensers could be presumably due to the comparatively reduced pheromone impregnation coupled with insufficient release of (Z)-9-tricosene. It was interesting to note variations in the attraction of male and female house flies towards the different dispensers. In the present study, more number of females were attracted than male flies towards circular plastic green lure, polyvial and silicone septa. This variation in the numbers of attracted male and female flies towards dispensers could be presumably due to the variation in the chemical ingredients of the dispensers as well as the colour, odour and physical structure that influences fly attraction. Female bias in the tested fly population could also be another reason for the increase in number of female flies. In addition, physiological and nutritional status of flies, environmental factors such as rheotaxis, sound, moisture and wind

velocity also could have contributed to this sex variation. Overall, the efficacy of the dispensers especially circular plastic green lure and polyvial indicates their ability to

retain the pheromone without deterioration and evaporation thereby help in sustained release of (Z)-9-tricosene and luring of house flies.

Figure – 3. House flies attracted towards circular plastic green lure



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Design and Operational Performance of Needlefish Gillnets along the Coast of Ramanathapuram District, Southeast Coast of Tamil Nadu

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ABSTRACT

A study undertaken to analyse the design and operational aspects of Needlefish gillnets of four different fishing villages of Ramanathapuram district of Tamilnadu, revealed the existence of notable difference with respect to catch rate despite having many common design features. Relatively shallow and broader continental shelf boarded with coral reef was found to serve as an ideal habitat for Needlefishes along the coastal villages of this district. Six species viz., *Ablenneshians*, *Tylosurus crocodiles*, *T. choram*, *T. agus*, *Strongylura strongylura* and *S. leiura* were found to constitute the fishery of Needlefish in gill nets with the dominant species being either *A. hians* or *T. crocodiles*. *A. hians* was dominant in the gillnet catches Gulf of Mannar while *T. crocodiles* showed its dominance in the gillnet catches from the fishing villages of Palk bay. Trawling was found to be a common disturbing activity for Needlefish gillnetting in all the four fishing villages studied. Among four villages, Mundal was found to be notable for Needlefish gillnetting as relatively longer gill nets involving more number of fishing crafts were found to be operated from this village. The catch composition of gillnets revealed that the mean Catch Per Unit Effort (CPUE) of Needlefishes ranged from 23 to 25 nos/boat/day while the CPUE of commercially important fishes such as seer fish, barracudas, mackerels, flying fish, queen fish and sail fishes altogether ranged from 15 to 19 nos/boat/day. The study suggests evolving a selective pelagic longline gear for capturing Needlefishes considering the rich resource of Needlefish along the coast of Ramanathapuram district of Tamil Nadu

Key Words: Gillnet, Needlefish, Vallam, CPUE

INTRODUCTION

Needlefish popularly called as ‘Mural’ in Tamil has a wide distribution in the

tropical and sub-tropical regions of the world. Needlefish belong to the family Belontiidae is represented by 32 species under 10 genera. However, only eight species belonging to different four genera such as *Ablennes hians*, *Platybelone argalus*, *Strongylura incise*, *S. leiura*, *S. strongylura*, *Tylosurus acus*, *T. crocodiles* and *T. choram* have been reported in the gillnet catches of Indian seas (FAO, 1995; Anon, 2011).

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Ramanathapuram is a notable maritime district of Tamil Nadu and occupies the first position in marine capture fish production of the state. This district has a coast line of 237 km and offers livelihood for nearly about 22% of the total fisher population and accounts for about 20% of the fish landings of the state (Johnson *et al.*, 2013).

Needlefishes form a sporadic bycatch in the gillnets operated all along the coast of Tamilnadu. However, Ramanathapuram coast is known for its Needlefish fishery through pelagic drift gillnets. The Needlefish fishery of this coast is constituted by seven species such as *A. hians*, *S. leiura*, *S.strongylura*, *P. argalus*, *T. acus*, *T. crocodiles*, and *T. choram*. The present study was undertaken with an objective analysing the present status of design and operational details of Needlefish gillnets of Ramanathapuram district.

MATERIALS AND METHODS

The study was carried out for four months from March 2016 to June 2016 covering four important coastal fishing villages of Ramanathapuram district *viz.*, Mundal (Lat.09°06'7"N; Long.78°35'6"E), Velayuthapuram (Lat.09°28'5"N; Long.78°89'6"E), Pamban (Lat.09°27'8"N; Long.79°22'9"E) and Mandapam (Lat.09°27'7"N; Long.79°12'5"E). Both design and operational details of Needlefish gillnets were collected. The survey included collection of information on the main specifications of fishing crafts engaged and the design details of Needle fish gillnets covering 17 parameters, drawn as per the FAO guidelines formulated Nedelec (1975). The operational details of the nets and

catch- effort particulars were also collected from the selected fishing villages.

RESULTS AND DISCUSSION

Design description of Needlefish gillnets

A design detail of different types of Needlefish gillnets operated from four different fishing villages of Ramanathapuram coast are given in Table - 1. The nets showed notable differences with respect to hung depth despite having common design features. Regarding the material of construction, the webbing made up of both multi-filament nylon twine with the specification of 23tex x2x3 and mono-filament nylon twine with the thickness of 1.0mm dia. were found to be used in Velayuthapuram. However, in all other fishing villages, nets with the webbings fabricated using multi-filament nylon twine of the specification 23tex x2x3 were alone found to be used. The gill nets were either green or yellow in colour, however, green was found to be the predominant by used colour of the net. The Head Rope Length (HRL) of Needlefish gillnets ranged from 40.5 to 104.0 m and the hung depth was notably small ranging from 1.5 to 2m owing to the pelagic nature of the fish.

In present investigation the mesh size of the Needlefish gillnets was found to range from 30 to 52 mm. Gill nets used in Mundal were relatively longer with 4,000 meshes along length in contract to (2,700 meshes in the gillnets of Velayuthapuram. Regarding hung depth also, it was as high as 3.4m in the gillnets of Mundal and was 1.7m in the gillnets at Velayuthapuram. Fishermen were found to use 8 to 14 gill nets per trip. During the study period, floats made up of plastic

and cork were used in Velayuthapuram, whereas plastic floats were alone used in other three fishing villages. The plastic floats each weighing 20 g with the extra buoyancy of 30 g in seawater were found used in the gillnets invariably in all the four villages.

