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Review article

CUTANEOUS RECONSTRUCTION 1: TENSION-RELIEVING TECHNIQUES

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INTRODUCTION

There are many options for coverage of cutaneous defects in dogs and cats. In comparison to horses and humans, our small companion animals have the type of skin that can be extensively manipulated to achieve a robust and enduring closure, rewarding for both veterinarian and client. Whenever a significant reconstructive effort is contemplated, the veterinarian must ensure that,

1. the owner is committed and invested,
2. the animal can tolerate the proposed reconstruction, and
3. you possess the skill to optimize a successful outcome.

This review to introduce you to a few of the more commonly utilized tension-relieving techniques used in veterinary specialist practice. It is designed to be appropriate for a skilled practitioner, and as such does not include more advanced techniques. Not all wounds will be able to be closed by tension-relieving techniques, many will be amenable to these closures and complications such as wound dehiscence will be minimized.

SKIN

Virgin skin is a relatively thin, bilaminate structure consisting of a very thin epidermis of progressively flattened epithelial cells, and a strong, thick dermis (20-30x thicker than epidermis) which contains fibrous, nervous, vascular, lymphatic, follicular and glandular elements. The skin's main function is protection, although it has other roles including thermostatic and immunologic. Skin is viscoelastic, which means it will have a tendency to both retract when wounded or incised, but also to adapt to applied stress forces. We can take advantage of this when it comes to relieving tension on the primary suture line. No wound should be closed by simple approximation of wound edges if the resulting tension will lead to ischemia and subsequent necrosis of the tissue. Tension-relieving techniques allow local tissues to be mobilized to cover a cutaneous defect *without* raising a flap or harvesting a graft. Before considering any reconstructive closure technique, however, the wound must always be managed until the wound is free of infection, has a good blood supply and the periwound tissueis healthy.

TENSION LINES

All portions of a dog's skin are not equally pliable in different directions. Tension lines in the skin of a dog are formed by the predominant pull of the fibrous tissue within the skin. The skin tension lines in dogs (especially those with tighter skin) should be considered when making an incision and when closing a defect. One of the best ways of assessing the amount of tension when closing a wound is to manipulate the edges several ways into apposition, then carefully ascertain if the tension is within physiological limits, and in which direction the suture line should run to have the least amount of tension. As a rule, closing the wound parallel to tension lines will place less tension on the sutures, minimize puckering and "dog ear" formation, and reduce the incidence of "biological tourniquet".

TECHNIQUES COMMON TO ALL TENSION RELIEVING PROCEDURES

Undermining

Undermining is the use of scissors or a scalpel to separate the skin from underlying tissue. This allows the full elastic potential of the skin to be utilised to cover a wound. On areas of the body that have a panniculus carnosus muscle (e.g., cutaneous trunci, platysma), undermining should be performed deep to the muscle, to preserve the deep subdermal plexus. When undermining, the surgeon should try to preserve any direct cutaneous vessels that supply the skin.

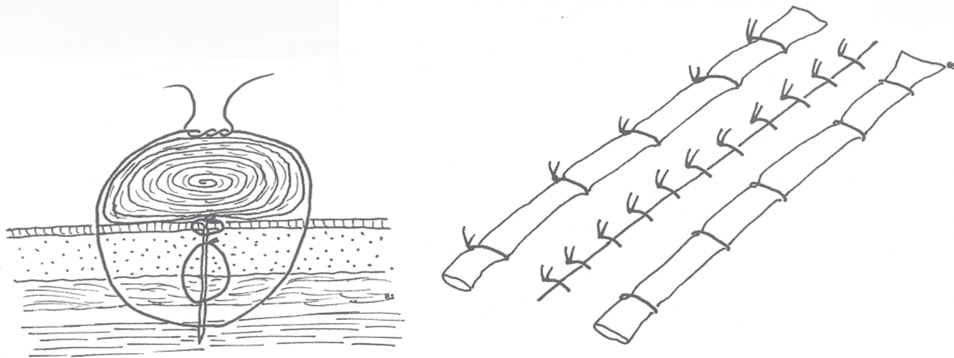
Strong Subcutaneous Sutures

We should never underestimate the contribution of a strong subcutaneous suture line. It can significantly reduce tension on the skin sutures of the primary suture line by redistributing the tension onto 2 layers. Ensure that the bites are taken in the fibrous layer of the hypodermis, which is stronger. Subcutaneous sutures can be interrupted or continuous – use interrupted in areas that may be susceptible to dehiscence. Following a good subcutaneous layer, the skin edges should be almost touching, thus allowing the fine skin sutures to concentrate on apposition rather than tension relief.

TENSION-RELIEVING SUTURES

Stent & Bolster Sutures

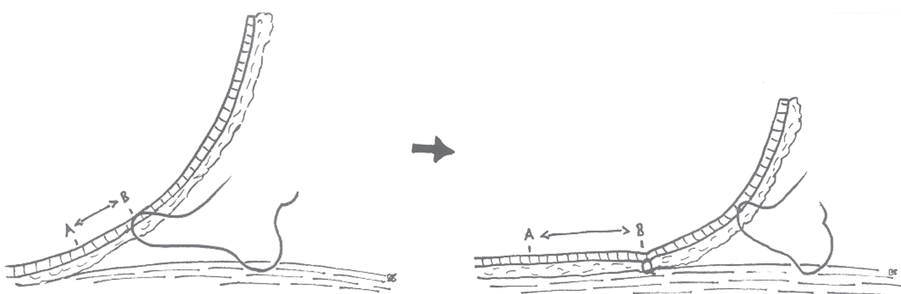
Stent sutures are usually in the form of vertical mattress sutures, supported with some form of bolster to prevent the suture cutting into the skin. These sutures are usually pre-placed deep into the tissues, at some distance from the wound edges. The padding material beneath the suture loops must be soft and extend the length of the wound, or be short lengths under each suture. Wide Penrose drains or bolsters of bandage roll are ideal, buttons are not (they do not disperse the tension widely enough, and can cause pressure necrosis underneath the button). Once the stent sutures are placed, the primary suture line is closed in two layers, then the stent sutures snugged down as necessary. Stent sutures should be removed on the 3rd or 4th day post-operatively, once stress relaxation of the skin has occurred. Note that these stent sutures should NOT be horizontal mattress sutures – these can compromise blood flow to the wound edge.



Stent Sutures

Walking Sutures

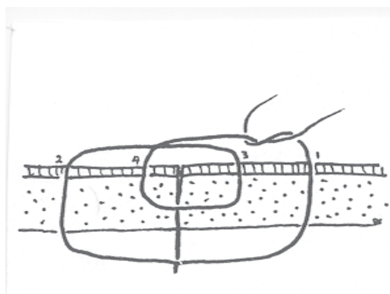
Walking sutures are interrupted sutures of 2-0 or 3-0 absorbable suture material (with a swaged needle), placed from the deeper portion of the dermis to the underlying fascia. They are generally used following undermining and serve to spread the tension evenly across the skin, away from the edges. Walking sutures are placed in staggered rows on both sides of the wound, moving the skin across the defect and allowing it to meet in the middle with minimal resulting tension. Final skin closure is then accomplished with routine subcutaneous and skin sutures. If walking sutures are correctly placed, i.e., with the bite taken through the dermis, the overlying skin will appear dimpled at that point. Be careful not to penetrate the skin with the walking sutures, as this may introduce contamination and lead to subcutaneous infection. This technique is easier to perform in dogs with a thick dermis, and may not be appropriate to cats and thin-skinned dogs.



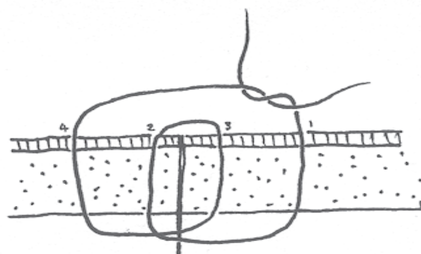
N.B. Although walking sutures reduce tension on the primary suture line, they can be transiently uncomfortable, and can also compromise the vascular supply to the skin (so be careful in flaps). In addition, increased suture material in the wound is not desirable in contaminated wounds. The skin dimpling is a temporary cosmetic problem.

FNNF and FFNN Sutures

These sutures are indicated for closure of wounds with *just a little bit* of increased tension, or in which tension on the wound edges cyclically increases and decreases during movement, e.g., a flexion surface or lacerated paw pad. Far-near-near-far and far-far-near-near sutures are combinations of tension and approximating sutures, and refer to entry or exit distance from the primary suture line. Both FNNF and FFNN sutures are placed in the order that their names indicate. The "far-far" component provides tension relief, while the "near-near" component is appositional. All entrance and exit points of these sutures are linear, and placedmeticulously perpendicular to the skin edges.



Far-Far-Near-Near Suture



Far-Near-Near-Far Suture

SKIN STRETCHING TECHNIQUES

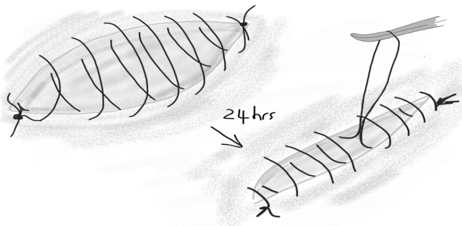
Mechanical creep is a phenomenon of the skin's viscoelasticity that allows it to elongate under constant short-term loading. Within the extracellular matrix of the loaded dermis, the convoluted superhelices of the coiled triple helix collagen fibers readily straighten and realign in a more parallel orientation under load, releasing water molecules and increasing skin viscosity. The delicate elastic fibers, also in the extracellular matrix, become fractured and lose their elasticity. Thus, over time, the skin will elongate (mechanical creep) and lose its tendency to recoil when the load is removed (stress relaxation).

Pretensioning

Pretensioning is a valuable tool to consider when contemplating closure of a large defect, even one that may require a flap. This valuable yet underused technique takes advantage of the previously mentioned phenomena of mechanical creep and stress relaxation over the 24 to 72 hours before definitive wound closure. Pretensioning sutures are placed to influence an existing wound; presuturing refers to sutures that are placed to have an effect on an area of planned excision. The technique works particularly well in truncal and proximal limb regions of dogs and cats, because of their weak hypodermal attachments. It can also be effective around the hock joint, antebrachium, and carpus in some animals. Pretensioning can be used when the surgeon can only achieve partial closure because of concern for biological tourniquet formation. When contemplating pretensioning or presuturing, it is essential that the periwound tissues are healthy.

There are several different ways of performing pretensioning and presuturing:

- Insertion of a simple continuous suture line through skin and superficial hypodermis of the wound edges with 2-0 or 0 nonabsorbable monofilament suture material:



- Using a continuous horizontal intradermal running pattern around the edges of the wound using 3-0 or 2-0 nonabsorbable monofilament suture material secured through a button at both ends. This technique is best suited to skin with a thick and healthy dermis. This suture pattern can be left in for longer than the usual 72 hours, acquiring a degree of biological creep.

The sutures are tightened to the point of mild and tolerable tension on the skin, one that is not likely to cause suture "cut-out" or great discomfort. Slippage of suture material can be avoided through the application of a lead split shot compressed over the suture ends. The area is then protected for 8, 12 or 24 hours and then reassessed. At that time, the sutures will be noticeably looser because the initial loading tension has dissipated because of stress relaxation. The suture line can be gently loaded again, gradually drawing the skin edges closer. Periodic loading can be performed every 8, 12, or 24 hours over 2 to 3 days, by which time even quite large defects can often be approximated directly. It is always surprising how much skin can be 'persuaded' to close over a few days.

At time of definitive closure, the pretensioning sutures are removed completely, and the area is prepared for surgery. Closure is still performed in two layers but requires minimal undermining and minimal disruption of the granulating tissue bed (which contributes to the contraction process).

Pre-suturingz

Presuturinginvolves insertion of sutures through the skin in a Lembert fashion, plicating the intact skin over an area of planned excision. This is generally performed 24-48 hours **before** surgery, tightened once or twice over that time, then removed immediately prior to surgery.

Post-tensioning

Externally applied skin stretching devices such as Velcro pads can be very effectively used postoperatively to minimize incisional tension. This technique is particularly useful if the surgeon becomes concerned about the tension on the incision after the wound has been closed.Velcro pads are glued to the skin and Velcro straps applied to relieve the load on the skin on either side of the incision.

Acute (Intra-operative) Skin Stretching

Intraoperative skin expansion takes the opportunity to obtain some degree of stress relaxation and limited mechanical creep during the surgical procedure. By loading the skin edges following undermining, adequate tension relief on the primary suture line may be obtained. The skin can be loaded using skin hooks, towel clamps or stay sutures to provide constant tension on the undermined skin for 30 – 45 minutes. This technique will not obtain the same degree of stress relaxation as pretensioning over several days, and may not even provide any advantage over simply undermining, but can be useful when positioning loose-skinned animals.

Chronic Skin Expansion

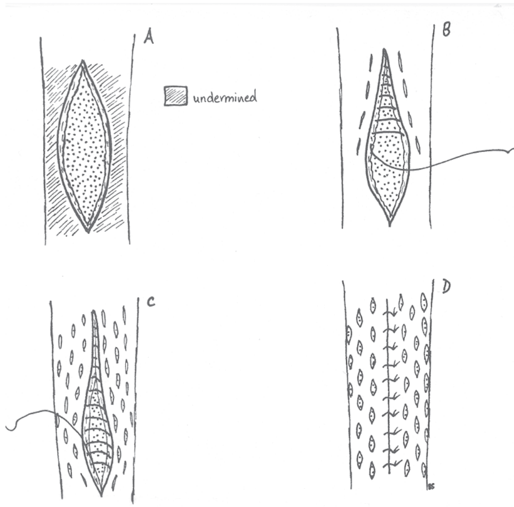
The use of skin expanders has been reported intermittently but consistently in the veterinary literature. This technique is worth mentioning because in certain situations where skin loss is significant (e.g., severe burns), this may be the only technique that will enable robust, full thickness re-coverage. Skin expansion is not suited to acutely traumatized skin and is usually undertaken as part of a delayed or staged reconstructive effort. The technique takes advantage of the phenomenon of ‘biological creep’, which is defined as the creation of new dermal and epidermal components following prolonged constant loading. An inflatable or expandable silicone elastomer device of predetermined volume (e.g., 100 mls) is surgically or endoscopically placed in the subcutaneous tissues of pliable skin adjacent to an existing or proposed defect. After an initial healing period of several days, the device is expanded by 10-15% of final volume every 2 or 3 days until final volume is achieved. During expansion, subcutaneous fat decreases, dermal thickness decreases, and epidermal proliferation occurs. A dense, fibrous capsule forms over the implant, thus the skin is not as pliable or elastic as normal skin. Skin perfusion is enhanced, however. Following completion of the expansion period, the addition of a static maintaining period following expansion appears to improve the quality of the expanded skin.

