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(Bi-monthly)

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Vol. 47 **September - October 2018** **No. 5**

Review article

Full length articles

Short Communications

Review article

Cutaneous reconstruction 2: skin flaps

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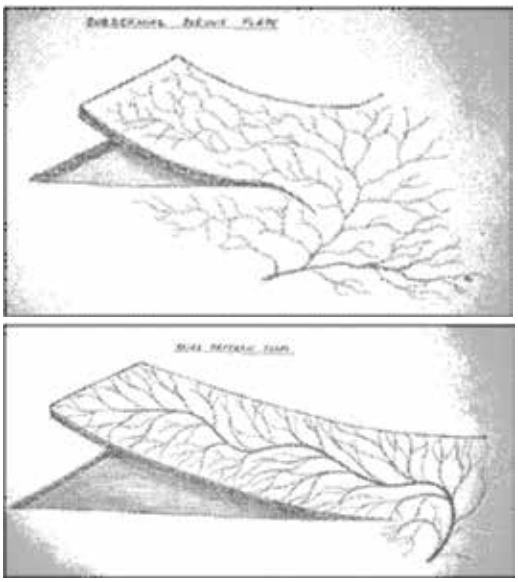
DEFINITION

A skin flap (= cutaneous pedicle graft) is portion of skin moved from one area of the body to another with an intact vascular attachment to its donor site. Skin flaps are indicated for large cutaneous defects where tension-relieving techniques will not provide adequate skin for closure by direct apposition. Skin flaps are, by definition, vascularized, and thus capable of enhancing the blood supply to a wound, covering body wall defects and areas difficult to immobilize (like joints or commissures). They also provide immediate protection with hirsute skin.

CLASSIFICATIONS OF SKIN FLAPS

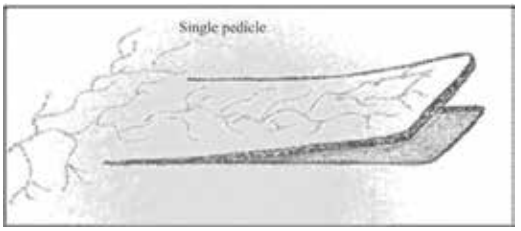
Blood Supply - Subdermal plexus flaps (random flaps) are elevated without intentional inclusion of direct cutaneous vessels and rely on perfusion via the subdermal plexi. Axial pattern flaps, however, incorporate a specific direct cutaneous artery and vein, using established guidelines for flap development. These flaps have an excellent blood supply and allow much larger flap development compared with subdermal plexus flaps. These flaps are not dependent on a cutaneous pedicle, and island arterial flaps are often created, thus enhancing mobility.

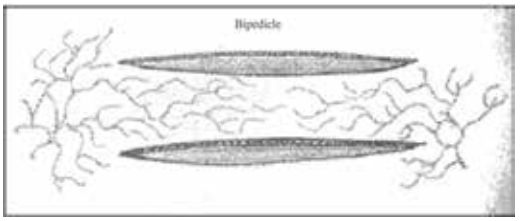


Distance/direction from donor site - Local flaps are elevated adjacent to the defect and are advanced or rotated into place. Distant flaps are almost always employed for closure of defects involving the limbs. Distant direct flaps (pouch flaps and hinge flaps) are transferred by elevating the affected limb to the donor site prepared on the lateral thorax or lower abdomen.

Attachment to donor site – A single pedicle flap has one cutaneous pedicle. A bipedicle flap has two cutaneous pedicles and allows a longer flap to be created, although is limited in mobility. Bipedicle advancement flaps can be used as tension-relieving technique, and in pouch flaps. An

island flap has no cutaneous attachment to the donor site, just a vascular pedicle for perfusion (e.g., island axial pattern flap).





Composition – Composite or compound flaps incorporate skin and other tissues such as muscle, fat, bone or cartilage. Composite flaps are used clinically in the dog, e.g., gracilis musculocutaneous flaps, labial advancement.