The inter distance between two floats was 0.45 m in the gillnets of Velayuthapuram whereas, it was 1.3 m for the gillnets of the other three fishing villages studied (Table - 2 and Figure 1 and 2).

Table - 1. Specifications of Needlefish gillnets operated in the selected fishing villages of Ramanathapuram district

Sl.No	Parameters	Fishing villages				
		Mundal	Velayuthapuram		Mandapam	Pamban
1	Material of construction	Multi- filament Nylon twine	Multi- filament Nylon twine	Mono- filament Nylon twine	Multi filament Nylon twine	Multi- filament Nylon twine
2	Twine specification (Tex numbering / Ø in mm)	23 tex x 2 x 3	23 tex x 2 x 3	1.0	23 tex x 2 x 3	23 tex x 2 x 3
3	Colour	Green	Green & Yellow	Green & Yellow	Green	Green
4	Head rope length (m)	104.0	40.5	40.5	97.5	97.5
5	Hung depth (m)	3.4	1.7	1.7	2.7	2.8
6	Mesh size (mm)	51	32	32	52	51
7	No. of meshes in length	4,000	2,700	2,700	3,750	3,750
8	No. of meshes in depth	66	52	52	55	55
9	Hanging coefficient (Horizontal)	0.50	0.47	0.47	0.50	0.51
10	Hanging coefficient (Vertical)	0.86	0.88	0.88	0.86	0.86
11	Head rope material	Nylon	Polypropylene	Polypropylene	Nylon	Nylon
12	Head rope thickness(mm)	2.0	2.5	2.5	2.0	2.0
13	Float material	Plastic	Plastic & cork	Plastic & cork	Plastic	Plastic
14	Float weight (g)	20	20	20	20	20
15	Buoyancy of the float(g)	30	30	30	30	30
16	Float Interval (m)	1.3	0.45	0.45	1.3	1.3
17	No of units operated/ boat	8-14	8-12	8-12	10-14	10-12

Both FRP boats and ‘*Vallam*’ were found used for operating Needlefish gillnets in the villages surveyed. In general, smaller fishing crafts with an Over All Length (OAL) ranging from 4 to 8 meters were found engaged for Needlefish gillnetting owing to the distribution of Needlefishes in the near shore fishing grounds located

just 12 to 25 nm from the shore. The exclusive use of ‘*Vallam*’ by the fishermen of Pamban in contrast to the other fishing villages may be attributed due to rough sea conditions prevailing in this region which prevented the operation of lighter FRP boats (Table - 2).

Table - 2. Specifications of crafts involved in Needlefish gillnets in the selected fishing villages of Ramanathapuram district

Sl.No	Parameters	Fishing villages						
		Mundal		Velayuthapuram		Mandapam		Pamban
1	Craft type	Vallam	FRP boat	Vallam	FRP boat	Vallam	FRP boat	Vallam
2	Length (m)	7.0 – 8.0	4.0	7.0-8.0	4.0-5.0	6.0-7.0	4.0	7.0-8.0
3	Beam (m)	2.0	1.5	2.0	1.5	2.0	1.5-2.0	1.5
4	Depth (m)	1.8 – 2.0	1.0-1.5	2.0	1.5	1.5-2.0	1.5	2.0-2.5
5	Total number of crafts	35	20	20	10	15	9	17
6	Total number of fishermen involved in Needlefish gill netting	180-200		100- 120		100-110		70-80

During the study period, six species of Needlefishes such as *A. hians*, *T. crocodilus*, *T. choram*, *T. agus*, *S. strongylura*, and *S. leiura* were found to constitute the catches of gill nets. *A. hians* was found to be the most dominant among the Needle fishes caught in the gill nets operated from Mundal and Velayuthapuram with the percentage contribution of 49% and 50% in the total Needlefish catch respectively.

However, *Tylosurus crocodilus* was the most dominant species in the gillnet catches of Mandapam and Pamban with the percentage composition of 60% and 70% of Needlefish catch respectively (Table 4). Similar observations have been made by Kasim *et al.* (1996) who reported *Ahiansas* the most dominant species followed by *T. crocodilus* of Needle fishes in the drift gill net catches of Thoothukudi coast.

Table - 4. Catch composition Needlefish gillnets of selected fishing villages of Ramanathapuram district

Sl. No	Name of the fishing village			
	Mundal	Velayuthapuram	Mandapam	Pamban
1	<i>Ablenneshians</i> (45%)	<i>Ablenneshians</i> (50%)	<i>Tylosuruscrocodilus</i> (60%)	<i>Tylosuruscrocodilus</i> (70%)
2	<i>Tylosuruscrocodilus</i> (27%)	<i>Tylosuruscrocodilus</i> (22%)	<i>Tylosurusacusmelanot</i> (20%)	<i>Tylosurusacus</i> (15%)
3	<i>Tylosurusacus</i> (14%)	<i>Tylosurusacus</i> (17%)	<i>Ablenneshians</i> (12%)	<i>Ablenneshians</i> (8%)
4	<i>Strongyluraleiura</i> (7%)	<i>Strongyluraleiura</i> (5%)	<i>Strongylurastrongylura</i> (3%)	<i>Strongylurastrongylura</i> ((4%)
5	<i>Strongylurastrongylura</i> (5%)	<i>Strongylurastrongylura</i> (4%)	<i>Strongyluraleiura</i> (3%)	<i>Strongyluraleiura</i> (2%)
6	<i>Platybeloneargalus</i> (2%)	<i>Platybeloneargalus</i> (3%)	<i>Platybeloneargalus</i> (2%)	<i>Platybeloneargalu</i> (1%)
7	Seerfishes, Barracudas, Mackerals & Sailfish	Flyingfish, Barracudas, Mackerals and half beaks	Seerfish, Barracudas, Mackerals and Queenfishes	Seerfish, Barracudas, Mullets, and Mackerals.