TENSION-RELIEVING INCISIONS

Mesh Expansion

Mesh expansion, or multiple punctate relaxing incisions are small (1cm, 1/2 inch), parallel, staggered incisions made in the skin adjacent to the wound to relieve the tension associated with wound closure. This technique is particularly useful to relieve tension associated with wound closure in the extremities below the elbow and stifle, the ear, and tail, but can also be used on proximal limb and trunk. The skin around the wound is undermined, and tension on the wound edges assessed. If tension is considered unacceptable for closure, an initial row of 1cm stab incisions is created, using a #15 or #11 scalpel blade, 1cm away from the wound edge with 1 cm space between each incision. Tension on the wound edges is reassessed, to decide if further rows are required. Depending on the amount of tension, one, few, or many rows may be placed. Rows should be staggered (i.e., offset from each other) and 1cm space allowed between the rows. A limb can be

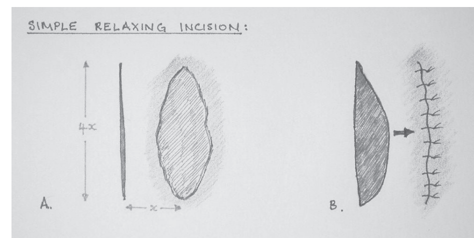
undermined and meshed circumferentially if necessary.



When meshing the skin, it is prudent to have a support under the skin, such as a sterile tongue depressor or gauze. This controls mesh placement and prevents inadvertent laceration of underlying muscles or vessels. Following mesh expansion and primary suture line closure, a non-adherent, semi-occlusive dressing and bandage are applied until the meshes are epithelialized, in approximately 7 days. When the mesh expansion is substantial, the meshed skin may appear compromised (red-purple discoloration) for several days, but healing and final outcomes are usually excellent. The surgeon should be careful, however, not to make the individual mesh incisions too large, as this can cause vascular compromise to the meshed skin. Mesh expansion of skin flaps are not recommended as they will compromise the circulation through the dermis.

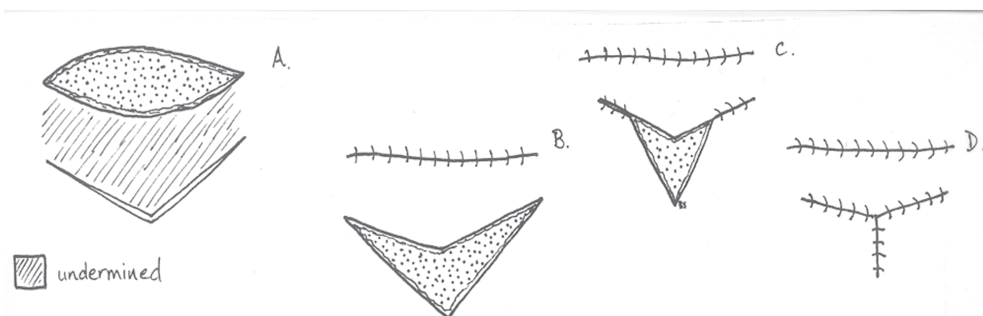
Simple Relaxing Incision

A simple relaxing incision, which is also called a bipedicle flap, is a useful tension-relieving option in several situations, including closing a defect near an orifice to prevent distortion, covering vital structures such as exposed joints, tendons, nerves, and orthopedic implants. It can also be used to close a chronic wound where edges are scarred and nonpliable, as it can mobilize adjacent, more elastic skin. An incision is created parallel to the long axis of the defect with the width of the skin bridge (or bipedicle flap) being equivalent to the width of the wound, and the length of the relaxing incision no longer than the length of the defect. The surgeon should keep in mind that if the length to width ratio of the bipedicle flap exceeds 4:1, vascular supply to the central skin bridge may become compromised. The bipedicle flap is undermined and advanced into the defect, which can then be closed without tension. The relaxing incision can allowed to heal by second intention, or closed with or without tension sutures. Although this procedure creates a new wound (the relaxing incision), generally this incision is in a less critical area, in healthy, pliable skin, and not challenging to address. Bilateral relaxing incisions, (i.e., one on either side of the wound) can be performed in areas that permit, and are especially useful when closing a chronic, fibrotic wound.



V to Y-Plasty

Similar to a simple relaxing incision, the V-Y plasty provides a flap of skin that can be advanced into a defect or provide tension relief adjacent to an orifice. The procedure is indicated for closing defects that are chronic and surrounded by inelastic skin, and closing wounds near structures that would be distorted by closure under tension, such as the eye. A chevron-shaped incision is made in the skin adjacent to the defect, with the point of the chevron away from the defect. The skin between the chevron and the defect is undermined and advanced over the defect. The resultant new defect (with the appearance of an open chevron) is then closed in the shape of a 'Y'. Suturing commences at the arms of the Y and continues until tension is apparent to the surgeon. The remaining open defect is closed to form the stem of the Y.



Although we have discussed a fair number of techniques, there are more procedures available to relieve tension on the primary suture line. Once you have become adept at these discussed above, your skills can be further honed to include other techniques such as Z-plasties, M-plasties, O-to-S closures and incisional negative pressure wound therapy. With some cutaneous defects, however, there is a point where simple tension relief on the primary wound edges will still not be adequate to achieve closure. To close these large defects, we must look to development of a cutaneous pedicle graft (skin flap) or harvesting a free skin graft.

Full Length Articles

EFFECT OF STANDARD TANNIN ON *IN VITRO* RUMEN METHANE REDUCTION AND RUMEN FERMENTATION CHARACTERISTICS

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ABSTRACT

Methane is normally emitted by ruminants and represents a loss of feed energy by 8-12 %. Various feeding strategies are used to reduce the methane emission from ruminants for sustainable animal production. In this context an experiment was conducted to find out the effect of a standard tannin on rumen methane reduction and rumen fermentation characteristics for dairy cattle by *in vitro* gas production technique (*IVGPT)*. The *IVGPT* was carried out by incubating the Cumbu Nappier hybrid (CO4) grass and rumen liquor with standard tannin at varying level viz. 0, 1.03, 2.06, 3.09 and 4.12 % of substrate in six replicates in shaking water bath for a period of 24 hours. After 24 hours the total gas production and pH were measured and methane was estimated in Gas Chromatography. The *in vitro* true dry matter digestibility (*IVTDMD*) was estimated and methane emission (ml) per 100 mg truly digested substrate was calculated. The rumen fermentation characteristics were also studied. The total gas production was significantly ($p<0.05$) decreased in 2.06 %, 3.09 % and 4.12 % standard tannin supplemented groups than other treatment groups. The methane production was significantly ($p<0.05$) decreased by 11.98 %, 29.34 %, 33.47 % and 34.71 % in 1.03 %, 2.06 %, 3.09 % and 4.12 % tannin levels, respectively than control. A highly significant ($p<0.01$) reduction of methane was observed at minimum level of standard tannin (2.06 %) per 100 mg of truly digested substrate, when compared to control. The rumen ammonia nitrogen, total bacteria and total protozoa were significantly reduced in tannin treated groups when compared to control. The other fermentation characteristics viz. *IVTDMD*, pH, TVFA, acetic acid, propionic acid, butyric acid and acetate propionate ratio were not differed among treatment groups. It was concluded that at minimum concentration of 2.06 % of standard tannin significantly reduced the methane emission, percentage of methane on total gas production and methane (ml) per 100 mg of truly digested substrate ($p<0.01$) than control without any adverse effect on rumen fermentation characteristics by *IVGPT*.
Key words: Tannin, methane, *in vitro* rumen fermentation, dairy cattle

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Introduction

The estimated methane (CH_4) emission from rumen fermentation was 15–20 % (Moss *et al*. 2000) causes global warming. Methane produced under anaerobic fermentation as a path way for disposal of metabolic carbon and hydrogen ion produced during microbial fermentation. The methane production is represents a loss of feed energy by 8-12 % leads to lower animal production. Therefore, decreasing methane production is desirable for reducing methane emission with improved efficiency of the digested energy utilization (Johnson and Johnson 1995). A decrease in methane emission is also desirable for increasing animal production in terms of growth, milk production, are also reducing green house gas emission in the environment, which decreases the global warming. There are many more feeding strategies used to reduce the methane emission. Changes in the feeding pattern and feeds can help to mitigate methane emission. Further, the use of antibiotics like ionophore compounds such as monensin, lasolocid and many other chemical feed additives have been shown to decrease methane emission in ruminants. Plants also produce a diverse array of plant metabolites such as tannins, saponins, organic acids, essential oils and organosulphur compounds that have been shown to selectively modulate the rumen microbial populations resulting in an improvement of rumen fermentation and nitrogen metabolism, and a decrease in methane production (Patra and Saxena, 2011). Further, a novel approach using the plant metabolites like saponin (Bharathidhasan *et al*., 2013), tannin (Bharathidhasan *et al.,* 2014) and organic acids (Bharathidhasan *et al.,* 2016) modulates the rumen microbial populations and reduces the methane production. Tannins have the capacity to form complexes mainly with proteins due to the presence of a large number of phenolic groups and have been found to be toxic for some of the rumen microbes, especially ciliate protozoa, fiber degrading bacteria and methanogenic archaea, and as a result methanogenesis in the rumen can also be reduced. Ramirez-Restrepo and Barry (2005) reported that the condensed tannin containing leguminous forages reduced methane. Hess *et al*. (2006) also reported that reduction in methane emissions when feeding high tannins. Hence, the present experiment was conducted to study the effect of standard tannin on reduction of methane emission and rumen fermentation characteristics by *in vitro* gas production technique in forage based diet of dairy cattle.

Materials and methods

The *in vitro* gas production technique (Menke and Steingass, 1988) was aimed to evaluate the effect of standard tannin at different levels viz. 1.03, 2.06, 3.09 and 4.12 % of substrate in six replicates on rumen methane production (Table 1). The standard tannin was procured from Sigma chemicals. The substrate Hybrid Cumbu Nappier (CN- CO4) grass (*Pennisetum purpureum* x P*ennisetum glaucum*) was used for this study. The *in vitro* gas production study was carried out with rumen fluid collected by using rumen extraction pump from three cattle maintained on grazing and it was squeezed through four layers of muslin cloth in to an Erlenmeyer flask under continuous

flushing with CO_2 and it was maintained at the temperature of 39 ^oC. Then rumen fluid was mixed with buffer as described by Menke and Steingass (1988). The substrate Hybrid Cumbu Nappier grass (CN-CO4) was dried and milled to pass through 1 mm sieve and 200 mg was weighed and taken in 100 ml calibrated syringes and weighed quantity of tannin at 0, 2.06 mg (1.03 %), 4.12 mg (2.06 %), 6.18 mg (3.09 %) and 8.24 mg (4.12 % of substrate) were added to the substrate in the syringes in six replicates. Then 30 ml of rumen inoculum was anaerobically transferred to glass syringe and it was incubated in a shaking water bath at 39 ^oC for 24 hrs. At the end of the incubation period the total gas was measured and pH also determined in fermentation fluid.

Table 1. Experimental design to identify the level of standard tannin needed to reduce methanogenesis

Treatment*	Inclusion level of standard tannin (% of substrate)	Quantity of standard tannin included to 200 mg of substrate inoculated
1 (Control)	0	0 mg
2	1.03	2.06 mg
3	2.06	4.12 mg
4	3.09	6.18 mg
5	4.12	8.24 mg

*Each treatment was carried out with six replicates

The gas samples were collected in vacuotainer for estimation of methane and fermented fluid was collected for the estimation of ammonia nitrogen, volatile fatty acids, true dry matter digestibility, bacterial and protozoal count.

Estimation of methane

Methane concentration was estimated using Gas Chromatography (Perkin Elmer, Claurus 500 model) fitted with Flame Ionization Detector (FID) and capillary column (30 meter length and 250 micrometer diameter). Helium was used as carrier gas with oven temperature at 60° C, injector temperature at 100°C and detector temperature at 110°C. Methane concentration in samples (%) was calculated using the following formula.

$$\text{Methane concentration (\%)} = \frac{\text{Peak area of sample gas}}{\text{Peak area of standard gas}} \times \text{Methane concentration in standard (\%)}$$

$$\text{Methane emission (ml)} = \frac{\text{Methane concentration (\%)}}{100} \times \text{Net gas production (ml)}$$

In vitro true dry matter digestibility (_IVTDMD_) estimation

The fermented fluid was centrifuged and the residue was transferred into sintered glass crucible and fitted in Fibretec and 100 ml of Neutral Detergent Solution (NDS) was added and it was refluxed for one hour after which the residue was recovered. The true digestibility was calculated as the weight of substrate incubated minus the weight of the residue after NDS treatment (Van Soest and Robertson, 1988).

Ammonia nitrogen

The ammonia nitrogen was estimated by steam distillation as per the method of Makkar and Becker (1996).

Estimation of total volatile fatty acids (TVFA)

The volatile fatty acids were estimated as per the method of Chase (1990). 2.5ml of fermented medium from each syringe was mixed with 0.5ml of 25 % meta phosphoric acid and centrifuged at 20000g for 30 minutes at 4°C and clear supernatant was collected in GC vials. The 1µl of supernatant was injected into Gas Chromatography (Perkin Elmer, Claurus 500 model) fitted with Flame Ionization Detector (FID) and capillary column (30 meter length and 250 micrometer diameter). Helium was used as carrier gas with oven temperature at 175° C, injector temperature at 220°C and detector temperature at 240°C.

Total Bacterial count

Total bacterial count was carried out using gram's staining as per the method of Gall e*t al.* (1949). One ml of fermented fluid was diluted to 5 ml using 10 % formal saline. One ml of diluted fluid was again diluted with 10 % formal saline to 100 ml. This fluid of 0.01ml was spread over marked area of 1 square centimeter on a clean glass slide. The smear was air and flame dried and stained with gram's staining. The bacteria were counted in 30 fields from all over the smear. The total bacterial load/ml effluent was calculated as follows,

Area of each field: πr^2
'r' – Radius of the microscopic fluid measured by using micrometer
Area of the smear: 1 square cm (or) 100 square mm
No. of fields/100 square mm: 100/ πr^2 = H
Total nos. field counted: 30
Average number of bacteria/field: N
Volume of fermented fluid used: 0.00002 ml
No. of bacteria in 1 ml of fermented fluid = $\frac{(H \times N \times 10^5)}{2}$

Total Protozoal count

Total protozoal count was calculated using Moir (1951) technique. One ml of fermented fluid was diluted to 5 ml with 10 percent formal saline. Two percent Eosin stain was added to the diluted fluid at the rate of one drop per 5 ml. Five to ten minutes time was given for the protozoa to take up the stain and the contents were

mixed thoroughly and the haemocytometer was charged. The protozoa were counted in all the eight WBC chambers.