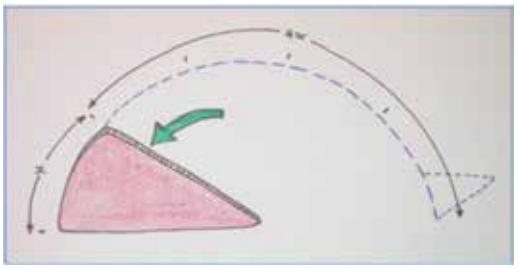
SUBDERMAL PLEXUS FLAPS

Local	Rotating	Rotation Transposition Interpolation
	Non-rotating	Single pedicle advancement H-plasty Bipedicle advancement flap
	Skin Fold Flaps	
Distant	Direct	Pouch (bipedicle) Hinge (single pedicle)
	Indirect	Tubes

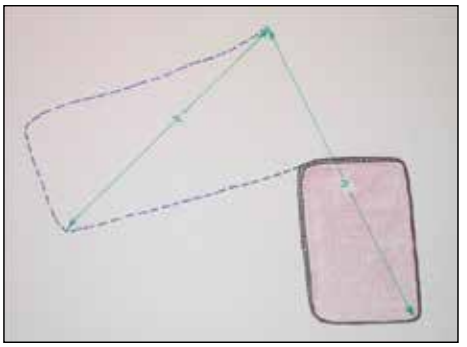
This table lists the subdermal plexus flaps that are most often used in veterinary teaching hospitals. These flaps are based on the random perfusion of the skin from the deep subdermal plexus, which arborizes through the middle and superficial plexi to all layers of the dermis and deeper epidermis. These flaps show consistent survival and are feasible to do with good instrumentation and surgical facilities in practice. These flaps provide a solid reconstructive repertoire for the competent surgeon.

Rotation Flap

A semi-circular flap of skin that rotates about a pivot point across a defect. Ideal for triangular skin defects. The incision should be long enough to create a flap that has no tension on it when sutured into place: at least four times the space through which the flap is to be moved. The flap can be created progressively, undermining until adequate skin is available to cover the defect. Avoid back cuts into the base of the flap. Can also use bilateral rotation flaps for square defects.



Rotation flap



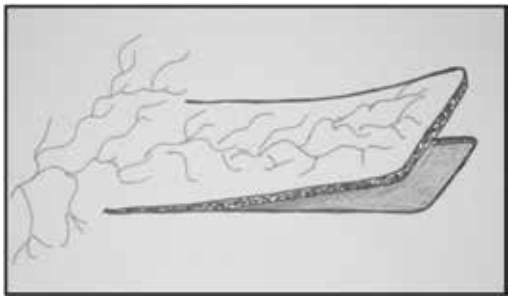
Transposition flap

Transposition Flap

A rectangular flap of skin that turns on a pivot point (less than 90^0) to cover an adjacent defect. The flap becomes shorter as it is transposed, so the flap must be developed longer than defect. Dog ears tend to develop as well. This flap worksbest when developed directly adjacent to the defect and is useful around the face and rump, but needs to be carefully planned and measured.

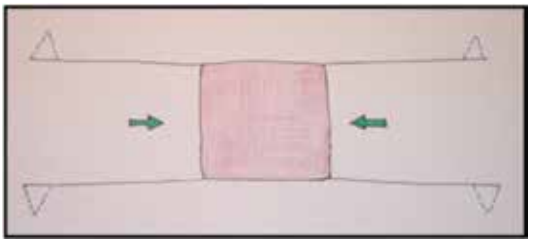
Single Pedicle Advancement Flap

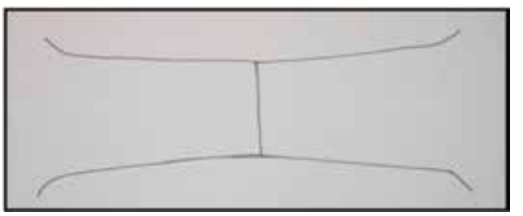
A flap of skin that is mobilised by undermining and then advanced into a defect, taking advantage of elasticity of skin. The flap should be at least as long as the defect - the longer the flap the less the tension on the suture line - but usually not more than 2x the width. Dog ears adjacent to the base of the flap can be excised after flap advancement and try to make the base wider to optimize blood supply to the leading edge of the flap. Large flaps may require a drain.