Among the four villages studied, Mundal was found to be a notable fishing village for Needlefish gill netting as evidenced through highest catch rate of 1, 00,870 numbers of Needlefishes and 67,223 number of other commercially important

fishes for a total fishing effort of 4,434 fishing days during the study period (Table - 3). In terms of total fish catch, Mundal was followed by Velayuthapuram, Mandapam and Pamban (Table - 5, 6,7 and 8) and (Figure 3) .

Table - 3. Operational details of Needlefish gillnets in selected fishing villages of Ramanathapuram district

Sl. No	Parameters	Name of the fishing village			
		Mundal	Velayuthapuram	Mandapam	Pampban
1	No of fishing trips per month	25	25	16-20	20-25
2	Depth of operation (m)	8 – 9	12 – 13	8 - 9	6 – 7
3	Nature of operation	Pelagic; drift	Pelagic; drift	Pelagic; drift	Pelagic; drift
4	Distance to fishing ground (Nm)	25	14	12	25
5	Fishing season	Throughout the year	Throughout the year	Throughout the year	Throughout the year
6	Peak fishing season	Oct-Jan	Oct-Jan	April-July	April-July

Table - 5. Catch and effort particulars of Needlefish gillnets of Munda

Sl. No	Month	Average number of boats operated per day (a)	Number of Fishing days (b)	Monthly fishing effort (boat days) (c)	Mean number of Fishes landed / boat / day (d)		Monthly mean Catch / boat (b) x (d)		Total catch estimated (c) x (d)	
					Needle fishes	Other Fishes	Needle fishes	Other Fishes	Needle Fishes	Other Fishes
1	March	46.50	24	1,116	19.5	13.00	468	312	21,762	14,508
2	April	47.25	24	1,134	23.5	14.75	564	354	26,649	16,727
3	May	46.50	24	1,116	22.0	15.50	528	372	24,552	17,298
4	June	44.50	24	1,068	26.1	17.50	624	420	27,907	18,690
Total effort group wise catch (Nos.)				4,434					1,00,870 CPUE= 22.75	67,223 CPUE= 15.16
Total effort /catch (Nos.)				4,434					1,68,093 CPUE = 37.91	

Table - 6. Catch and effort particulars of Needlefish gillnets of Velayuthapuram

Sl. No	Month	Average number of boats operated per day(a)	Number of Fishing days (b)	Monthly fishing effort (boat days) (c)	Mean number of Fishes landed / boat / day (d)		Monthly mean Catch / boat (b) x (d)		Total catch estimated (c) x (d)	
					Needle fishes	Other fishes	Needle fishes	Other Fishes	Needle Fishes	Other Fishes
1	March	26.5	26	687	20	18	520	468	13,780	12,402
2	April	27.5	26	715	24	16	624	416	17,160	11,440
3	May	27.0	26	702	22	20	572	520	15,444	14,014
4	June	27.5	26	689	26	22	676	572	17,914	15,158
Total effort group wise catch (Nos.)				2,795					64,298 CPUE= 23.00	53,040 CPUE= 18.97
Total effort /catch (Nos.)				2,795					1,17,338 CPUE= 41.98	

Table - 7. Catch and effort particulars of Needlefish gillnets of Mandapam

Sl. No	Month	Average number of boats operated per day(a)	Number of Fishing days (b)	Monthly fishing effort (boat days) (c)	Mean number of Fishes landed / boat / day (d)		Monthly mean Catch / boat (b) x (d)		Total catch estimated (c) x (d)	
					Needle fishes	Other fishes	Needle fishes	Other Fishes	Needle Fishes	Other Fishes
1	March	20.0	21	420	26	18	546	378	10,920	7,560
2	April	21.0	21	441	24	15	504	315	10,584	6,615
3	May	20.0	21	430.5	22	14	462	294	9,471	6,027
4	June	21.0	21	441	27	17	567	357	11,907	7,497
Total effort group wise catch (Nos.)				1,732.5					42,882 CPUE = =24.75	27,699 CPUE = 15.99
Total effort /catch (Nos.)				1,732.5					70,581 CPUE = 40.73	

Table - 8. Catch and effort particulars of Needlefish gillnets of Pamban

Sl. No	Month	Average number of boats operated per day (a)	Number of Fishing days (b)	Monthly fishing effort (boat days) (c)	Mean number of Fishes landed / boat / day (d)		Monthly mean Catch / boat (b) x (d)		Total catch estimated (c) x (d)	
					Needle fishes	Other fishes	Needle fishes	Other Fishes	Needle Fishes	Other Fishes
1	March	16	22	352	23	15	506	330	8,096	5,280
2	April	15	22	330	25	17	550	374	8,250	5,610
3	May	17	22	374	20	14	440	308	7,480	5,236
4	June	15	22	330	22.5	15	495	330	7,425	4,950
Total effort group wise catch (Nos.)				1,386					31,251 CPUE = 22.54	21,076 CPUE = 15.20
Total effort /catch (Nos.)				1,386					52,327 CPUE = 37.75	

Operational details of Needlefish gillnets

Fishermen belonging to the four fishing villages surveyed were found to operate the gill nets for 16 to 25 days per month (Table - 3). Further, fishermen of the Mandapam were found to restrict their number of fishing days not exceeding 20 per month owing excessive disturbances caused by the operation of trawlers. The reason for higher abundance of Needlefishes along the coastal fishing villages of Ramanathapuram district

may be attributed to the availability of wide and shallow continental shelf bordered with coral reef which is found to be an ideal natural living habitat for Needlefishes. Though the Needlefish gillnetting was observed throughout the year, the peak fishing season was from October to January in Mundal and Velayuthapuram and April to July in Mandapam and Pamban may be attributed to spatial difference. As the fishing villages such as Mundal and Velayuthapuram are

located in Gulf of Mannar while Mandapam and Pamban are located in Palk bay. Owing to the difference in the oceanographic features such as current, wind etc., notable

difference in the Needlefish fishing season could be observed between the villages although all the four fishing villages are located closer to each other.