The total protozoal count/ml effluent was calculated as follows.

Dilution ratio: 1:5

Volume of 1 WBC chamber: 0.0001 cmm

0.0001 cmm of diluted effluent contained: A number of protozoa

Number of protozoa in 1 ml of fermented fluid =

$$\frac{A \times 5}{0.0001} = A \times 5000$$

The data collected on various parameters was statistically analyzed as per the method of Snedecor and Cochran (1989).

Results and Discussion

The effect of standard tannin on total gas (ml), methane (ml), percentage of methane on total gas production and methane (ml) per 100 mg of truly digested substrate are presented in Table 2. The total gas production was significantly ($p<0.05$) decreased in 2.06 %, 3.09 % and 4.12 % standard tannin supplemented group than control. The total gas was lowered by 21.68 %, 25.59 % and 25.75 % in 2.06 %, 3.09 % and 4.12 % standard tannin supplemented groups respectively than control. Similar decrease in total gas production was also observed by Getachew *et al*. (2008), who reported that the addition of purified quebracho tannins to alfalfa hay on *in vitro* gas production technique decreased the rate of gas production significantly with increased level of tannin from 0 to 150 mg/kg DM. Further they observed that the addition of gallic acid and tannic acid reduced the rate of gas production but increased the potential gas production.

Table 2 Effect of standard tannin on total gas (ml), methane (ml), percentage of methane on total gas production and methane (ml) per 100 mg of truly digested substrate (Mean# ± S.E)

Treatment	Inclusion level of standard tannin (% of substrate)	Total gas (ml)*	Methane (ml)**	Percentage of methane on Total gas production**	Methane (ml) per 100 mg of truly digested substrate **
1	0 (Control)	12.27 ± 0.19 [b]	2.42 ± 0.01[b]	19.75 ± 0.37[b]	2.02 ± 0.01[b]
2	1.03	10.87 ± 0.32 [ab]	2.13 ± 0.12[b]	19.64 ± 0.65[b]	1.89 ± 0.11[b]
3	2.06	9.61 ± 0.32 [a]	1.71 ± 0.07[a]	17.86 ± 0.31[a]	1.51 ± 0.10 [a]

4	3.09	9.13 ± 0.32 [a]	1.61 ± 0.07[a]	17.62 ± 0.25[a]	1.41 ± 0.02[a]
5	4.12	9.11 ± 0.21[a]	1.58 ± 0.06[a]	17.24 ± 0.33[a]	1.36 ± 0.06 [a]

[#] Mean of six observations; [NS] Not significant, Means bearing different superscripts in the same column differ significantly (*p<0.05), (**p<0.01)

The decrease in the rate of gas production and concomitant increase in asymptotic gas production when gallic acid and tannic acid was used might suggest that rumen microbes are capable of degrading gallic acid and tannic acid or are able to tolerate the effects of tannic acid. On the other hand condensed tannin such as quebracho tannin was largely resistant to microbial degradation. This is the reason which was responsible for the decrease in the total gas production recorded in the present study. Vieira and Borba (2011) also reported that the effect of tannin in the form of *Quebracho* extracts from the plant extract of *Trifolium repens, Lotus corniculatus* and *Lolium perenne* at 2.5 % and 5.0 % levels significantly decreased the total gas production than control. On the contrary to the present findings Pellikaan *et al*. (2011) reported that the addition of condensed tannin (CT) and hydrolysable tannin (HT) at 100g/kg feed did not affect the total gas production by *in vitro*. They also observed that the condensed tannin rich sources like grape seed, Quebracho had lower gas production and higher hydrolysable tannin sources like green tea and myrabolan tended to increase the gas production.

The methane was significantly (p<0.01) decreased in 2.06 %, 3.09 % and 4.12 % standard tannin added group than control and 1.03 % tannin added group. The reduction in methane was 29.34, 33.47 and 34.71 % respectively in treatment 3, treatment 4 and treatment 5 when compared to control. The minimum level that reduced maximum methane was 2.06 %. The significant reduction in methane emission was observed in this experiment is in confirmed with earlier reports by Jayanegara *et al*. (2010), who also reported a significant negative relationship between total tannins and methane production. They also observed that simple phenolics like cinnamic, caffeic, p-coumaric and ferulic acids decreased methane production significantly when added at 5 mM and addition of purified chestnut and sumach (hydrolysable tannin) at 1mg/ml to *in vitro* rumen fermentation system containing hay: concentrate (70:30) decreased methane production by 6.5 and 7.2 %, respectively. Bhatta *et al*. (2009) observed that the addition of *quebracho* tannin (7.62 % hydrolysable tannin and 1.33 % condensed tannin) at 5, 10, 15, 20 and 25 % of substrates in timothy hay (65): concentrates (35) decreased the methane production by 10.2 to 41.7 % in in *vitro* gas production technique.

The highly significant (p<0.01) decrease in methane (%) on total gas production was observed in 2.06 %, 3.09 % and 4.12 % standard tannin supplemented groups than control. The minimum level of 2.06 % of standard tannin was able to

reduce the methane on total gas production by 9.57 % when compared to control. Similarly Pellikaan *et al*. (2011) also reported that the addition of condensed tannin and hydrolysable tannin at 100g/ kg reduced *in vitro* methane emission on total gas production by 16.30 % and 15.85 % respectively when compared to control by *in vitro* study. The authors also opined that the addition of poly ethylene glycol increased the total methane emission on total gas production equivalent to control.

The methane per 100 mg of truly digested substrate was also significantly (p<0.01) reduced in 2.06 %, 3.09 % and 4.12 % tannin added groups than control. Standard tannin at 2.06 % level was reduced the methane per 100 mg of truly digested substrate by 25.25 % when compared to control. The present study was in agreement with the earlier findings of Castro –Montoya *et al*. (2011) who reported that the purified condensed tannin like *quebracho* tannin and mimosa tannin at 0.5, 0.75 and 1.0 mg/ ml decreased the methane emission per 100 mg true dry matter digestibility by 25, 30.77 and 36.54 % in *quebracho* tannin and 23.08, 32.69 and 40.38 %, respectively in mimosa tannin than control. They also found that the purified hydrolysable tannin like sumach tannin and chestnut tannin at 0.5, 0.75 and 1.0 mg/ml decreased the methane emission (ml) per 100 mg true dry matter digestibility by 17.31, 23.08 and 30.76 % respectively in sumach tannin and 13.46, 17.31 and 21.25 % respectively in chestnut tannin than control. Similarly the addition of condensed tannin and hydrolysable tannin at 10 % reduced *in vitro* methane emission per gram of organic matter by 24.48 % and 17.88 % respectively than control (Pellikaan *et al*., 2011). The decrease in methane emission might be due to the effect of tannin on suppression of protozoa and methanogenic bacteria. The effects of tannin on ruminal fibre digestion may be attributed to decrease in number of cellulolytic bacteria (Mc Sweeney *et al*., 2001), formation of tannin cellulose complexes that are resistant to enzymatic digestion (Makkar *et al*.1995) or impairment in substrate adhesion by fibrolytic microbes (Bento *et al*., 2005), which would reduce hydrogen availability and lessen methanogenesis (Carulla *et al*., 2005). Furthermore tannin is known to reduce the protozoal numbers which might decrease in methane production (Makkar *et al*., 1995).

The ammonia nitrogen content in all tannin treated groups was significantly (p<0.01) reduced than control. Similarly, Alexander *et al*. (2008) reported that the addition of 2 mg/ ml of *Moringa oleifera* aqueous methanol extract which contained 1.11 % of HT decreased the total ammonia nitrogen by 13.63 % than control. Pellikaan *et al*. (2011) also reported that the ammonia nitrogen was significantly decreased by 35.18 and 33.38 % in CT and HT addition at 100g/kg substrate than control. The decreased ammonia nitrogen might be due to tannins binds with protein, which resisted the rumen degradation and reduced ammonia nitrogen in the rumen (Vieira and Borba, 2011). The reduced ammonia concentrations in the rumen are typical when protozoal growth is inhibited, presumably as a result of depressed bacterial lysis (Pen *et al*., 2006).

Table 3. Effect of standard tannin on Ammonia nitrogen, (mg/100ml), Bacterial count (per ml), Protozoal count (per ml), *In vitro* true dry matter digestibility (*IVTDMD*) and pH (Mean# ± S.E)

Treatment	Inclusion level of standard tannin (% of substrate)	Ammonia Nitrogen (mg/100ml)	Bacterial count (X 10^8) (per ml)	Protozoal count (X 10^5) (per ml)	*In vitro* true dry matter digestibility NS (*IVTDMD*)	pH NS
1	0 (Control)	35.29 ± 0.40 ^{b}	4.37 ± 0.05 ^{c}	3.47 ± 0.03 ^{c}	59.93 ± 0.43	7.05 ± 0.08
2	1.03	34.56 ± 0.38 ^{a}	3.98 ± 0.06 ^{b}	2.97 ± 0.04 ^{b}	56.73 ± 1.36	6.90 ± 0.11
3	2.06	33.44 ± 0.34 ^{a}	3.76 ± 0.04 ^{a}	2.88 ± 0.03 ^{ab}	57.07 ± 1.53	6.77 ± 0.03
4	3.08	33.27 ± 0.25 ^{a}	3.73 ± 0.03 ^{a}	2.79 ± 0.03 ^{a}	57.17 ± 1.52	7.03 ± 0.07
5	4.11	33.52 ± 0.57^a	3.77 ± 0.03^a	2.78 ± 0.03 ^{a}	57.27 ± 1.13	7.10 ± 0.10

Mean of six observations; NS Not significant, Means bearing different superscripts in the same column differ significantly ($p<0.01$)

There was no significant difference on *in vitro* dry matter digestibility due to supplementation of tannin in forage based diet. Sliwinski *et al*. (2002) also observed that the addition of *Castanea sativa* wood extracts containing the hydrolysable tannin at 0.5 and 2.5 g per kg dry matter to the basal diet with grass silage, barley grain and grass hay did not influence the organic matter degradation among the treatment groups by *RUSITEC*. Similarly, Castro –Montoya *et al*. (2011) reported that the purified CT like quebracho tannin and mimosa tannin and HT like sumach tannin and chestnut tannin at 0.5, 0.75 and 1.0 mg/ml of *in vitro* rumen fermentation study did not influence the true dry matter digestibility in all treatment groups.

The bacterial count in all tannin treated groups was significantly ($p<0.01$) reduced than control. The minimum dose with maximum reduction of bacterial load by 13.96 % was observed in 2.06 % tannin treated group than control. Tannins are generally regarded inhibitory to the growth of the rumen micro organisms. The early work of Tagari *et al*. (1965) showed that the growth of cellulolytic and proteolytic bacteria was inhibited by carob tannins in an artificial rumen. It has been stated that tannins from carob pod extract changed the morphology of bacteria to produce antimicrobial activity (Heins *et al*., 1964). Hence, inhibitory activity of tannins against bacteria has been implicated due

to the ability of tannins to form complexes with the cell wall and membrane causing morphological changes of the cell wall and the extracellular enzymes secreted (Smith *et al*., 2005). Sliwinski *et al*. (2002) observed that the addition of *Castanea sativa* wood extracts containing the hydrolysable tannin at 0.5 and 2.5 g per kg dry matter to the basal diet with grass silage, barley grain and grass hay reduced the bacterial count numerically decreased by 3.14 and 11.55 % in 0.5 and 2.5 g HT added groups than control in *Rusitec.*

The protozoal count was significantly (P<0.05) reduced in all tannin supplemented groups than control. The reduction in protozoal count was 14.4 %, 17.00 %, 19.59 % and 19.88 % in 1.03, 2.06, 3.09 and 4.12 % of tannin supplemented groups, respectively than control. Makkar *et al*. (1995) reported that the quebracho tannin significantly reduced the numbers of total protozoa, Entodiniomorph and holotichs, the effect being higher in Holotrichs which may increase the efficiency of microbial protein synthesis in the rumen. Briceno *et al*. (2005) screened the defaunating properties of 15 tree fodders containing tannins but inhibitory effect on protozoa was observed in *Acacia farnesiana, Calliandra calothyrsus and Lysiloma latisiliquum.* Patra *et al*. (2006) observed that the tannin extracted with ethanol and methanol from *Terminalia chebula* decreased the numbers of total protozoa. Anti protozoal properties of tannins from different plants have been reported in many studies with *Lotus striata* and *Lotus cuneata* (Animut *et al*., 2008) Quebracho and mimosa tannin (Bhatta *et al.,* 2009).

No significant difference was observed in pH among the treatment groups. The result of this study was in agreement with the earlier findings such as the addition of condensed tannin through *Medicago sativa* and *Lotus pedunculatus* did not influence the pH by *in vitro* gas production technique (Tavendale *et al*., 2005). But, Alexander *et al*. (2008) and Pellikaan *et al*. (2011) reported that the pH significantly decreased by addition of tannin. The tannin supplementation decreased the number of methanogens which leads to reduced methane production and increased the accumulation of hydrogen ions. The unchanged pH in the present study was due to the carbohydrate fermenting bacteria utilize other mechanism of reducing equivalent particularly elimination of hydrogen ions and there by the pH is unaltered (Kessel and Russel, 1996).

The TVFA, acetic acid, propionic acid, butyric acid and A/P ratio were not differed among tannin supplemented groups. Sliwinski *et al*. (2002) observed that the addition of *Castanea sativa* wood extracts containing the hydrolysable tannin at 0.5 and 2.5 g per kg dry matter to the basal diet with grass silage, barley grain and grass hay did not influence TVFA, acetic acid, propionic acid, butyric acid and A/P ratio among the treatment groups in *Rusitec*.