Single pedicle advancement flap

H-Plasty: An H-plasty is simply made up from two opposing single pedicle advancement flaps. This technique can be useful in chronic wounds where the adjacent skin is less elastic due to fibrosis.





Skin Fold Flaps

The elbow fold flap and flank fold flap utilise the folds of skin from the elbow to the trunk, and from the hind leg to the trunk. A fold can be developed into a flap

by retaining just one of the four possible pedicles: medial leg, lateral leg, dorsal trunk or ventral trunk. Once conceptualized, these flaps are robust and versatile, and can be used to reconstruct wounds on both trunk and proximal extremities. However, it does take some time to get familiar with how these flaps are used. We will go through several examples of using both the elbow fold and the flank fold to cover cutaneous defects on the trunk or proximal extremity.

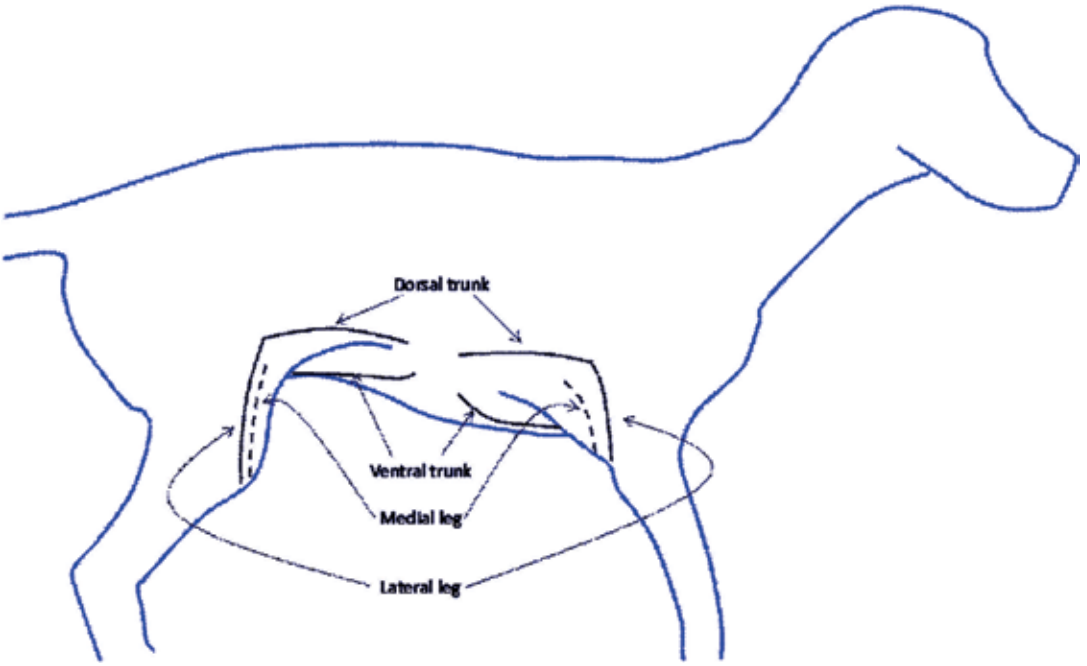


Diagram illustrating the pedicles (=bases) of the flank fold

DISTANT FLAPS

Distant flaps refer to the situation where skin from another part of the body (i.e., distant to the wound) is developed and used to cover the wound. Distant Direct flaps indicate that the wound is brought in contact to the donor site, and is utilized most commonly for distal extremities – forelimb, hind limb, ear pinna, etc. Distant Indirect Flaps consist of tubed pedicle flaps that are moved in several stages by 'caterpillaring', 'waltzing', or 'tumbling' towards the defect. Distant Indirect Flaps are not common in veterinary medicine today. There are two types of Distant Direct Flaps that we occasionally use today, and they are the pouch flap and the hinge flap:

Pouch Flap: is a double pedicle flap created on the body to reconstruct a defect on an extremity by temporarily moving the extremity to the flap. It is important to ensure that the animal will tolerate a limb bandaged to the body for 14 days; sometimes bandaging for 24 hours before the surgery is a good test. Note that in hind limb pouch flaps, the hair will grow in a reverse direction. For limb defects that are not full 360^{o}, the skin edges under the flap can be

sutured, or a drain may be placed. These flaps can benefit from delay procedures, i.e., the pedicles can be cut down in stages. Careful bandaging is required to ensure that the flap is not under tension from the leg, and movement is not excessive.

Hinge Flap: is similar to a pouch flap, but only involves one pedicle on the body, usually dorsal.

Length to width ratios for subdermal plexus (random) flaps: generally do not exceed 2:1 for single pedicle flap, or 4:1 for bipedicle flaps.

The Delay Phenomenon – augmenting flap survival.

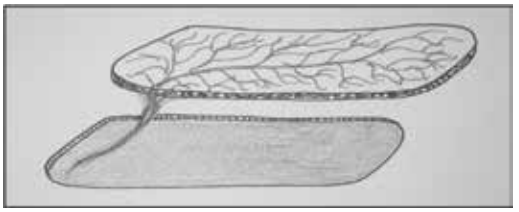
Large subdermal plexus flaps can be raised in several stages before transfer. This actually enhances perfusion of the flap by "training" the blood supply to go through the pedicle. These flaps are more likely to survive than are flaps transferred in one stage – and can also be made a little larger. Both subdermal plexus flaps and axial pattern flaps can be delayed in this fashion. This method of augmenting flap survival is called the "delay phenomenon". This is not as frequently performed now due to axial pattern flaps and myocutaneous flaps (but understanding the delay phenomenon may help in selecting drugs which mimic this physiological response, thus help in salvaging failing flaps).Both denervation and ischemia are the stimuli for effective delay, so flaps with adequate circulation do not benefit from this procedure. The mechanism is probably through sustained vasodilation, initially thru denervation, then by locally released neurohumoral substances. Angiographic and clearance studies have shown that delay appears to lead to improved circulation through the skin. Angiogenesis is not responsible for circulatory improvement.One week is too early to transfer a delayed flap. Some suggest 2 weeks, but 3 weeks is probably best. The pedicle can be divided by halves or thirds every 3 days.

AXIAL PATTERN FLAPS

Axial pattern flaps were initially described in the mid-1980s by MM Pavletic in veterinary medicine, and over the years they have slowly revolutionized reconstructive surgery in cats and dogs. Due to their unique blood supply, based on a single vascular pedicle, these flaps enable the surgeon to transfer skin areas of considerable dimensions in a single stage safely without the necessity of a delay procedure. These flaps are based on the premise that in certain anatomical areas on the skin of the dog or cat, there are direct perforating cutaneous arteries (with accompanying veins) that consistently perfuse a large area of skin (angiosome). Not only can that area of skin be developed in to a flap, but through the ability of small anastomotic connections (choke vessels), a certain amount of skin in the adjacent angiosome can also be used in flap development.