Figure - 1. Needlefish gillnet of Velayuthapuram

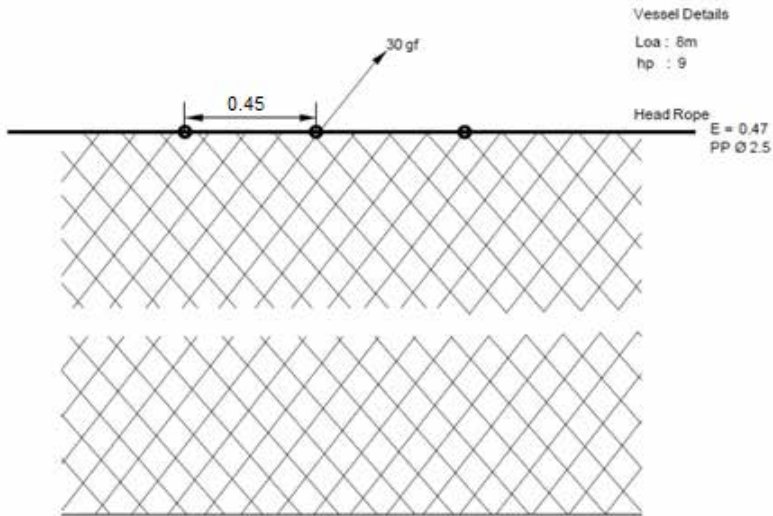


Figure - 2. Needlefish gillnet of Mundal, Mandapam and Pamban

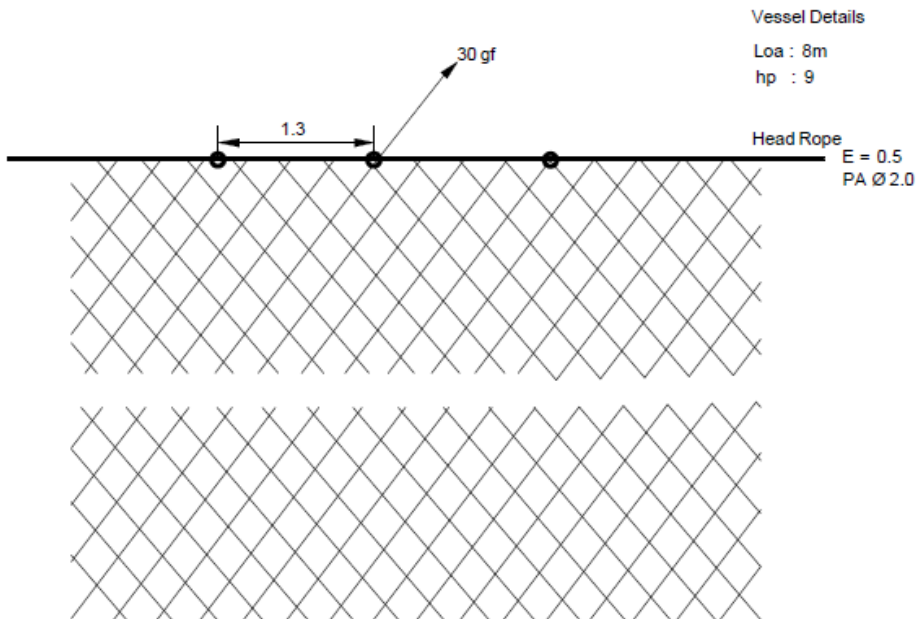
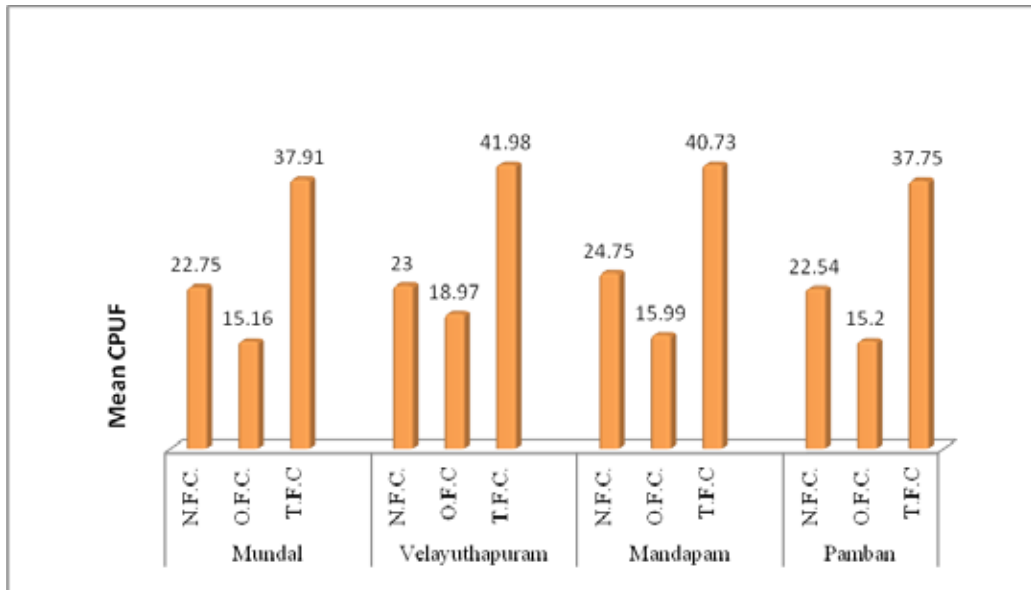


Figure - 3. Catch and effort particulars of Needlefish gillnets of different fishing villages



Note: N.F.C – Needle fishes ; O.F.C – Other fishes ; T.F.C – Total fish catch

Mean CPUE = Average no. of fish caught / boat / day

CONCLUSION

Catch per unit effort

The catch composition of gillnets revealed that the mean CPUE of Needlefish ranged from 23 to 25 nos/boat/day while CPUE of all other commercially important fishes such as seer fish, barracudas, mackerels, flying fish, queen fish and sail fishes altogether ranged from 15 to 19 nos/boat/day. Further, the mean total fish catch ranged from 38 to 42 nos/boat/day. Hence, the pelagic drift gill nets are termed as Needle fish gill nets owing to the domination by Needlefishes

Higher abundance of Needlefishes along the coastal fishing villages of Ramanathapuram district was found to be due to wider continental shelf area bordered with coral reefs which serves as an ideal habitat for Needlefishes. Drift gillnetting was found to be an ideal fishing method for the capture of Needlefishes. However, the study suggests that developing pelagic longlines for the selective capture of Needlefishes is essential considering the rich resource of Needlefishes along the coast of Ramanathapuram district.