Similarly, Tavendale *et al*. (2005) reported that the addition of condensed tannin through *Lotus pedunculatus* (10.7% CT) and *Medicago sativa* (0.02% CT) did not influence the total VFA, acetic acid, propionic acid, butyric acid and acetate propionate ratio (A/P ratio) levels by *in vitro* fermentation study. Similarly, acetic acid,

propionic acid, butyric acid and acetate propionate ratio were also significantly not differed in tannin supplemented groups by *in vitro*. Pellikaan *et al*. (2011) also reported that the propionic acid production and butyric acid production were not affected by the addition of CT (20.2 %). Hess *et al*. (2006) observed that the acetate to propionate ratio was not influenced by supplementation with increasing proportion of low tannin legume *Cratylia argentea* and rich tannin legume *Calliandra calothyrsus* supplement or in combination between of these forages in *Rusitec.*

Table 4. Effect of standard tannin on Total volatile fatty acid (mg/dl), Acetic acid, Propionic acid, Butyric acid and A/P ratio (Mean# ± S.E)NS

Treatment	Inclusion level of standard tannin (% of substrate)	TVFA (mg/dl)	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)	A/P ratio
1	0 (Control)	64.98 ± 0.34	65.33 ± 0.42	23.33 ± 0.49	11.33 ± 0.72	2.81 ± 0.06
2	1.03	64.94 ± 0.25	65.00 ± 0.37	23.33 ± 0.33	11.67 ± 0.33	2.79 ± 0.05
3	2.06	65.08 ± 0.31	64.50 ± 0.99	24.00 ± 0.52	11.50 ± 0.62	2.70 ± 0.10
4	3.08	65.09 ± 0.24	67.00 ± 0.36	24.17 ± 0.48	10.83 ± 0.31	2.70 ± 0.06
5	4.11	65.44 ± 0.59	66.33 ± 0.56	22.83 ± 0.48	10.84 ± 0.17	2.91 ± 0.08

Mean of six observations; NS Not significant

It was concluded that the minimum concentration of 2.06 % of tannin significantly caused a maximum reduction in the total gas production, methane emission, percentage of methane on total gas production and methane (ml) per 100 mg of truly digested substrate. The rumen ammonia nitrogen, total bacteria and protozoa were significantly reduced in tannin treated groups when compared to control. The other fermentation characteristics viz. *IVTDMD*, pH, TVFA, acetic acid, propionic acid, butyric acid and A/P ratio were not differed among tannin treated groups. The energy saved through decrease in methane emission will be utilized for milk or meat production and the global warming also reduced.

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DETECTION OF *PESTE DES PETITS RUMINANTS* VIRUS ANTIBODIES IN SERA OF CATTLE *

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Abstract

A total of 120 serum samples of cattle collected from six districts in Tamil Nadu were screened for PPRV antibodies. Competitive enzyme linked immune sorbent assay and micro serum neutralization test were used for seromonitoring of PPRV antibodies. In areas with no previous history of PPR incidence and PPRV vaccination in goats and sheep under field/ farmers holdings, the serum samples collected from selected cattle herds showed 8.3% positivity for PPRV antibodies by both cELISA and micro SNT, whereas in areas with previous history of PPR incidence and PPRV vaccination in goats and sheep, the cattle herds showed 18.3% positivity.

Key words: PPR, Vaccination, Tamil Nadu, Cattle, Immune response.

INTRODUCTION

Peste des petits ruminants virus (PPRV) causes natural disease in goats and sheep. Gibbs *et al.* (1979) had first classified PPRV as the fourth member of the genus *Morbili virus*. The genus *Morbilli virus* comes under the family *Paramyxoviridae* of the order *Mononegavirales* (International Committee on Taxonomy of Viruses, 2017). The virus is closely related to Rinderpest virus (RPV), another member of *Morbilli virus* genus, which causes similar disease in large ruminants (Anderson *et al*.,1990 ; Couacy-Hymann *et al*., 1995). It is generally considered that cattle alone are naturally infected sub-clinically, although in the 1950s, disease and death were recorded in calves experimentally infected with PPRV-infected tissue (OIE, 2012).

Antibodies against PPRV in cattle can provide cross protection against RPV (Taylor, 1979). Because of its close relationship to RP, PPR was suggested to be taken into account in the rinderpest control programmes (Lefevre and Diallo, 1990). Infection of cattle with PPRV also interferes with immune response against RPV (Anderson and McKay, 1994). With the successful eradication of rinderpest, global strategy for the control and eradication of PPR is being contemplated (Singh *et al.,* 2009 / 2011; OIE–FAO, 2015; Banyard and Parida, 2016; FAO-OIE, 2016). The aim of the present study is to seromonitor PPRV antibodies in cattle sera in Tamil Nadu.

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MATERIALS AND METHODS

During the study period, the serum samples were collected from selected herds of cattle in Thiruvallur, Kancheepuram and Tiruvannamalai districts in those areas there was no previous history of PPR incidence and PPRV vaccination in goats and sheep. The details of the samples collected are given in table 1.

Table 1. Details of cattle sera collected under field / farmers holdings in areas with no previous history of PPR incidence and PPRV vaccination in goats and sheep

Species	No. of serum samples		
	Thiruvallur District	Kancheepuram District	Tiruvannamalai District
Cattle (below 1 year)	10	10	10
Cattle (above 1 year)	10	10	10

During the study period, the serum samples were collected from Pudukottai, Thoothukudi and Tirunelveli districts in the selected herds of cattle from areas with previous history of PPR incidence and PPRV vaccination in goats and sheep. The details are given in table 2.

Table 2. Details of cattle sera collected under field / farmers holdings in areas with previous history of PPR incidence and PPRV vaccination in goats and sheep

Species	No. of serum samples		
	Tirunelveli District	Thoothukudi District	Pudukottai District
Cattle (below 1 year)	10	10	10
Cattle (above 1 year)	10	10	10

The PPR virus competitive enzyme linked immunosorbant assay (cELISA) test kit was procured from Indian Veterinary Research Institute (IVRI), Mukteswar, India and the test was carried out as per the protocol. The principle of competitive ELISA test is based on the inhibition of binding of monoclonal antibody to antigen in the presence of PPR antibody in the test sera. This results in reduced colour development, when anti-mouse antibody conjugated to HRPO is used for tracing the binding of monoclonal antibody. This monoclonal antibody is directed against a neutralising epitope of Hemaggultinin (HA) protein of PPR virus. As this is a solid phase assay, washing is required between each step to ensure removal of unbound reagents.

Plates were read in a microplate reader µQuant (Biotek) at 492 nm filter. The reader was connected to a computer loaded with

KC software specific for the ELISA plate reader which was used to acquire the readings. The optical density (OD) values were converted to percentage inhibition by using the following formula:

PI=100-(absorbance of the test sample /absorbance of Cm wells) X100

Test Sera showing more than 40% PI of mean OD values of the Cm (monoclonal antibody control) wells were taken as positive for PPR antibodies.

The micro serum neutralisation test (mSNT) was performed in Vero cells for the detection of antibodies to PPRV with a fixed dilution of 1:8 for each test sera sample as per the method described in Rossiter *et al* (1985); Balamurugan *et al* (2012) and OIE (2012).

RESULTS AND DISCUSSION

cELISA responses (Mean ± SE) of serum samples collected for seromonitoring of PPRV antibodies in selected herds of cattle in the areas without previous history of PPRV disease incidence and PPRV vaccination in goats and sheep under field/ farmers holdings are presented in Table 3.

Table 3. PI values of cELISA for PPRV antibodies in cattle sera collected from areas with no previous history of PPR incidence and PPRV vaccination in goats and sheep

Species	PI values (Mean±SE) for PPRV antibodies in cELISA		
	Thiruvallur District	Kancheepuram District	Tiruvannamalai District
Cattle (below 1 year)	Negative	45.76	Negative
Cattle (above 1 year)	46.83±0.43	49.31±0.52	45.98±0.57

The mean PI values was calculated only from those sera samples that showed positive PI values (>40) in cELISA

cELISA responses (Mean ±SE) of serum samples collected for seromonitoring of PPRV antibodies in selected herds of cattle in the areas with previous history of PPRV disease incidence and PPRV vaccination in goats and sheep under field/ farmers holdings are presented in Table 4.

Table 4. PI values of cELISA for PPRV antibodies in cattle sera collected from areas with previous history of PPR incidence and PPRV vaccination in goats and sheep

Species	PI values (Mean±SE) for PPRV antibodies in cELISA		
	Tirunelveli District	Thoothukudi District	Pudukottai District
Cattle (below 1 year)	51.52	48.63	Negative
Cattle (above 1 year)	59.73±0.41	58.24±0.43	60.16±0.48

The mean PI values was calculated only from those sera samples that showed positive PI values (>40) in cELISA

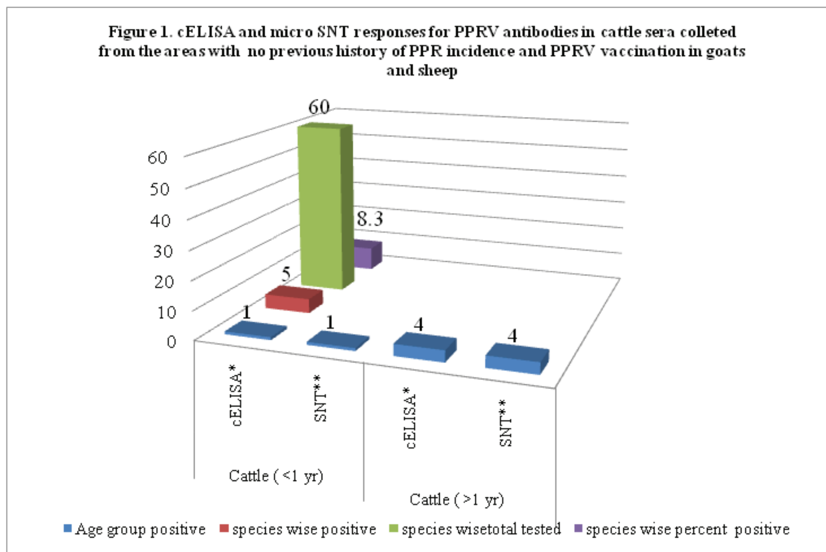
cELISA and micro SNT responses of serum samples collected for seromonitoring of PPRV antibodies in selected herds of cattle in areas without previous history of PPRV disease incidence and PPRV vaccination in goats and sheep under field/ farmers holdings are presented in Table 5 and shown in Figure 1 which showed 8.3% positivity .

Table 5. cELISA and micro SNT responses for PPRV antibodies in cattle sera collected from areas with no previous history of PPR incidence and PPRV vaccination in goats and sheep

Species	Test	No. of animals positive/ No of animals tested			Age group wise Total No.	Species wise Total Positive by both	Species wise percent
		Thiruvallur District	Kancheepuram District	Tiruvannamalai District			
Cattle (< 1 yr)	cELISA*	0/10	1/10	0/10	1/30	5/60	8.3
	SNT**	0/10	1/10	0/10	1/30		
Cattle (> 1 yr)	cELISA*	2/10	1/10	1/10	4/30		
	SNT**	2/10	1/10	1/10	4/30		

* No. of sera positive / No. of animals tested in cELISA

** No. of sera showing ≥ 1 in 8 titre in micro SNT / No. of animals tested



cELISA and micro SNT responses of serum samples collected for seromonitoring of PPRV antibodies in selected herds of in the areas with previous history of PPRV disease incidence and PPRV vaccination in goats and sheep under field/ farmers holdings are presented in Table 6 and presented in Figure 2 which showed 18.3% positivity.

Table 6. cELISA and micro SNT responses for PPRV antibodies in cattle sera collected from areas with previous history of PPR incidence and PPRV vaccination in goats and sheep

Species	Test	No. of animals positive/ No of animals tested			Age group wise Total No. Positive / Total No. tested	Species wise Total Positive by both cELISA and SNT / Total No. Tested	Species wise percent positive
		Tirunelveli District	Thoothukudi District	Pudukottai District			
Cattle (< 1 yr)	cELISA*	1/10	1/10	0/10	2/30	11/60	18.3
	SNT**	1/10	1/10	0/10	2/30		
Cattle (> 1 yr)	cELISA*	3/10	4/10	2/10	9/30		
	SNT**	3/10	4/10	2/10	9/30		

* No. of sera positive / No. of animals tested in cELISA

** No. of sera showing ≥ 1 in 8 titre in micro SNT / No. of animals tested

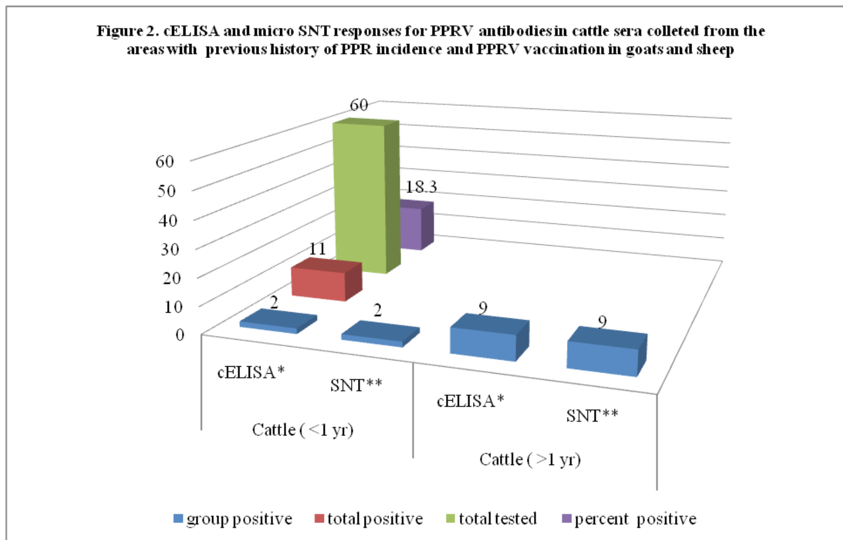


Figure 2. cELISA and micro SNT responses for PPRV antibodies in cattle sera colleted from the areas with previous history of PPR incidence and PPRV vaccination in goats and sheep

Cattle can be infected, but they do not seem to develop clinical signs and are not known to transmit PPRV to other animals, which appear to be dead-end hosts (www.cfsph.iastate.edu, 2015). Although cattle are unable to transmit the disease to another host, sero-conversion against the PPRV H protein has been observed (Khan *et al*., 2008).

Interestingly, epidemiological surveillance studies carried out in different enzootic regions have revealed PPRV seroprevalence in other ruminants including cattle, buffalo and camel. This seroprevalence can be as high as 67% in case of cattle. Cattle are considered as potential dead-end hosts for PPRV. It appears however that this virus, for reasons not yet elucidated, can occasionally overcome the innate resistance of these species, resulting in the development of clinical signs (Naveen kumar *et al*., 2014).