Furthermore, unlike subdermal plexus flaps, axial pattern flaps are not dependent on their cutaneous base for their blood supply. In fact, once the vascular pedicle

has been carefully identified and preserved, one can transect the cutaneous base and develop an island arterial flap:



These flaps are larger and have superior survival than subdermal plexus flaps of similar dimensions. They require a reasonable degree of surgical competency and experience before attempting. The commonest cutaneous arteries of the dog and cat used for axial pattern flap development are listed below, and some examples of these flaps to demonstrate their robust and consistent nature are detailed below:

1. Superficial temporal
2. Caudal auricular
3. Angularis oris
4. Superficial cervical branch of the omocervical
5. Superficial brachial artery
6. Thoracodorsal
7. Cranial superficial epigastric
8. Caudal superficial epigastric
9. Deep circumflex iliac artery
10. Genicular branch of the saphenous artery
11. Reverse saphenous conduit flap
12. Superficial lateral caudal

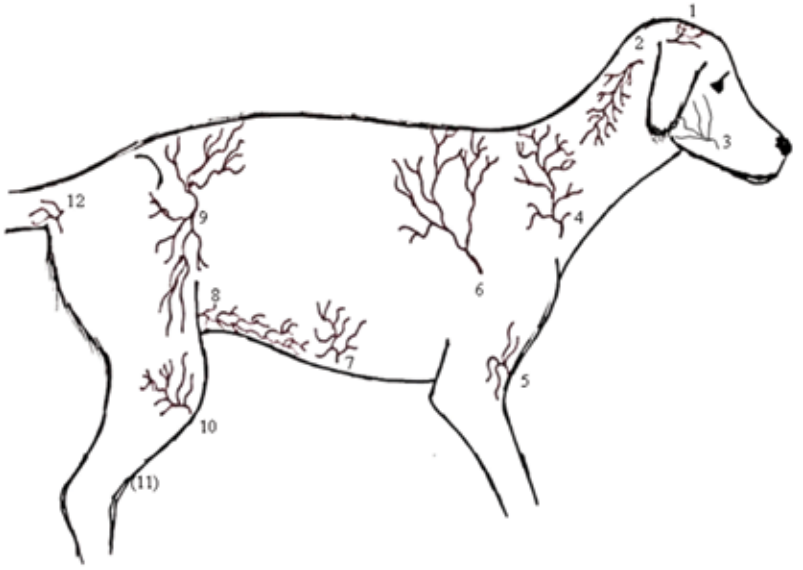


Diagram illustrating the major direct cutaneous arteries that are used in the development of axial pattern flaps. Numbers refer to the list above.

The most frequently used axial pattern flaps are probably: the caudal superficial epigastric, the thoracodorsal, the angularis oris and the deep circumflex iliac. The angularis oris is extremely beneficial for facial reconstruction.

Caudal Superficial Epigastric (CSE) Axial Pattern Flap development: These flaps are also robust and have an extremely anatomically consistent vascular pedicle – the caudal superficial epigastric artery (and vein) which arise from the external pudendal vessels as this pedicle exits the body through the inguinal canal. This is a long flap that will contain the caudal 3 or 4 mammary glands, depending on the size required. The medial landmark for this flap is the ventral midline; the lateral border is twice the distance from the midline to the nipple line. These medial and lateral incisions can be joined with a curved transverse incision cranially behind the first or second mammary gland. The flap is undermined down to the body wall and the vascular pedicle should always be identified. To island the flap, a transverse incision is made at the level of the cranial pubis. The CSE flap can be swung around to cover defects of medial and lateral thigh and stifle, caudal abdomen, prepuce and perineum. It is reliable and cosmetically acceptable in ovariectomized animals.

Deep Circumflex Iliac (DCI) Axial Pattern Flap development: This is a great flap that is developed from the DCI a&v, which perforates the body wall (directly off the aorta) just cranioventral to the wing of the ilium. The pedicle can provide tissue from its dorsal and/or ventral branch to cover rump defects, lateral thoracic defects, ventral abdomen and inguinal defects. In addition to a robust pedicle, it has excellent choke vessels to allow extension of the flap over the midline and well to the opposite side. The caudal border of the flap is about midway between the greater trochanter and the cranial wing of the ilium. The cranial border is the same distance as the caudal border to the vascular pedicle of the DCI a&v. This flap often requires repositioning of the animal during the procedure.