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Socio – personal and Economic profile of Dairy Farmers in Palakkad District of Kerala

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ABSTRACT

The present study was conducted in Palakkad district of Kerala as a research activity of ASRTC Trust. A Schedule was developed to record socioeconomic profile of the respondents. Data was collected from 240 dairy farmers using questionnaires by personal interview method and was analysed with statistical tools like frequency and percentage. The distribution of farmers according to age observed in the present study was 51.7 per cent in middle age, 46.8 per cent in old age and 1.70 per cent in young age groups. Farmers with education of class X and less accounted for 75.88 per cent of respondents while 17.65 per cent were graduates and 4.12 per cent had post-graduation or higher education. Illiterate farmers accounted to 2.35 per cent of respondents. Majority of farmers were from OBC communities (46%) followed by general (39%) while SC/ST category accounted for 15 per cent of the respondents. Family size was small (less than 5) in 70.4 per cent of respondents and 27.9 per cent of farmers had medium sized families with 6-10 members. About 1.7 per cent of farmers had large families (more than 10 members). Majority of the respondents maintained small herds (65.8%) while 21.3 per cent had medium sized herds. Large herds were maintained by 12.9 per cent of respondents. Majority of the farmers (40.4%) belonged to medium income group, 33.8 per cent had low income and 25.8 per cent had high income. Most farmers had a landholding between 11 cents and up to one acre (45.08%). Almost equal proportions of farmers had less than 10 cents (27.98%) and more than 2 acres (26.94%). Biogas plant was installed by 14.2 per cent of the respondents

Key words: Dairy, Socio-personal, economic, Palakkad, livestock

INTRODUCTION

Palakkad, renowned as the ‘granary of Kerala’ is its largest district with an area of 4475.8 square kilometer. Palakkad lies between 10° 21’ North and 11° 14’ North latitudes and 76° 02’ East and 76° 54’ East longitudes. It borders with, Malappuram

district in the North West, Thrissur district in the south west and Tamil Nadu’s Coimbatore district in the east. The climate is humid to sub humid and the average annual rainfall recorded in the region is 2171 mm (Premakumar *et al.*, 2015). Paddy, coconut, vegetable and rubber forms the major crops cultivated in the district. The bovine population of Palakkad district was 1,75,088 heads including 9,178 buffaloes as per cattle census report of 2012. Agricultural census report (2007) brought out that the size of land holdings in Kerala had

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decreased from 0.28 ha in 1967 to 0.15 ha in 2007. Likewise, livestock census reports indicate that bovine population of the state declined from 33.96 lakhs (1996) to 14.31 lakhs (2012). The article attempts to bring out the differences in the socio economic profile of dairy farmers with emphasis on the standing of Palakkad district vis a vis other south Indian states.

MATERIALS AND METHODS

The present study was conducted among 240 purposively selected dairy farmers in Palakkad district of Kerala as part of a research activity of ASRTC Trust. Schedule was developed for analysing socio-economic profile of respondents in the study area. The parameters investigated

were age, education, community, family size, income generated, herd size and land holding. Questionnaire was given to dairy farmers. Some of the respondents were reluctant to disclose details of community, education and land holding. Information collected by personal interview method was analyzed with tools like frequency and percentage.

RESULTS AND DISCUSSION

The results from the present study on social profile of dairy farmers of Palakkad district is summarized in Table - 1. Community and education were not revealed by 16.67 per cent and 29.17 per cent of the respondents respectively.

Table - 1. Social profile of dairy farmers in Palakkad district of Kerala

Characteristics	Classification	Frequency	Percentage
Age (N=240)	Young age (20-40 years)	4	1.70
	Middle age (41-60 years)	124	51.60
	Old age (>61 years)	112	46.70
Family Size (N=240)	Small (<5)	169	70.40
	Medium (6-10)	67	27.90
	Large (>10)	4	1.70
Community (N=240)	General	78	32.50
	OBC	92	38.33
	SC/ST	30	12.50
	Not revealed	40	16.67
Education (N=240)	Up to High School	129	53.75
	Graduate	30	12.50
	Post graduate	7	2.91
	Illiterate	4	1.67
	Not revealed	70	29.17

Majority of respondents in the present study belonged to middle and old ages. Both these groups accounted for 98.3 per cent of sample size. The shift in farmer's age profile towards old age could be an indicator of the reluctance of younger generation to engage in dairying and allied industries; other than sampling bias, if any. However results of a study conducted in Wayanad district (Prasad *et al.*, 2017) of Kerala presented a higher level of involvement of young farmers (34%). Hence the age profile of farmers involved in dairying could be highly variable across the state. The probable causes besides socio economic reasons could be the availability of permanent pastures in Wayanad (Anon, 2011) and higher average productivity of crossbred animals calculated from data presented by Rath (2016). In Karnataka the inclusion of young farmers stood at 9.72 per cent (Mali *et al.*, 2014) to 17.5 per cent (Mahalakshmi *et al.*, 2016). Gopi *et al.* (2017) reported that 23.33 per cent of dairy farmers were in the young age group after conducting a study in Villupuram and Salem districts of Tamil Nadu. Therefore participation of youth in dairying appeared higher in other south Indian states than that in Palakkad district of Kerala (1.7%). The reasons for disengagement of younger generation from dairy farming remains largely obscure and should be studied separately as the remedial measures may involve policy decisions by law makers.

Majority of dairy farmers (70.4%) who participated in the study had small families with 1 to 5 members. In Bangalore North taluk of Karnataka small families operated 40 per cent of the farms (Sathyanarayan *et*

al., 2010) which is lower than that observed in the present study. Study conducted by Devasena *et al.* (2015) in Chittoor district of Andhra Pradesh indicated that 54.3 per cent of dairy farmers had small families. Gopi *et al.* (2017) observed that 73.3 per cent of the dairy farmers had small families in Villupuram and Salem districts of Tamil Nadu which was marginally higher than that observed in Palakkad. The findings of the present study agree well with national statistics which indicated that average family size (Anon, 2006) was lowest in Tamil Nadu, followed by Andhra Pradesh, Kerala and Karnataka, among south Indian states. However the participation of small families in Andhra Pradesh should have been more than that in Kerala. The discrepancy could be due to sampling error and warrant detailed investigation before drawing any conclusion.