The prevalence of PPR virus antibodies among domestic animals (goat, sheep, cattle and camel) populations in NE Nigeria was studied by El-Yuguda *et al.* (2013), using virus neutralisation test (VNT) and c-ELISA. In VNT, seroprevalence in cattle was 16.7%. Similar pattern of prevalence was noted when the sera were tested for PPR antibodies using c-ELISA.

Balamurugan *et al.* (2014) studied the prevalence of PPRV antibodies in cattle, buffaloes, sheep and goats carried out during the period 2011 using the serum samples randomly collected from different villages of five states in India. A total of 605 cattle serum samples were collected from 52 districts in five states (Andhra Pradesh, Gujarat, Jammu and Kashmir, Maharashtra and Rajasthan) of India and screened for PPRV-specific antibodies by using PPR monoclonal antibody-based competitive ELISA kit. Analysis of 605 samples showed PPRV seroprevalence of 11.07 % in cattle.

Muthuchelvan *et al.* (2015) reported seroprevalence rate of PPRV at country level in goats and sheep as 43.56 % and 4.58% in cattle and buffaloes, respectively.

CONCLUSION

Cattle sera showed the prevalence of PPRV antibodies as high as 18.3% in Tamil Nadu indicating infection of PPRV without clinical signs. The presence of PPRV antibodies demonstrated that bovines are exposed to PPRV infection and it implied the importance of cattle as subclinical hosts for the virus, besides widespread presence of the disease in sheep and goats. Though the cattle are considered to be the dead end host, it is recommended to vaccinate the cattle when they are reared along with sheep and goats in the premises for National PPRV control to be successful.

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SOCIO-ECONOMIC STATUS, HUSBANDRY PRACTICES FOLLOWED AND CONSTRAINTS FACED BY MADRAS RED SHEEP FARMERS IN THEIR FIELD FLOCKS IN KANCHIPURAM DISTRICT

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ABSTRACT

A detailed study was conducted to analyze the socio-economic status, husbandry practices followed and constraints faced by Madras Red sheep farmers in their field flocks in Kanchipuram district. The study was conducted on 100 farmer's flock from 19 villages included in Network Project on Sheep Improvement, PGRIAS, Kattupakkam (NWPSI). Socio-economic status of the Madras Red sheep farmers revealed thatmajority(99%) of the Madras Red sheep farmers belonged to Hindu religion, more than half of them (56.0%) belonged to most backward community, followed by backward community (25.0%), majority of sheep rearers (69.0%) located in rural areas, more than half (64%) of the sheep rearers were in the age group of > 50 years and majority(74%) of them were rearing goat along with sheep. The husbandry practices revealed that majority of the farmers (72%) provided shelter to their sheep near their residence, most of the sheep sheds (91%) were having earthern floor, half of the sheds were (56%) made with thatched roofing material, majority of the sheep rearers (56%) stored manure away from the sheep, nearly half of sheep farmers (42%) were allowing animals for a grazing duration of 8 and 8 hours 30 minutes, All most all (100%) of them were following flock mating type breeding and all (100%) were following ram exchange program, majority of sheep rearers (96%) followed deticking technique for sheep. Constraints perceived from sheep farmers revealed that most prevailing problem faced was lack of grazing land during summer followed by low sale price per animal and threat of stray dogs.

Key Words: Sheep rearers, extensive system of management, field flocks& constraints

INTRODUCTION

Tamilnadu possesses ten defined sheep breeds **(**Ganesakala and Rathnasabapathy, 1973; Acharya, 1982) namely Kilakarsal, Mecheri, Madras Red, Ramnad White, Vembur, Katchaikatty Black, Nilagiri, Coimbatore, Chevvadu and Tiruchi Black with 4.79 million population, ofwhich Madras Red sheep is a medium-sized hairy sheep, well adapted to the agro-climatic conditions of the North-Eastern region of Tamil Nadu (Acharya., 1982). It is a meat

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type breed mainly distributed in Thiruvallur, Kancheepuram, Chennai, Vellore and Villupuram districts of the state (Raman et al., 2003). Breeding and performance characters (Devendran*et al.*, 2009; Balasubramanyam*et al.*, 2010), most of these reports were mainly based on animals maintained on the organized farms or in adopted farmers flocks. Information about the socio economic statusof sheep farmers and husbandry practices followed and constraints faced by farmers in its breeding tract is scanty. Therefore the present study was undertaken to evaluate Madras Red sheep under actual field conditions. Moreover, knowledge about the constraints in sheep farming will help policy makers and planners in making suitable corrective and remedial measures.

MATERIALS AND METHODS

Survey on socioeconomic status of the Madras Red sheep farmers was done and information such as age of the farmer, religion, community, type of family, size of family, land holding pattern, flock size possessed by famer and other animals for integrated farming and the husbandry practices *viz.* housing management, grazing management, manure management, health care management, marketing management & reproductive management were obtained from Madras Red sheep farmers in the breeding tract through structured questionnaire. A total of 11 constraints perceived by the Madras red sheep farmers through survey questionnaire were given rank as 1 to 11.The most severe problem faced by the farmer was given rank1 likewise increasing order the least severe problem was given as rank 11.

RESULTS AND DISSCUSSION

1) SOCIO- ECONOMIC STATUS OF THE FARMER

The socio-economic status of the Madras Red sheep farmers are given in Table 1. Majority (99%) of the Madras Red sheep farmers belonged to Hindu Religion, only 1 percent of them belonged to Christian religion and no Muslims were found.Majority (85.83%) of sheep farmers belonged to Hindu religion while only 14.17% of sheep farmers were Muslimsamongthesheep farmers in Rajasthan(Choudary*et al.,*2012).99.32% of the sheep farmers in the Telangana region of Andhra Pradesh were Hindus followed by 0.52% Christians and 0.17% Muslims(Rajanna*et al.,*2012).

Among Madras Red sheep farmers majority of them (56.0%) belonged to most backward community, followed by backward community (25.0%), SC (19.0%) and no ST were found.71.11% of sheep farmers of AndhraPadesh were belonging to Backward caste (BC) followed by 11.67% Scheduled Castes(Mastanbi*et al.,* 2017).

Majority of Madras Red sheep rearers (69.0%) located in rural areas but few farmers (31.0%) were located in Sub urban areas. 40.83% of sheep farmers in Kancheepuram district (were) belonged to old age and 21.67% belonged to young age group (Thilakar and Krishnaraj., 2007). which was also the case with Madars Red sheep farmers. In Telangana region of Andhra Pradesh the average age group of Sheep farmers was 42.69 years and majority (70.31%) of the respondents belonged to middle age(Rajanna*et al.*, 2012).

In the breeding tract of the Madras Red sheep majority of them (50%) were illiterates followed by primary school (27%) educational qualification, higher secondary school (22%) educational qualification and only 1% of them were graduates.These findings are in agreement with the observations Rajanna*et al.* (2012), Maheswaran (1993), Suresh *et al.* (2011) and Choudary*et al.* (2012). However Tilakar and Krishnaraj (2007) observed that more than 64 per cent of sheep farmers were literate with primary to secondary education.Majority of respondents (77.26%) in Telangana region had agriculture as main occupation and 22.74% of respondents had animal husbandry as their main occupation (Rajanna*et al.*, 2012). which was likesheep rearing was the primary occupation for 86 percent of Madras Red farmers whereas for 14 percent of them it was secondary occupation.

Most of the families (66%) of Madras Red sheep farmers were of nuclear type with a small number (34%) of joint type of families. Majority of the sheep farmers (61%) were land less where as 35% of them had 0.1 to 2.0 acre land.60.10% of shepherds in North coastal zone of Andhra Pradesh had less than 2.5 acres land (Rao*et al.*, 2013), 90% of Coimbatore sheep farmers were landless and among those who owned land the average holding size was 5.28 acres (Devendran*et al.*, 2009).In contrast to present findings Rajanna*et al.* (2012) observed that majority of sheep farmers in Telangana region were land owners.

73% of Madras Red sheep farmers in the study area had>20 years of experience in sheep rearing. Similarly in Telangana region of Andhra Pradesh (64.58%) of sheep farmers had 17 to 41.3 years of experience (Rajanna*et al.,* 2012) and in Prakasam district of Aandhrapradesh62.22% of sheep farmerspossesed medium experience in sheep farming (Mastan bi *et al.,*2017)

2) INTERGRATION WITH OTHER FARM ANIMALS

Integration with other farm animals and flock size of sheep in field flocks were presented in Table 2. 65% of Madras Red sheep farmers in Kanchipuram district were rearing cattle along with sheep, 18% were rearing buffalos and majority(74%) of them were rearing goat along with sheep. Similar observations were made byVenkataramanan*et al*. (2017) in Madras Red sheep as farmers reared goats (78.4%), cows (53.8%) and buffaloes (27.7%).

The study on flock size maintained by sheep farmers revealed that majority of them (51.0%) had a flock size of 26-50 animals, followed by 51-100 animals (25%), 1-25 animals (15.0%), 101-150 animls (7%) and more than 150 animals (2%). Similarly varying flock sizes were reported for other Indian breeds *viz.* 57-64 in Cholka sheep farmers of West Rajasthan (Kushwala*et al.,* 1999) and 20 -68 sheep with the mean size of 38.6 in Vembur sheep (Chandran*et al.,* 2009).

3) HOUSING MANAGEMENT

Housing practices followed by Madras Red sheep farmers in Kanchipuram district were given in Table 3. Half of the (50%) farmers provided closed type shed with adjoining paddock, followed by both open and closed type (32%) shelter, open type

(11%) with tree shade and fencing and closed type (7%) shelters. Most of the sheep sheds (91%) were having earthern floor followed by floor covered with tarpaulin sheet (7%), with only 2% having cement type of floor. Tiruvenkadan*et al.* (2007) reportedfor Mecheri sheep in its breeding tract of Tamil Nadu that 94.84 sheep sheds are open type, 2.73 percent were semi open and 2.43 percent were closed type and type of flooring observed were 99.6 percent sheds with kutcha flooring and only 0.04 percent were having pucca flooring. Yadav and Tailor (2010) reported that 44% of sheep farmers maintained their flock in open housing system. Madras Red sheep in their breeding tract revealed that the partition is made with dried thorny bushes and 'all open' type housing is seen in the villages of Kanchipuram (Balasubramanyam*et al*., 2012).

TailorandYadav(2010) reported that housing had tile as the roofing material (57.3%). But observationsin the study area of Madras Red sheep were half of the sheds were (56%) made with thatched roofing material.

Among Madras Red sheep farmers 67% followed soil replacement in their sheds, 12% farmers followed filling the depressions on the floor and 21% neither follow soil replacement nor follow depression filling. Most of the farmers (65%) did not disinfect their sheds, whereas 35% only disinfected their sheds.

4) MANURE MANAGEMENT

Manure storage, usage and sale price/ tractor load of manure followed by Madras Red sheep farmers are presented in Table 4. Majority of the sheep rearers(56%) stored manure away from the sheep shed but, 44% stored near the shed. 96% of them stored manure in the form of a heap and 4% of them applied directly into their agricultural fields.88.02% of sheep farmers in north coastal zone of Andhra Pradesh stored their manure in open method (Rao*et al.,*2013).

Balasubramanyam*et al*. (2012) revealed that in Villupuram and Thiruvannamalai districts, it is a common practice that the sheep are kept overnight in the fields for manuring purpose.55.72% of farmers used the manure for the own farms and 13.95% of farmers sold the manure. Similarly in the present study,majority of sheep farmers were landless, 49% of them were selling their manureand 32% were using sheep manure for their own agricultural purposes.

5) FEEDING MANAGEMENT

Feeding management followed by Madras Red sheep farmers are depicted in Table 5. Nearly half of the sheep farmers (42%) were allowing animals for a grazing duration of 8 to 8hours 30 minutes followed by 7 to7 hours 30 minutes grazing (41%), 6 hours to 6 hours 30 minutes grazing (11%) and 9 hours to 9 hours 30 minutes grazing by 6% of farmers. Majority of sheep farmers (89%) covered a distance of 3 to 5 km for grazing, 10% farmers covered 1 to 2 km distance and 1% farmers were taking more than 5 km distance.Studies inMadras Red sheep(Balasubramanyamet al., 2012) in Kancheepuram, Thiruvallur, Villlupuram and Thiruvannamalai districts, the animals are mainly reared on extensive grazing

as no fodder is cultivated separately for feeding the sheep. SimilarlyTiruvenkadanet al. (2007) reported that Mecheri sheep in its breeding tract of Tamil Nadu mainly depended on grazing for 7 to 8 hours daily and sheep were taken for grazing up to a distance of 3 – 4 km. But,Aroraet al. (2014) in Malpura sheep said that sheep farmers moved their flock daily up to 10 km for grazing.

Kumar *et al.* (2015) revealed that 80 percent of goat keepers in Rajasthan adopted grazing on community pasture land. Only 7% of Madras Red sheep farmers were allowing animals in forest areas and others (93%) were allowing in common grazing lands, river sides, Real estate lands and harvested agricultural fields .

Balasubramanyam*et al.* (2012) reported that Madras Red animals are also fed with different fodder tree leaves and dried ground nut haulms. Similar results are observed in this survey as 35% sheep farmers were provided groundnut haulms as supplementary feed during lean period of summer, 3% farmers provided concentrate feed and remaining (62%) were not provided any supplementary feed during lean period.

6) REPRODUCTIVE MANAGEMENT

Reproductive data perceived from field flocks of Madras Red sheep are given in Table 6. All the farmers (100%) were following flock mating type breeding and as farmers were included in NWPSI, all farmers (100%) followed ram exchange program.The ram is retained in theMadras Redflockall thethe day (Balasubramanyam*et al.*, 2012) and about 85.59% sheep farmers replaced breeding rams from their own flock (Rajanna*et al.*, 2012).

The mean age at first mating observed in the Northcostal zone of Andhrapradesh was 12.85 $\pm$ 0.10 months(Rao*et al.*, 2013). Field flocks of Southern and North eastern agro climatic zone of Tamil Nadu revealed that the age of ewes at first mating (months) was 12.90 and 13.41(Kumarvelu and Pandian, 2012). The present study revealed that in majority of the flocks (52%) age at first mating was 1.5 – 2 years, followed by 1 – 1.5 years of age (36%) and 2 to 2.5 years of age (12%).

The mean age at first lambingobserved in the Northcostal zone of Andhrapradesh was 17.75$\pm$ 0.10 months (Rao*et al.*, 2013). Field flocks of Southern and North eastern agro climatic zone of Tamil Nadu revealed that the age of ewes at first lambing (months) was 18.19 and 18.97.In the present study age at first lambing of 2-2.5 years was observed in 52% flocks, 1.5 – 2 years in36% flocks and 2.5 – 3.0 years was observed in 12% flocks.