Thoracodorsal Axial Pattern Flap development: The cutaneous branch of the thoracodorsal artery and vein arise from the caudal shoulder depression. The cranial border is defined by the spine of the scapula, and the caudal border by a parallel line twice the distance from the cranial border to the shoulder depression. The dorsal border of the flap can be an L shape (hockey stick configuration), or straight (peninsular configuration), and typically extends no more than halfway down to the contralateral pedicle. Dissection should proceed deep to the cutaneous trunci muscle and the vascular pedicle should always be identified. Once elevated, this flap can be islanded or maintained with a cutaneous pedicle ventrad. The ventral border of the flap (if being islanded) is drawn at approximately the level of the shoulder joint. The thoracodorsal axial pattern flap can be used to cover large defects of forelimb (almost down to carpus), axilla, elbow, sternum, shoulder and lateral neck. Animals with large amounts of subcutaneous fat can experience partial flap necrosis distally.

Angularis Oris Facial Axial Pattern Flap development: This flap has only recently been described, but we have used it for a variety of successful reconstructive efforts

around the face, and it has largely replaced the use of the superficial temporal axial pattern flap. The flap extends caudally from the commissure of the lips, with the dorsal border being the zygomatic arch, and the ventral border in line with the mandible. The flap is recommended to be developed to a length, level with the vertical ear canal, but we have extended this flap (several times) to the wing of the atlas. Once developed, the flap can be rotated at the commissure of the lips to cover face and muzzle easily, even in dolichocephalic breeds. It can also be used in palate reconstruction.

General guidelines for Axial Pattern Flap development:

Read the landmarks for borders. Careful positioning is required to prevent distortion of anatomical landmarks and ensure inclusion of direct cutaneous artery. As such large flaps can be developed, the patient may require repositioning once flap is developed. Flaps can be rotated adjacent to donor site, taken to a distant site via a bridging incision or tubing of the flap. Closure of the defect can be performed with undermining and a secure two layer closure.

PLANNING RECONSTRUCTIVE SURGERY

The aim of the wound care practitioner and reconstructive surgeon is to cultivate the wound bed to a stage where it is free of infection, has a healthy blood supply and all vital structures (bone, joints, ligaments, tendons, nerves, implants, etc) are covered. A healthy granulation bed will satisfy these requirements, and provides an ideal surface for epithelialization. However, as we have discussed, there are times when the wound should be closed, either by direct apposition or skin flap. This can be employed at any point along the continuum of wound management. Although the decision to close a wound can be based upon many factors, the timing of that closure will depend largely on the degree of contamination of the wound, and the status of the periwound tissue.

- Consider owner commitment and likelihood of compliance
- Consider the patient's ability to tolerate the procedure.
- Consider host factors, e.g., geriatric, renal, hepatic disease, endocrinopathies, etc.
- Manage the wound open until it is ready to close, e.g., healthy wound and periwound area.
- Consider all alternatives for wound closure.
- Consider staging the reconstruction.
- Consider partial closure.
- Consider potential stresses (tension, movement) on the area, and the activity level of the patient.
- Know the vascular supply to the area.
- Imagine it, play with the periwound, manipulate the wound edges → develop a flexible plan.

PRINCIPLES OF FLAP DEVELOPMENT

- Clip the periwound skin generously, then clip more. Free drape the leg if it close to the reconstruction.

- Manipulate the wound edges again, confirm your plan and have an alternative plan.
- Anticipate location of drains.
- Consider donor site closure.
- Always use a sterile marking pen.
- Undermine beneath the panniculus layer, preserving any direct cutaneous vessels.
- Be meticulous about hemostasis, and utilize an atraumatic technique.
- Avoid backcuts into flap.
- Minimal tension – approximate edges and "negotiate" the flap, spreading tension evenly.
- Generally perform a two layer closure: subcutaneous layer, skin.