Analysis of educational profile of dairy farmers in Palakkad district indicated that 70.83 per cent were literate. About one third (29.17 %) of the respondents had refrained from revealing their educational status. Higher education (graduation) was gained by 17.65 per cent of farmers with about 4.12 per cent of them being postgraduates. In Kolar district of Karnataka 39 per cent of resource poor dairy farmers were illiterate (Mahalakshmi *et al.*, 2016) and in Chittoor district of Andhra Pradesh 42.5 per cent of dairy farmers were illiterate (Devasena *et al.*, 2015). Nearly half of the dairy farmers (45%) in Salem & Villupuram districts of Tamil Nadu were illiterate according to Gopi *et al.* (2017). Results from the present study indicate that literacy was higher among dairy farmers of Palakkad district

in comparison with similar regions of south Indian states. Higher literacy among dairy farmers of Palakkad district could be a reflection of it being the state with highest literacy and human development index in India.

The results from the present study on economic profile of dairy farmers in Palakkad district is summarized in Table - 2. Information about land holding was not revealed by 19.58 per cent of the farmers sampled for the study.

Table - 2. Economic profile of dairy farmers in Palakkad district

Characteristics	Classification	Frequency	Percentage
Herd Size (N=240)	Small (<5)	158	65.80
	Medium (6-10)	51	21.30
	Large (>10)	31	12.90
Monthly income (N=240)	Low income (<Rs. 5,000)	81	33.80
	Medium income (Rs. 5,000 to 15,000)	97	40.40
	High income (>Rs. 15,000)	62	25.80
Land holding (N=240)	< 10 Cents	54	22.50
	10 Cents to One Acre	87	36.25
	> 2 Acres	52	21.67
	Not revealed	47	19.58
Biogas Plant (N=240)	Installed	34	14.20
	Not installed	206	85.80

Present study identified that 65.8 per cent of farmers owned small herds and were engaged in Livestock rearing as a livelihood activity. The number of animals maintained by a farmer may remain correlated with per capita land holding since more than one fifth (22.5%) of the respondents from Palakkad district owned less than 10 cents of land. The observations could be justified in the light of the report by Shaharban and Shabana (2015) that 70 per cent of farmers in the state were marginal farmers. The proportion of farmers with medium herd size remained more or less similar in the study area (21.3%) and Chittoor district of Andhra Pradesh (23.7%) as reported by Devasena *et al.* (2015). The same study suggested that farmers maintaining small

herds accounted for 74.3 per cent of the total dairy farmers, which is higher than that in Palakkad district. However majority of the farmers (73.3%) in Tamil Nadu had medium sized herds (Gopi *et al.*, 2017). Analysis of the results in comparison with other south Indian states indicated that herd size was lower in Palakkad (Kerala) and Chittoor (Andhra Pradesh) whereas farmers in Salem and Villupuram districts (Tamil Nadu) maintained larger herds.

As inferred from the present study, 40.4 per cent of the farmers in Palakkad district belonged to medium income group while 33.8 per cent earned low income and 25.8 per cent earned high returns. In contrast, 96.7 per cent of farmers in Bangalore north

taluk of Karnataka (Sathyanarayan *et al.*, 210) and 76.6 per cent of contract dairy farmers of Namakkal district of Tamil Nadu (Kalaivani *et al.*, 2017) earned low income. However another study conducted in Vijayapur and Bagalakote districts of Karnataka identified that 32.69 per cent of farmers were in high income group and 48.08 per cent were low earners (Mallu and Teggi., 2017). Therefore the earnings of dairy farmers in the present study could be comparable to certain regions of other south Indian states.

Biogas utilization observed in the present study is lower than that reported from Wayanad district (56%) of Kerala by Prasad *et al.* (2017). Lower adoption of biomethanation technology could be due to economic reasons or the lack of penetration of extension services and government support. Biogas being a renewable resource has large potential for development and utilization in Palakkad district.

Most of the dairy farmers in Palakkad had a landholding of more than 11 cents to one acre (36.25%). Almost equal proportions of farmers had less than 10 cents (22.50%) and more than 2 acres (21.67%) of land. Nearly half of the dairy farmers in Belgaum district of Karnataka (45.83%) had large land holding (Mali *et al.*, 2014). In Bengaluru rural district, 89 per cent of the farmers were marginal farmers who owned less than one hectare of land, while 5 per cent were landless (Chandrasekar *et al.*, 2017). About two-fifth (39.16 per cent) of the dairy farmers in Chittoor district of Andhra Pradesh were marginal land owners (Gopi *et al.*, 2017). With reference to agriculture in Kerala, it was reported that

landholding size declined due to population pressure, climate change and decline in profitability (Shaharban and Shabana, 2015). The average land holding of dairy farmers in Palakkad district could be lower than that in other south Indian states. About a quarter of dairy farmers in Chittoor district (27.1%) of Andhra Pradesh and 22.5 per cent of dairy farmers in Villupuram district of Tamil Nadu were observed as landless by Devasena *et al.* (2015) and by Gopi *et al.* (2017) respectively. The real proportion of landless farmers in Palakkad district remained unclear because as many as 47 respondents did not reveal details about their land holdings.

CONCLUSION

The age profile of livestock farmers indicated that the new generation in Palakkad district is moving away from dairying and allied work areas. The reasons for disengagement of younger generation from dairy farming remain largely obscure. The neighboring states have higher involvement of youth in this sector. Higher proportion of dairy farmers in study area had smaller families than those in some comparable regions of other south Indian states. Dairy farmers in Palakkad district maintained smaller herds than that in Tamil Nadu. The average land holding of dairy farmers in Palakkad district could be lower than that in other south Indian states. The participation of landless farmers could not be ascertained as some participants refrained from responding to such questions. About one third of the farmers earned low income from dairy farming in Palakkad district. Adoption of biogas utilization remained low in the study area.