Life time number of lambing up to which ewes were kept was 5-6 in 38% flocks, 4 to 5 in (30% flocks), >6 in 18% flocks and 2-4 in 14% flocks.Only 9% sheep farmers reported twinning in their flock. Majority (85%) of them did not prefer the ewes that had twinning.

7) HEALTH MANAGEMENT

Health care practices followed by Madras Red sheep farmers are given in Table 7. Majority of sheep rearers (96%) followed deticking technique for sheep, of them 37%

followed pour on method of deticking, 40% followed spraying method and 19% followed dipping method.Lahoti and Chole (2010) reported that 94.00% farmers were not following and 6% followed deticking. Only 55.33% of Sonadi sheep farmers followed the deworming practices (Tailor et al., 2010).

Most of the Madras Red shepherds (92%) were not following any traditional method of treatment, whereas a small proportion (8%) was practicing traditional method of treatment. Majority of farmers (90%) disposed dead animals from the flock by burying and only 10% farmers thrown in to bushes.

8) MARKETING MANAGEMENT

Marketing management of the Madras Red sheep farmers are given in Table 8. Majority (93%) of sheep farmers were selling Ram lambs (RL) at the age of 6-12 months and only 7% farmers sold at 12-18 months age. Majority (57%) of them were getting a sale price of Rs. 3001-4000/ animal, followed by Rs 4001-5000 (40%) and 3% were getting Rs.1000- 3000/animal. 98% farmers only sold their aged ewes @ Rs.1001-2000/animal (43%), Rs.100-1000/ animal (30%) and>Rs. 2000 (25%).The lambs of Ramnad white were sold at the age of 5-6 months at the rate of Rs 1500-2000 and selling price of the animal was based on the body size(Raja *et al*., 2012)

9) CONSTRAINTS

Constraints perceived by Madras Red sheep farmers WERE obtained through structured questionnaire by direct interviews and score was given according to the farmer's perception and the constraints were ranked by Friedman ranking test and presented in Table 9. Most of the farmers felt insufficient grazing during summer as most severe constraint,followed by threat of stray dogs, low sale price of animal, unavailability of loans, availability of quality breeding stock, middle men exploitation , safety while grazing, lack of proper scientific knowledge to manage sheep and sudden disease incidence.Similarlymajor constraints hindering in Garole sheep rearing by the farmers were lack of grazing facility (73.64%) (Sagar and Biwas, 2008) and in contrast Rao*et al.*(2013) revealed that the major problems faced by sheep farmers were disease outbreaks (85.95%) and lack of veterinary facilities (83.81%).

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Table1: Socio-economic status of Madras Red sheep rearers

S. no	Category	Sub category	Percentage
1	Religion	Hindu	99.0
		Christian	1.0
2	Community	BC	25.0
		MBC	56.0
		SC	19.0
3	Location of the farmer	Sub urban	31.0
		Rural	69.0
4	Age of farmer	Below 30 years	4.0
		31-40 years	10.0
		41-50 years	22.0
		51-60 years	41.0
		Above 60 years	23.0
5	Educational qualification of the farmers	Illiterate	50.0
		Primary school	27.0
		Higher secondary school	22.0
		Graduate	1.0
6	Sheep farming as	Primary occupation	86.0
		Secondary occupation	14.0
7	Family type	Nuclear	66.0
		Joint	34.0
8	Land holding	No land	61.0
		0-2.0 acres	35.0
		2.1-5.0 acres	4.0
9	Experience in sheep rearing	1-5 years	14.0
		6-10 years	13.0
		11- 20 years	39.0
		>20 years	34.0

Table2: Integration of sheep farming with other animals& flock size in field flocks

S. no	Category	Sub category	Percentage
1	Integration with cattle	No cattle	35.0
		Integration with C	65.0
2	Integration with buffalo	Not having buffalo	82.0
		Integration with B	18.0
3	Integration with goat	Not having goat	26.0
		Integration with G	74.0
4	Integration with C+B+G	No integration	9.0
		Integration with C+B	30.0
		Integration with C+G	53.0
		Integration with B+G	8.0
5	Poultry	No poultry	52.0
		Have poultry	48.0
6	Other species(Duck/Rabbit)	No	99.0
		Yes	1.0
7	Flock size	1-25	15.0
		26-50	51.0
		51-100	25.0
		101-150	7.0
		>150	2.0
8	Sheep rearing system	Semi intensive system	3.0
		Extensive system	97.0
9	Dog as grazing companion	No grazing companion	97.0
		Grazing companion	3.0

Table3: Housing system followed in the field flocks of Madras Red sheep

S. no	Category	Sub category	Percentage
1	Location of the shed	Near the residence	72.0
		Away from the residence	18.0
		Both	10.0
2	House type	Open	11.0
		Closed	7.0
		Closed with open space	50.0
		Both open and closed	32.0
3	Type of floor	Earthen floor	91.0
		Cement floor	2.0
		Earthen floor with tarpaulins cover	7.0
4	Roof type	No roof	10.0
		Gable	71.0
		Lean to type	18.0
		Both gable and lean to type	1.0
5	Roofing material	No roof	10.0
		Thatched	56.0
		Asbestos	16.0
		Galvanized iron	3.0
		Tarpaulins	3.0
		Thatched with tarpaulin sheet	12.0
		Tiles	1.0
6	Housing of lambs	Housed separately	19.0
		Along with ewe	81.0

7	Type of lamb enclosure	No special house	81.0
		Movable structure	6.0
		Partition inside ewe shed	4.0
		Kept in farmers house	9.0
9	Height of roof at ridge	No roof	10.0
		5-10 ft	78.0
		>10 ft	12.0
10	Height of roof at eves	No roof	9.0
		1 – 2 ft	22.0
		3 – 7 ft	63.0
		8ft & above	6.0
11	Soil replacement	No replacement	21.0
		Once a year	43.0
		Twice a year	18.0
		Thrice a year	6.0
		Fill the depressions	12.0
12	Disinfection	Yes	35.0
		No	65.0

Table4: Manure management in the field flock

S. no	Category	Sub category	Percentage
1	Place of storage of manure	Near the shed	44.0
		Away from the shed	56.0
2	Method of Storage of manure	Kept as open heap	96.0
		Applied directly in agriculture field	4.0
3	Mode of use of manure	Sale	49.0
		Own purpose	32.0
		Both sale & own purpose	12.0
		Relatives	4.0
		Exchange for dry fodder	3.0
4	Frequency of disposal of manure	Need basis	39.0
		6 months once	11.0
		Yearly once	49.0
		2 years once	1.0
5	Sale price of manure/tractor load	No sale	39.0
		<Rs.500	20.0
		Rs.501-1000	39.0
		>Rs.1000	2.0

Table5: Feeding management in field flocks

S. no	Category	Sub category	Percentage
1	Duration of grazing	6 and 6.30 hours	11.0
		7 and 7.30 hours	41.0
		8 and 8.30 hours	42.0
		9 and >9 hours	6.0

2	Grazing distance	0-2 km	10.0
		3-5 km	89.0
		>5 km	1.0
3	Grazing area for sheep	Common land	26.0
		Forest area	6.0
		Common land & forest area	28.0
		Forest area & harvested field	20.0
		Common land+forest area+harvested field	3.0
		Real-estate land & harvested field	9.0
		Real-estate & river side area	7.0
		Forest area for lease	1.0
4	Supplementary feed offered during summer	No other feed	62.0
		Concentrate feed	3.0
		Groundnut haulms	35.0
5	Provision of water in summer	Yes	64.0
		No	36.0

Table6: Reproductive data from field flocks

S. no	Category	Sub category	Percentage
1	Detection of anestrous ewe	Yes	62.0
		No	38.0
2	Age at first mating	1 to 1.5 yrs	36.0
		1.5 to 2.0 yrs	52.0
		2 to 2.5 yrs	12.0
3	Age at first lambing	1.5 to 2 7 yrs	36.0
		2 to 2.5 yrs	52.0
		2.5 to 3 yrs	12.0

4	No. of lambings in the life time	2 to 4	14.0
		4 to 5	30.0
		5 to 6	38.0
		> 6	18.0
5	Twinning observed among flocks	Yes	9.0
		No	91.0
6	Twinning preference	Yes	15.0
		No	85.0
7	Breeding method	Flock mating	100.0
8	Ram exchange	Yes	100.0

Table7: Health care management in the field flock

S. no	Category	Sub category	Percentage
1	Deticking	Yes	96.0
		No	4.0
2	Method of deticking	Application	37.0
		Spraying	40.0
		Dipping	19.0
		No deticking	4.0
3	Traditional method of treatment	Yes	8.0
		No	92.0
4	Mode of disposal of died animals	Burying	90.0
		Thrown into bushes	10.0

Table 8: Marketing management of Madras Red sheep in field flock

S. no	Category	Sub category	Percentage
1	Sale price of ram lambs	Rs.1000-rs.3000	3.0
		Rs.3001-rs.4000	57.0
		Rs.4001-rs.5000	40.0
2	Marketing age of ram lambs	6 to 12 months	93.0
		12 to 18 months	7.0
3	Sale price of aged ewe	Rs.100- rs.1000	30.0
		Rs.1001-rs.2000	43.0
		>rs.2000	25.0
		No sales	2.0

CARCASS CHARACTERISTICS OF LARGE WHITE YORKSHIRE GROWER PIGS MAINTAINED UNDER ROOF INSULATION AND WATER FOGGING SYSTEM DURING SUMMER SEASON

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ABSTRACT

A study was carried out to assess the effect of heat stress amelioration practices such as roof insulation and water fogging on the carcass characteristics of Large White Yorkshire grower pigs in summer season. Forty numbers of weaned pigs were assigned to five treatment groups randomly. It was observed that the groups maintained under insulated roofing with 10 and 5 minutes fogging had significantly ($P<0.05$) higher pre slaughter weight, dressing percentage, loin eye area, muscle percentage and meat bone ratio, while hot carcass weight, carcass length and fat percentage were highly significant ($P<0.01$) in these treatments when compared to control.

Key words : Heat stress, Fogging, Enriched roofing, Carcass traits, Large White Yorkshire Pig

INTRODUCTION

One of the most important factors affecting welfare throughout the stages of growth is the environment in which animals are maintained (Nasirahmadi *et al.,* 2015). Pigs raised in tropical climate are always under stress, where the environmental temperatures are frequently above the zones of thermo neutrality (Myer *and* Bucklin, 2001). Reduction of heat load in environment is the primary management tool during summer season for most of the productive animals in tropics which are at greatest risk of heat stress and requires most attention.. The modified thermal environment usually provides increased productivity (Gangwar, 1988). Therefore, any attempt directed towards the improvement of the managemental practices will yield substantial increase in pork production. The most economic means of reducing heat stress for swine may be through evaporative cooling and modified shelter arrangements, so that the maximum expression of genetic potential of pigs can be achieved. With the above circumstances, the present research work has been taken up

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to study the carcass characteristics of Large White Yorkshire pigs raised under enriched housing and water fogging system

MATERIALS AND METHODS

The study was carried out at Post Graduate Research Institute in Animal Sciences, Kattupakkam, TANUVAS, Kancheepuram district in Tamil Nadu. Forty weaned Large White Yorkshire piglets of both sexes at the age group of four months were allotted randomly to five treatment groups and reared with equal floor space allowance of $1m^2$./piglet under asbestos roofing during the hot months of March to May. T_1 was maintained as control. For T_2 and T_3 treatment groups, the roof was enriched with dry fodder waste and maintained under controlled fogging for 5 and 10 minutes respectively. T_4 and T_5 were maintained under controlled fogging for 5 and 10 minutes respectively. The foggers were having a flow range of 5.5 to 7.5 l/h which sprayed fine water droplets once in 45 minutes period (6 times a day) by automatic timers from 11:00 to 15:00 hrs daily. Two animals from each treatment group were selected at random and slaughtered after the end of the trial for the evaluation of carcass traits, Pigs were kept off feed for a period of 12 hours prior to slaughter but given *ad libidum* access to water. The statistical analysis was carried out by using IBM SPSS® Version 20.0 for Windows®.

RESULTS AND DISCUSSION

The pigs reared under enriched roofing with 5 and 10 minutes of fogging (T_2 and T_3) showed numerically higher pre slaughter live weight of 66.85±0.45 and 66.60±0.10 kg respectively than the control group (61.70±1.30 kg). The results were in agreement with the finding of Gnanaraj *et al.* (2002) and Joshi *et al*. (1997) who had reported heavier live weight in the pigs that were protected from heat stress with cooling system. The hot carcass weight after the slaughter of grower pigs were 41.75±1.25, 46.75±0.25, 48.25±0.25, 45.75±0.25 and 46.50±0.50 kg for T_1, T_2, T_3, T_4 and T_5 groups respectively. Statistical analysis showed highly significant ($P<0.01$) difference between the five treatment groups. Carcass weight (kg) of pigs of T_3 group maintained under enriched roofing with 10 minutes fogging system (48.25±0.25 kg) was higher, followed by T_2 group under enriched roofing with 5 minutes fogging system (46.75±0.25 kg). This indicated that the pigs under enriched roofing and fogging were able to convert feed into meat better than the pigs kept without any heat stress protection systems. Moreover, the dressing percentage was significantly ($P<0.05$) higher in T_3 group under enriched roofing with 10 minutes fogging system (72.18±0.86 per cent) when compared to control group (67.65±0.60 per cent). The highest carcass length was recorded in T_3 group under enriched roofing with 10 minutes fogging system (90.75±0.25cm), followed by T_2 group under enriched roofing with 5 minutes fogging system (89.75±0.25 cm), T_5 group under 10 minutes fogging system (89.25±0.25 cm), T_4 group under 5 minutes fogging system (88.75±0.25cm) and least was recorded in control group (86.75±0.75 cm). These results concurred with the findings of Joshi *et al.* (1997). No significant difference in back fat thickness (cm) of Large White Yorkshire grower pigs

maintained under enriched roofing, fogging and control was noticed. The result of the present study was in agreement with that of Wu *et al*. (2016) with regard to effect in back fat thickness. Loin eye area (cm^2) was significantly (P<0.01) larger in T_3 group under enriched roofing with 10 minutes fogging system (25.20±0.69cm^2), followed by T_2 (23.46±0.01cm^2), T_5 (23.25±0.10cm^2). Significantly higher loin eye area in the treatment groups under enriched roofing with 5 and 10 minutes fogging point to the suitability of this combined system to provide comfortable environment. Moreover, T_2, T_3, T_4 and T_5 groups had significantly (P<0.05) higher meat percentage compared to the control group. The result of increased meat percentage under cooling system was in agreement with the findings of Bridges *et al.* (1998). Similarly, significantly (P<0.01) higher fat percentage was observed in T_2, T_3, T_4 and T_5 groups when compared to the control group. The present finding of reduced fat percentage in heat stressed pigs was in agreement with Rinaldo *et al.* (2000) and Bellego *et al.* (2002). However, the statistical analysis of bone percentage had not revealed any significant difference between the five treatment groups. This was in agreement with the findings of Lefaucheur *et al.* (1991). The meat bone ratio was significantly (P<0.05) higher in T_2, T_3, T_4 and T_5 groups (3.93±0.01, 3.97±0.02, 3.98±0.02 and 3.96±0.01) when compared to T_1 group (3.75±0.07). The higher meat bone ratio in the third and second group under enriched roofing and water fogging system points to the possibility of efficient meat production under this heat stress alleviation measure.

Table 1 Mean±SE of various carcass characteristics of Large White Yorkshire grower pigs maintained under enriched roofing and water fogging system

Carcass character	T_1	T_2	T_3	T_4	T_5	'F' value
Live Weight (kg)	$61.70^{a}\pm1.30$	$66.60^{b}\pm0.10$	$66.85^{b}\pm0.45$	$65.00^{b}\pm1.00$	$65.95^{b}\pm0.95$	5.760*
Hot carcass weight (kg)	$41.75^{a}\pm1.25$	$46.75^{bc}\pm0.25$	$48.25^{c}\pm0.25$	$45.75^{b}\pm0.25$	$46.50^{bc}\pm0.50$	14.875**
Dressing Percentage	$67.65^{a}\pm0.60$	$70.19^{b}\pm0.27$	$72.18^{b}\pm0.86$	$70.40^{b}\pm0.70$	$70.51^{b}\pm0.26$	7.636*
Carcass length (cm)	$86.75^{a}\pm0.75$	$89.75^{bc}\pm0.25$	$90.75^{c}\pm0.25$	$88.75^{b}\pm0.25$	$89.25^{bc}\pm0.25$	13.538**
Back fat thickness (cm)	$3.20^{a}\pm0.03$	$3.29^{b}\pm0.02$	$3.32^{b}\pm0.04$	$3.23^{ab}\pm0.02$	$3.25^{ab}\pm0.02$	3.717^{NS}
Loin Eye area (cm^2)	$20.88^{a}\pm0.93$	$23.46^{bc}\pm0.01$	$25.20^{c}\pm0.69$	$21.80^{ab}\pm0.15$	$23.25^{bc}\pm0.10$	10.122*
Meat percentage	$49.82^{a}\pm0.05$	$52.08^{b}\pm0.68$	$52.23^{b}\pm0.06$	$51.80^{b}\pm0.06$	$51.51^{b}\pm0.02$	9.848*
Bone percentage	13.30±0.28	13.26±0.14	13.16±0.04	13.01±0.04	13.01±0.03	0.930^{NS}
Fat percentage	$31.02^{a}\pm0.09$	$33.16^{c}\pm0.39$	$33.57^{c}\pm0.03$	$32.35^{b}\pm0.04$	$33.55^{c}\pm0.07$	34.278**
Meat bone ratio	$3.75^{a}\pm0.07$	$3.93^{b}\pm0.01$	$3.97^{b}\pm0.02$	$3.98^{b}\pm0.02$	$3.96^{b}\pm0.01$	7.609*

* Significant at five percent (P<0.05) ; ** Significant at one percent (P<0.01)

Figures having different superscripts in a row differ significantly

CONCLUSION

With the results obtained in the present study, it could be concluded that heat stress control measures such as insulation of roof with dry fodder waste along with 5 or 10 minutes controlled fogging provided beneficial effect on growing pigs in terms of better carcass yield. It also indicated that an intervention in the housing management system by insulating the roof with dry fodder waste and skin wetting with fogging system is the best practice that could be adopted in hot regions as a thermal stress amelioration measure

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Short Communications

SURGICAL MANAGEMENT OF COMPLETE UTERINE PROLAPSE IN A CAT

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Uterine prolapse is an uncommon and infrequent complication of parturition in cat. It occurs immediately or over a period of 48 hours after delivery of the last neonate. Uterine prolapse is an eversion of the organ which turns inside out as it passes through the cervix into the vagina. The prolapse can be complete, with both horns protruding from the vulva, or limited to the uterine body and one horn (Deroy *et al*., 2015). The etiology of uterine prolapse is unknown.

It was thought to occur as a result of decreased myometrial tone that might allowed the uterus to fold in and permit part of the wall to move towards the pelvic inlet (Murphy and Dobson, 2002).

A 9 months old Domestic shorthair female stray cat was presented with the history of prolapsed uterus through the vulva. Physical examination revealed the cat was dehydrated. Prolapsed uterus was necrotic with laceration and infested with live maggots. The condition was diagnosed as complete uterine prolapse based on clinical observation (Figure 1). Preoperative haematology, serum biochemistry values were within the normal range. Cefotaxime @ 20 mg/kg body weight was given intramuscularly. The cat was premedicated and induced with xylazine 0.5 mg/kg and ketamine @ 10 mg/kg body weight intramuscularly. Endotracheal intubation was done after topical application of 2% lignocaine. General anaesthesia was maintained with 2% isoflurane with 100% oxygen. Ringer's lactate was administered at 10 ml/kg/hour intravenously. The cat was positioned in sternal recumbency. The live maggots were removed and the dirt and debris were flushed and irrigated with normal saline. A clamp was applied on the prolapsed uterine body, proximal to the clamp ligation was made with PGA 2-0, and the prolapsed uterine portion was amputated distal to the suture and then the proximal portion with ligation were repositioned into the vagina. Cefotaxime @ 20 mg/kg and butorphanol @ 0.2 mg/kg body weight was

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given intramuscularly for three days follow up. Cat recovered uneventfully.

Uterine prolapse is relatively uncommon in cats and accounting for 0.6% of the maternal cause of dystocia. Clinical signs include vaginal discharge, straining, restlessness, pain and protrusion of a mass from vulva and signs may progress to shock and toxemia (Deroy *et al.,* 2015). Uterine prolapse can be associated with uterine rupture (Carreira *et al.,* 2012). Uterine prolapse is an obstetrical emergency and require immediate attention to decrease the risk of uterine artery rupture or avulsion from the internal iliac leading to fatal haemorrhage (Miesner, 2008). The treatment for uterine prolapse depends upon the severity of damage to the uterus. The prognosis following treatment for a uterine prolapse is guarded depending on the timing of veterinary intervention (Deroy *et al.,* 2015).

In the presented case, the occurrence of uterine prolapse was unknown as it was stray cat and the prolapsed uterus was contaminated and infested with live maggots. Hence manual reduction of the uterus was not attempted. It is concluded that surgical amputation of the prolapsed uterus was successful in managing complete uterine prolapse in cat.

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Fig.1 Complete uterine prolapse

FUNCTIONAL ANALYSIS OF CUMULUS CELLS ASSOCIATED GENES RELATED TO THE QUALITY OF *IN VITRO* FERTILIZED CAPRINE EMBRYOS

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As successful embryonic development is dependent on a time- and site-specific expression of appropriate genes, the studies on differential gene expression in cumulus oocyte complexes (COCs) could potentially elucidate the signaling pathways involved in the crosstalk between the oocyte and its somatic associates during maturation and the developmental processes (Mourad *et al*., 2008). Communication between the oocyte and the cumulus ells is accomplished mainly through the gap junction communications and the cumulus cells were thought to be the mediators of oocyte paracrine signals and developmental potential. Hence, transcriptomic analysis of cumulus cells could be a non- invasive method to assess developmental competence of the oocyte. Certain genes in cumulus cells are predicted to be expressed differentially that would predict the quality of oocyte, its developmental competence and also the final embryo quality.

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Growth differentiation factor 9 (GDF9), a member of the transforming growth factor-β superfamily, was the first oocyte-specific factor shown to cause cumulus expansion (Elvin *et al*. 1999). GDF9 functions as an oocyte-secreted paracrine factor that regulates several key granulosa cell enzymes involved in cumulus cell expansion and creates a microenvironment optimal for acquisition of oocyte developmental competence (Pangas and Matzuk, 2005). The expression levels of GDF9 downstream target genes in the cumulus cells may reflect GDF9 activity and could ultimately predict oocyte health (McKenzie *et al*., 2004) and the grade of the resulting embryos (McKenzie *et al*., 2004, Zhang *et al*., 2005). Cyclo-oxygenase 2 (COX2) / prostaglandin endoperoxide synthase 2 (PTGS2), gremlin1 (GREM1) and hyaluronic acid synthase 2 (HAS2) are all downstream GDF9 target genes found in cumulus cells and have been evaluated as markers for oocyte developmental competence and the subsequent embryonic development (Cillo *et al*., 2007). Analysis of the above mentioned genes might help us to evaluate the developmental competence of the oocytes thus determining the quality of embryos. Based on this hypothesis the

present study was undertaken to identify the target genes in cumulus cells associated with developmental competence of caprine oocytes.

Goat ovaries were obtained from the slaughter house and transported to the Centralized Embryo Biotechnology Unit, Department of Animal Biotechnology Unit II, TANUVAS, Chennai – 51, in 0.9 per cent normal saline containing penicillin (100 IU/ml) and streptomycin (50 mg/ml) at 30-35° C in a thermos flask within 2 h of slaughter.

Cumulus oocyte complexes (COCs) retrieved by slicing of ovaries were screened and graded as A, B, C, D and E based on their cumulus cells investment and ooplasm homogeneity. Only COCs of grades A and B were washed in TCM 199 + 10 per cent fetal bovine serum (FBS - GIBCO: Invitrogen, USA) and finally in *in vitro* maturation (IVM) medium, composed of TCM-199 supplemented with 10 per cent FBS, 1 µg/ml of Folltropin (FSH), 0.02 IU/ml of Luteinizing Hormone (LH), 1 µg/ml of estradiol and 10 ng/ml of epidermal growth factor (EGF). A group of ten to fifteen COCs were transferred to a 50 µl droplets of maturation medium in a 35 mm petridish, pre-equilibriated with the IVM medium for 2h at 38.5°C under 5 per cent CO_2 in air and cultured for 27 hrs at 38.5°C in a humidified atmosphere of 5 per cent CO_2 in air. After 27 hours of incubation, the COC's were observed under stereo-zoom microscope (Nikon, Japan). Maturation rate was evaluated based on degree of cumulus expansion and extrusion of the first polar body (Degree 2- cumulus cells homogeneously expanded, Degree 1-cumulus partially expanded with few clustered cells, Degree 0 - No morphological change).

The Degree 2 and Degree 1 COC's were washed separately in OCM in 35mm petri dishes by vigorous pipetting. This process detached the cumulus cells from the oocytes. Pipetting was carried out until all the cumulus cells were removed. The degree 1 and degree 2 cumulus cells were collected. They were washed in PBS twice and were stored in PBS in 1.5 ml microfuge tubes at -20°C for RNA isolation.

Sperms were extracted from the cauda epididymis of buck testes from the slaughter house. The motile sperms were separated by swim up method. Concentration of the final sperm pellet was determined with a haemocytometer and the sample was diluted with spTALP (sperm tyrode's albumin lactate pyruvate) to yield a concentration of 1-2 $\times 10^6$ sperm/ml. The fertilization droplets of 75 µl of *in vitro* fertilization (IVF-TALP) medium supplemented with heparin (10µg/ml) in 35 mm petridish overlaid with sterile mineral oil was pre-equlibrated at 38.5˚C under 5 per cent CO_2 in air. The matured COCs were washed in pre-equilibriated spTALP and oocytes were washed in IVF TALP medium and transferred to the pre-equilibrated IVF droplets such that each droplet contained 10-15 oocytes degree 2 and degree1 matured oocytes separately. The motile sperm suspension obtained by swim up technique were inseminated into the IVF droplets containing oocytes to achieve the final concentration of 2 million sperm/ml and co-incubated for 18-24h at 38.5°C in a humidified atmosphere of 5 per cent CO_2 in air.

After 18 to 24 hours the plate was checked for fertilization process. The oocytes were removed from the droplets and were washed to remove the dead sperms from them. The cleaved cells were placed in SOF culture media drops covered with sterile mineral oil in 35mm petri dish till the morula stage. The culture media were changed once in 48 hours.

The Degree 2 and Degree 1 cumulus cells were thawed. They were centrifuged in a cooling centrifuge at 4°C, 7,500xg for 5 minutes. 0.25ml of sample (containing pellet) was used from each tube by discarding the supernatant. 0.75ml of Trizol reagent was added to 0.25ml of sample and mixed by pipetting. The samples were incubated at RT for 5 mins. 0.2 ml of chloroform was added to the above mixtures and mixed well. It was then incubated at RT for 2 to 3 minutes. After incubation, the samples were centrifuged at 4°C, 12,000xg for 15 minutes. The aqueous phases from the samples were transferred to fresh 1.5ml microfuge tubes. 0.5ml of isopropanol was added to each tube containing aqueous phases. The mixtures were incubated at RT for 10 minutes. Then they were centrifuged at 4°C, 12,000xg for 10 minutes. The pellet was washed with 75% ethanol at 7500xg for 5 minutes and the pellet air dried. The pellets were re-suspended in DEPC treated water/nucleus free water.

The extracted RNA was used for cDNA synthesis using a high capacity cDNA Reverse Transcriptase kit (Applied Biosystems Inc, USA) following the manufacturer's instructions. The cDNA obtained from RNA were used for predicting the expression of PTGS2, HAS2 and GREM1 genes in caprine cumulus cells by PCR amplification along with the β-actin as marker gene.