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Short Communication

Seroprevalence of foot and mouth disease in small ruminant population of Tamil Nadu

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ABSTRACT

Foot and mouth disease is an acute febrile highly contagious disease of cloven footed animals. India has a sizable proportion of small ruminants (135.17 million goats and 65.0 million sheep). Tamil Nadu, the eleventh largest state in India has a sizable proportion of sheep and goat populations (7.36 million sheep and 6.02 million goats). Sub-clinically infected small ruminants may pose a threat to cattle and buffalo in integrated livestock system. Small ruminants are neglected in FMD surveillance and control strategies in the country. In the present study, serological investigations against FMD were carried out to generate data on antibody prevalence in sheep and goat population of Tamil Nadu. Overall, 21.4% of sheep (83 out of 387 samples) and 23.5% of goats (81 out of 345 samples) tested were positive for FMD NSP antibodies and 14.7% of sheep and 18.3 % goats were positive for antibodies against virus structural proteins. The current study demonstrated the seroprevalence of FMD in the sheep and goat population of Tamil Nadu and suggests the need for surveillance activities and FMD control by vaccination in small ruminants alongside large ruminant population.

Key words: Seroprevalance, FMD.Small Ruminants, Control

INTRODUCTION

Foot and mouth disease (FMD) is an acute febrile highly contagious disease of cloven footed animals (Thomson, 2002). There are seven distinct FMD virus

(FMDV) serotypes prevalent in the world. The serotype O is responsible for majority of the outbreaks in India followed by A and Asia 1 (ICAR- DFMD Annual Report 2015). There are 135.17 million heads of goats and 65.0 million heads of sheep reared in India. Tamil Nadu, the eleventh largest state in India has sizable populations of sheep and goat (7.36 million sheep and 6.02 million goats). Cattle and buffalo are vaccinated regularly under FMD control programme (FMD-CP) in India. However, the sheep and goat population are not included in the

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FMD control programme (Patil *et al.*, 2002a; 2002b). Foot and mouth disease in small ruminants is mild or inapparent (Kitching and Hughes, 2002). In integrated livestock system, the sub-clinically infected small ruminants posed a threat to the cattle and buffalo population. Serology may be used to diagnose the disease in sheep and goats (Paton *et al.*, 2009). The current study was carried out to determine the seroprevalence of FMD in sheep and goat population of Tamil Nadu.

MATERIALS AND METHODS

A total of 732 random serum samples (387 sheep and 345 goats) from FMD outbreak and non-outbreak regions of Tamil Nadu were collected during 2010-2011 and screened for antibodies against structural protein (SP) and non-structural protein (NSP) of FMDV. The consideration of an out-break and non-outbreak region was based on the appearance and absence of classical signs of FMD among ruminant populations.

The serum samples were subjected to FMD structural antibody and non-structural antibody assay. Virus neutralisation tests (VNT) was performed for the sera in flat bottomed tissue culture grade microtiter plates (Nunclon™, Denmark) as described previously (Golding *et al.*, 1976). Antibody titres were expressed as the reciprocal of the final dilution of serum in the serum/virus mixture which neutralised an estimated 100 TCID₅₀ of virus at the 50% end-point (Karber, 1931). Foot and mouth disease antibody titres ≥ 32 were considered positive (Balinda *et al.*, 2009).

A foot and mouth disease NSP antibody was measured using PrioCHECK®FMDV NS kit (Prionics Lelystad B.V., The Netherlands) (Sorensen *et al.*, 1998). Briefly, The PrioCHECK®FMDV NS is a blocking ELISA. ELISA test plates were coated with 3ABC specific monoclonal followed by incubation with the 3ABC protein. The test was performed in two days. On day 1, 80 μ l of ELISA buffer was dispensed to all wells, then 20 μ l negative control, weak positive and positive controls were added to the appropriate wells. Twenty μ l test samples were added to the remaining wells and the test plates were sealed. The plates were incubated 16-18 hours at room temperature (20-25 °C). On day 2, emptied the test plate after the incubation period and washed the plates six times with washing fluid. Tapped the plates firmly after the last washing. Hundred μ l of working dilution of conjugate was added to all the wells and incubated the plates for one hour at room temperature (20-25 °C). After incubation, the plates were washed with washing fluid and 100 μ l of Chromogen/substrate mix was added and incubated for 20 minutes at the room temperature (20-25 °C). Hundred μ l stop solution was added to stop the reaction. Finally, optical density (OD) was measured at 450 nm within 15 minutes after the colour development stopped. The percentage inhibition (PI) of the controls and the test sera were calculated. The percentage inhibition (PI) ≤ 50 % was considered as negative and PI ≥ 50 % was considered as positive.

RESULTS AND DISCUSSION

Overall, 21.4% of sheep (83 out of 387 samples) and 23.5% of goats (81 out of

Table – 1. Details of sheep and goat serum samples and summary of SP and NSP antibody results

Districts	Number of samples collected		Per cent NSP Positive samples*		Percent SP positive samples**					
	Sheep	Goats	Sheep	Goats	Sheep			Goats		
					O	A	Asia 1	O	A	Asia 1
Kanchipuram	70	70	24.3	11.4	14.3	0	0	7.1	0	0
Thiruvannamalai	40	30	62.5	33.3	37.5	0	0	23.3	0	0
Dharmapuri	20	40	50.0	30.0	25.0	0	0	22.5	0	0
Salem	30	30	50.0	43.3	36.7	0	0	43.3	0	0
Namakkal	10	50	60.0	40.0	60.0	0	0	30.0	0	0
Erode	20	40	50.0	45.0	50.0	0	0	35.0	0	0
Pudukottai	70	30	0.0	0.0	0.0	0	0	0.0	0	0
Sivagangai	30	30	0.0	0.0	0.0	0	0	0.0	0	0
Virudhunagar	97	25	0.0	0.0	0.0	0	0	0.0	0	0
Total	387	345	21.4	23.5	14.7	0	0	18.3	0	0

SP- structural protein; NSP- non-structural protein; *%NSP positive serum samples;

**%SNT positive serum samples

345 samples) were positive for FMD NSP antibodies and 14.7% of sheep and 18.3 % goats were positive for structural antibodies. In sheep, the percentage NSP seropositivity varied widely from 24.3 in Kanchipuram district to 62.5 in Tiruvannamalai district. However, in goats the percentage NSP seropositivity varied from 11.4 in Kanchipuram district to 45.0 in Erode district. Similarly, in sheep, the neutralizing antibody response varied from 14.3% in Kanchipuram district to 60% in Namakkal district whereas in goats the neutralising antibody response varied from 7.1% in Kanchipuram district to 43.3% in Salem district. Moreover, sheep and goats were seropositive for FMDV type O structural antibody only (Table.1). These results suggested that there was FMD outbreak due

to type O in these districts. The current results is in accordance with the FMD outbreak data of Tamil Nadu where FMD type O outbreak was recorded at Thiruvannamalai, Dharmapuri, Salem, Namakkal and Erode districts in 2011(PD FMD Annual Report 2011).However, there were no NSP and SP reactors of sheep and goats in three districts (Pudukottai, Sivagangi and Virudhunagar) of Tamil Nadu suggesting that there were no FMD outbreaks. This is the first report of seroprevalence of FMD in small ruminant population in Tamil Nadu . In FMD control programme, sheep and goats are not vaccinated, hence, the presence of SP and NSP antibodies may be due to FMDV infection related seroconversion. So, serology may be used to diagnose the FMD in small ruminant population.

Madhanmohan *et al.* (2010a and 2010b) demonstrated that infected small ruminants transmitted the infection to cattle and buffalo and also showed that FMD oil adjuvant vaccinated small ruminants were protected from clinical disease from in-contact FMDV challenge (Madhanmohan *et al.*, 2010a; 2010b). Regular FMD vaccination reduces post-infection virus persistence in sheep and goats (Madhanmohan *et al.*, 2011).

Madhanmohan *et al.* (2012) demonstrated that the sheep and goats vaccinated with 1µg FMD serotype O antigen were protected from clinical disease. So, to reduce the cost of vaccination in small ruminants the authors suggested that i) the monovalent vaccine with reduced antigen payload may be practiced, ii) to reduce the logistic, combination vaccine may be practiced (i.e. In Sheep: FMD+ Blue Tongue+ Sheep Pox+ Enterotoxaemia+ Tetanus; In goats; FMD+ PPR + goat Pox + Enterotoxaemia+ Tetanus).

In conclusion, the study demonstrated that FMD is prevalent in the state as indicated by the presence of NSP antibodies in the small ruminant population. The absence of antibodies against serotype A and Asia 1 suggested that the small ruminants are excluded from the vaccination programme. So, these inapparent FMDV infected sheep and goats could pose a potential risk of FMD transmission to cattle and buffalo in the integrated livestock system. The current study suggests the need for surveillance activities and FMD control by vaccination in small ruminants alongside large ruminant population.

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Unihorn Pyometra in a Bitch

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Canine pyometra is the most common diestrual uterine disease of intact bitches and is characterized by accumulation of purulent material in the uterine lumen, typically occurring during or immediately following a period of progesterone dominance. Pyometra can be classified as open-cervix or closed-cervix, with the latter being a medical emergency requiring rapid intervention to prevent subsequent sepsis and potential patient death (Pretzer, 2008).

CASE HISTORY AND OBSERVATIONS

An eight years old nulliparous intact Labrador bitch (body weight:30.40 kgs.) was presented in lateral recumbency to the small animal outpatient unit of Gynaecology section, Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli with an anamnesis of distended abdomen, anorexia, polydipsia and purulent vaginal discharge for the past seven days.

Clinical examination revealed bilaterally distended abdomen and malodorous purulent vaginal discharge. The vital parameters were elevated (Temp: 39.8°C; Respiratory rate: 36 / min; Heart rate: 123 / min). Abdominal radiography

revealed distended radio-opaque uterine shadow. Ultrasonographical examination revealed anechoic to hypoechoic uterine sacculations of 43.5 to 63.9 mm diameter. Total blood count and biochemistry revealed neutrophilia and elevated levels of BUN (195.5 mg/dl), Creatinine (6.5mg/dl) and ALP (220 units) respectively. Based on the clinical examination, blood picture and imaging techniques, the case was diagnosed as open cervix pyometra.

TREATMENT AND DISCUSSION

Taking into consideration of the age and systemic illness of the animal, ovariohysterectomy was opted over empirical endocrine therapy. The patient was stabilized with fluid therapy and antibiotics to minimize the surgical risk.

The affected right cornua was massive weighing about 4.5 kg with four litres of pus (Figure - 1). Ovarian examination revealed multiple corpora lutea in the right ovary (Figure - 2), while the left ovary is devoid of any structures.

Previously, Raja *et al.* (2017) has reported a case of unilateral pyometra but they attributed the condition to partial evacuation of the fluid from one horn in response to prostaglandin treatment. During

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Figure -1. Massive unihornpyometra



Figure - 2. Multiple corpora lutea in the right ovary

diestrus, the dominance of progesterone usually increases endometrial gland secretory activity, endometrial proliferation and decreases the myometrial contractility (Hagman, 2018). The uniqueness of the present case is the massiveness of the uterine enlargement and fluid accumulation, which might be attributed to the increased response of that horn to elevated levels of progesterone secreted by multiple corpora lutea of ipsilateral ovary as suggested by Chaffaux and Thibier (1978). Inactive left ovary might explain the non-involvement of left uterine horn in the present case.

Histopathology of both right and left cornua revealed cystic endometritis, thickened endometrial wall with mononuclear cell infiltrations. Cystic endometrial hyperplasia usually develops after repeated progestational stimulation during the luteal phase of the oestrous cycle. These effects are cumulative after repeated

oestrous cycles, explaining the increased incidence in middle-aged to older bitches (Hardy and Osborne, 1974).

As a post operative management, fluid losses were replaced and antibiotic coverage was provided for five days. The blood and biochemical parameters returned to normal physiological limits within 10 days and the animal had an uneventful recovery. Proper utilisation of diagnostic aids and timely intervention with surgical procedure saved the bitch.

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