Primer Sequences

Gene Name	Primer Sequence	Annealing Temperature (º C)	Product Size
GREM1	5'-AACAGCCGTACCATCATCAAC-3'	55	65bp
	5'-TTCAGGACAGTTGAGAGTGACC-3'		
COX2	5'-CATGGGTGTGAAAGGGAGGAAAGA-3'	58	304bp
	5'-CCTTAGTGAAAGCTGGTCCTCGTT-3'		
HAS2	5'-ATAAATGTGGCAGGCGGAAGAAGG-3'	60	182bp
	5'-GTCTTTGTTCAAGTCCCAGCAGCA-3'		

Cycling conditions for PCR

Primer	Steps				
	1	2	3	4	5
GREM1	95°C/10 min	95°C/15 sec	55°C/45 sec	72°C/30 sec	72°C/10 min
HAS2	95°C/10 min	95°C/15 sec	60°C/45 sec	72°C/30 sec	72°C/10 min
COX2	95°C/10 min	95°C/15 sec	58°C/45 sec	72°C/30 sec	72°C/10 min

Step 2 to 4 was repeated for 50 cycles

After the run, the amplified products were analyzed by agarose gel electrophoresis

To date, non-invasive embryo selection had been based mainly on morphological and developmental criteria performed during *in vitro* development (Scott, 2003). The quality of the embryos depends on the oocyte quality which implies that with careful assessment of the oocyte quality, the quality of the embryos produced could be manipulated. The hindrance in assessing the oocyte quality might be overcome by the additional parameters that would support morphological and metabolic evaluation of the oocyte in order to appropriately select those that have the greater chance of fertilization and development. Given the essential nature of the interaction between cumulus cells and the maturing oocyte and that many aspects of cumulus function are regulated by the oocyte (Eppig, 2001, Matzuk *et al*., 2002), the analysis of cumulus cells appear a logical potential approach to the non-invasive assessment of oocyte developmental competence (Li *et al*., 2008). Growth differentiation factor 9 (GDF9), a member of the transforming growth factor-β superfamily, associated genes and their expression levels were considered as positive markers of developmental competence of oocytes.

In the present study, the expression of GDF9 associated genes (GREM1, HAS2 and COX2) was studied separately in grade A and grade B oocytes and compared with the cleavage rate and development of embryos. The cleavage rate/morula rate of grade A and grade B oocytes where found to be 44.29±1.31 / 28.60±1.78 and 34.83 ± 2.72 / 16.19 ± 1.79, respectively and all the three genes were expressed in both grades of oocytes. The results of the present study was in accordance with McKenzie *et al*. (2004), who stated that the expression of genes HAS2, PTGS2 and GREM1 could be correlated to morphological and physiological characteristics and might provide a novel approach to predict embryo development. In spite of their expression, the cleavage rate and morula rate were significantly different between grade A and grade B oocytes which might be due to the difference in the level of expression of genes (differential expression) between the two grades of oocytes. According to Anderson *et al*. (2009) genes HAS2 and PTGS2 had great level of expression in mature oocytes than immature oocytes with 4.7 fold increases in PTGS2 gene in mature cumulus. They also reported fertilization and early embryo

cleavage showed a positive relationship with PTGS2 and GREM1 expression stating that the quality of the embryos were high (graded by graduated embryo score (GES)) from oocytes in which these genes were differentially expressed.

From the present study it can be concluded that the expression of the candidate genes (HAS2, PTGS2 and GREM1) helps to predict the quality of the oocytes selected. Ultimately, the analysis of the level of expression of these genes would bring about a clear view in the determining the criteria for the selection of good quality embryos.

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MORPHOMETRY OF THE MANDIBLE AND UPPER JAW OF THE NATIVE DOGS OF TIRUNELVELI DISTRICT AND ITS CLINICAL VALUE DURING REGIONAL ANAESTHESIA

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Clinical anatomy is one of the principles of the clinical and surgical practice because it enables the clinician to visualize details of structures (Ommer and Harsahan, 1995). Morphologic and morphometric studies of the upper jaw and mandible not only reflect contribution of genetics and environmental components to individual development and describe the genetic and phenotypic variations but also are the foundation of clinical and surgical practices (Karimi *et al.*, 2011). The direction of the cranial nerves and their passages from different foramina in the upper jaw and mandible were of clinical importance in the regional anaesthesia of the head (Hall *et al.*, 2000).

The form and size of the skull shows breed and individual variation. The shape of the skull and the mandible is the most important criteria in the determination of dog breeds. However, there is a lack of information on the morphometric measurements of the upper jaw and mandible of native dogs of Tirunelveli district and its clinical value during regional anaesthesia. Therefore the work was taken to provide morphometry of the dog upper jaw and mandible and its application for the head regional anaesthesia.

The study was conducted on the upper jaw and mandibles without any apparent skeletal disorders of six adult native dogs (Non Descript breed) bones which were available at the Department of Veterinary Anatomy, Veterinary College and Research Institute, Tirunelveli. The following morphometric measurements were done in the upper jaw and mandibles using scale and vernier callipers.

Skull length – from the dorsal nasal cartilage to the external occipital protuberance and was subdivided into cranial and nasal length

Cranial length

Nasal length

Cranial width

Cranial index = Cranial width x 100 / Cranial length

Distance from the root of the premolar tooth to the midlevel of the infraorbital canal

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Distance from the midlevel of the infrorbital canal to the root of the canine tooth

Mandibular length – from the level of cranial extremity of the alveolar root of the incisor to the level of the caudal border of the mandible

Distance from the lateral alveolar root to the mental foramen

Distance from the mental foramen to the caudal mandibular border

Distance from the mandibular foramen to the base of the mandible

Distance from the caudal border of the mandible to below of the mandibular foramen

Distance from the condyloid fossa to the height of the mandible

Distance from the condyloid fossa to the base of the mandible

Maximum mandibular height – from the base of the mandible to the highest level of the coronoid process

Distance from the caudal border of the mandible to the level of mandibular foramen

Distance from the mandibular foramen to the mandibular angle.

The values were measured (Mean ± SE) and data obtained were analysed statistically (Snedecor and Cochran, 1994) and the results were presented in the Table I.

In the present study, the skull length, cranial length, nasal length and cranial width of the native adult dogs were 18.2 ± 0.21 cm, 10.7 ± 0.35 cm, 7.5± 0.02 cm and 7.0± 0.56 cm, respectively. The cranial index was 65.42. (Table I, Fig. 1). The above values were similar to the results on the adult Kangal dogs (Onar *et al.*, 2001) and Iranian adult dogs (Monfared, 2013) but were relatively different from the findings on the German Shepherd puppies (Onar, 1999). It may be due to the existence of significant differences in the skull shape and size between various breeds.

In adult native dogs, the distance from the root of the premolar tooth to the infrorbital canal and from the latter to the root of the canine tooth was 0.9 ± 0.85 cm and 2.8± 0.44 cm, respectively (Table I). The infraorbital foramen in the dog was over the alveolus of the third premolar tooth and infraorbital nerve emerge from this foramen. So this data was useful for tracking the infraorbital nerve and necessary for the desensitization of the skin of the upper lip, nostril and face on that side of the foramen (Ommer and Harshan, 1995). The injection of the local anaesthetic agents within the canal via the infraorbital foramen would lead to analgesia of the incisor, canine and first three premolars.

In this study, the distance from the lateral alveolar root to the mental foramen was 2.0± 0.66 cm (Fig. 2). This value was a guide in detection of the location of the mental nerve for regional nerve block in the adult dogs for lower lip. The injection of the local anaesthetic agents was to

be administered in the rostral aspect of the mandibular canal through the mental foramen for mandibular nerve block.

The mandibular length and height in the adult native dogs was 12.8 ± 0.12 cm and 6.1 ± 0.55 cm (Fig. 2) respectively which were greater than the values of German Shepherd puppies (Onar, 1999) but lesser than the values of Iranian adult dogs (Malfared, 2013).

The caudal border of the mandible to below of the mandibular foramen was 1.4 ± 0.33 cm in adult dogs. In addition the distance from the caudal border of the mandible to the level of the mandibular foramen and from the latter to the border of the mandibular angle was 1.5 ± 0.21 cm and 1.1 ± 0.66 cm (Fig. 3) respectively. This data was necessary for achieving the regional anaesthesia of the mandibular nerve effectively for desensitization of all the teeth in the lower jaw.

In conclusion, the morphometric values of the skull and the clinical anatomy of the head region of the native adult dogs provide an important baseline for further research in this field.

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Table I: Morphometrical parameters of mandibles and upper jaws of native adult dogs of Tirunelveli district

S. No.	Parameters	Mean ± SE (cm)
A	Skull length	18.2 ± 0.21
B	Cranial length	10.7 ± 0.35
C	Nasal length	7.5 ± 0.02
D	Cranial width	7.0 ± 0.56
E	Cranial index	65.42
F	Root of the premolar tooth to midlevel of infraorbital canal	0.9 ± 0.85
G	Midlevel of infraorbital canal to root of canine tooth	2.8 ± 0.44
H	Mandibular length	12.8 ± 0.12
I	Lateral alveolar root to mental foramen	2.0 ± 0.66
J	From the level of mental foramen to the extreme caudal border of the mandible	10.5 ± 0.89
K	Mandibular foramen to base of mandible	1.1 ± 0.75
L	Caudal border of the mandible to below of the mandibular foramen	1.4 ± 0.33
M	Condyloid fossa to the height of the mandible	2.6 ± 0.05
N	Condyloid fossa to the base of the mandible	3.4 ± 0.88
O	Maximum mandibular height	6.1 ± 0.55
P	Caudal border of mandible to the level of the mandibular foramen	1.5 ± 0.21
Q	Mandibular foramen to mandibular angle	1.1 ± 0.66

FIGURES

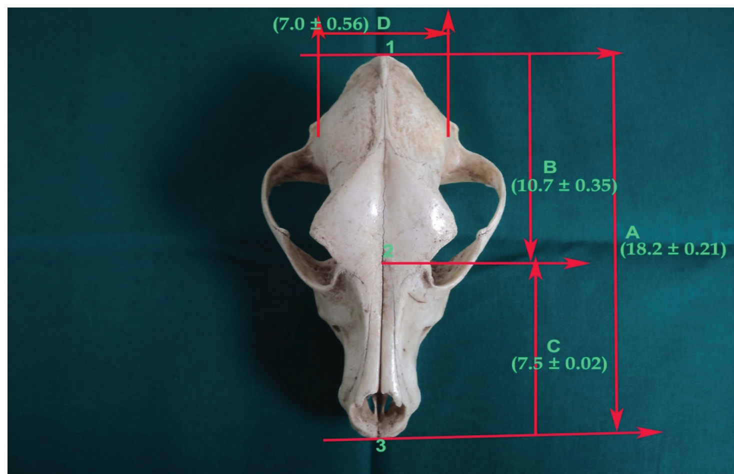

Fig. 1: Measurement of the cranium (Dorsal view) of native dogs of Tirunelveli district

1 – Central surface point of external occipital protuberance
2 – Junction of median plane of the right and left nasofrontal sutures
3 - Anterior end of interincisive suture
A. Skull length B. Cranial length C. Nasal length D. Cranial width

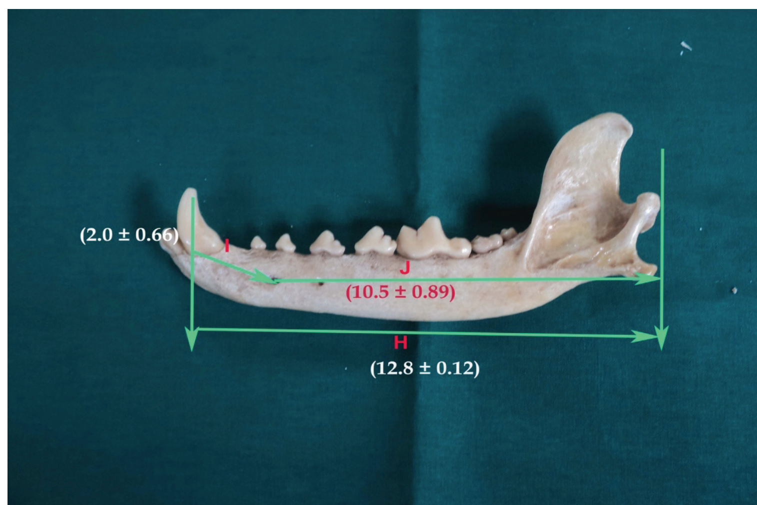

Fig. 2: Lateral aspect of the mandible showing distances

H- Mandibular length
I - Lateral alveolar root to mental foramen
J - From the level of mental foramen to the extreme caudal border of the mandible

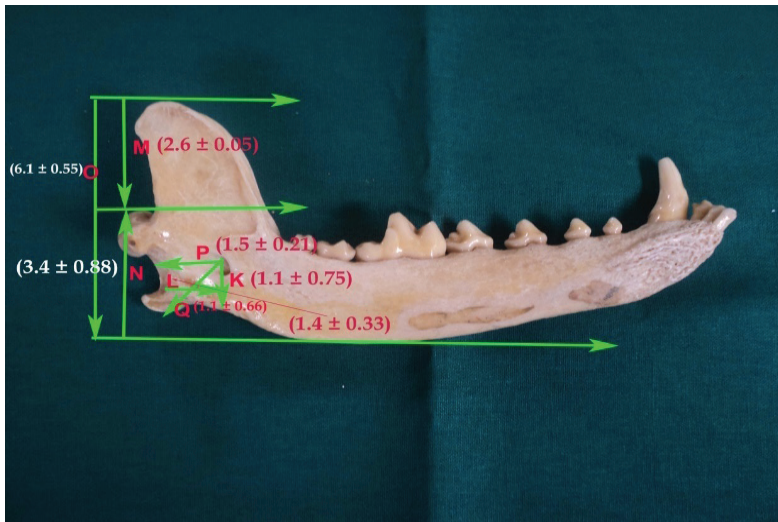

Fig. 3: Medial aspect of the mandible showing distances

K - Mandibular foramen to base of mandible
L - Caudal border of mandible to below of the mandibular foramen
M - height of the mandible to condyloid fossa
N - Condyloid fossa to the base of the mandible
O - Maximum mandibular height
P - Caudal border of mandible to the level of mandibular foramen
Q- Mandibular foramen to mandibular angle

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"Indian Journal of Veterinary and Animal Sciences Research" published six times in a year will consider original papers for publication on all aspects of animal and fisheries sciences. The scope of the journal includes animal and fisheries health, management, production and marketing of products. Acceptance of manuscript will be based on scientific merit as judged by referees and Editorial Board.

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Books and articles within edited books

Rundall, C.J. (1991). A colour Atlas of Diseases of the Domestic Fowl and Turkey. 2nd ed. London. Wolf Publishing Ltd. 175 p.

Handbooks, Technical bulletins, Thesis and Dissertations

Callow, L.L and Dalgliesh, R.J. (1982). Immunity and Immunopathology in Babesiosis. In: S. Choen and K.S. Warren (Ed) Immunology of Parasitic Infections. Blackwell, Oxford. pp 475-526.

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