Full Length Article

Evaluation of nutrient composition and phenolic compound of pomegranate fruit waste as a novel feed for dairy cattle

Patil Saroja Popat[1], A.Bharathidhasan, R. Karunakaran, K. Vijayarani and P. Tensingh Gnanaraj

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ABSTRACT

The pomegranate (*Punica granatum*) belongs to the *Punicaceae* family and is a nutrient dense food source rich in phytochemical compounds.About 50 % of the total fruit weight corresponds to the peel, which is an important source of bio active compounds such as phenolics, flavonoids, ellagitannins (ETs) and proanthocyanidin compounds which can be used as an alternative feed source for dairy cattle. Hence, the present study was carried out to evaluate the nutrient composition and polyphenolic compound of pomegranate fruit waste (PFW) for dairy cattle. Six samples of PFW were collected from six different places in India and screened for their nutritional composition and polyphenolic compound. The results showed that pomegranate fruit waste contained 12.72 % DM, 4.80 % CP, 12.26 % CF, 3.43 % TA, 1.47 % EE, 65.3 % NFE, 28.21 % NDF, 23.30% ADF, 4.91 % Hemicellulose, 13.79 % Cellulose and 5.14 % Lignin. Polyphenolic compound of PFW were 2.57 % total phenolic compound, 2.43 % total tannin content,0.13 % non-tannin phenolics, 1.75 % condensed tannin, 0.69 % hydrolysable tannin and 2.05% total flavonoid content.Thus, it is concluded that PFW is of high nutritive value which can be used for feeding to dairy cattle.

Key words : Pomegranate fruit waste, Nutrient composition, Polyphenolic compound.

INTRODUCTION

The pomegranate (*Punica granatum*) belongs to the *Punicaceae* family and is a nutrient dense food source rich in phytochemical compounds. Pomegranate fruit waste is a by-product of the pomegranate juice industry. PFW can be used as a relatively good agro-industrial by-product for ruminant nutrition (Mirzaei Aghsaghali *et al.,* 2011). Pomegranate fruit consists of seeds, aerials and peel which include the husk and interior network membranes. Pomegranate Fruit Waste (PFW) contains protein, carbohydrates, lipids, vitamins, minerals and water. India is one of the largest producers of pomegranate in the world. The annual production of pomegranate is about 13.46 lakh tonnes in India (NRCP, 2015). About 50 % of the total

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fruit weight corresponds to the peel, which is an important source of bio active compounds such as phenolics, flavonoids, ellagitannins (ETs) and proanthocyanidin compounds. Climatic condition and water scarcity had escalated the cost of animal feeds. Use of agricultural by-products obtained after processing of fruits such as pomegranate pulp by-product is one of the useful way of overcoming this problem. In the last few years there is an increasing interest of nutritionists in bio-active plant factors like phytochemicals as natural feed additives. Tannins are the ones that can modify the rumen fermentation processes, improve the protein metabolism, reduce ammonia production and curb methane emission to the atmosphere. In a livestock production system, pomegranate fruit waste is found to be appropriate for use in animal feeds due to the presence of various micronutrients. The addition of pomegranate fruit waste extraction using either water or solvent mixture positively altered some rumen parameters such as NH_3–N concentration, acetate-to-propionate ratio and protozoan growth; increased microbial protein and also improved the yield and quality of milk (Abarghuei *et al*., 2013). Hence, the present study was carried out to evaluate the nutrient composition and phenolic compound of pomegranate fruit waste for feeding dairy cattle.

MATERIALS AND METHODS

Six samples of PFW were collected from six different places in India viz. Chennai, Solapur, Sangli, Kancheepuram, Thanjavur and Tiruchirapalli. The PFW sample were dried in hot air oven at a temperature of 55-65°C to constant weight and ground to pass through 1mm sieve and stored in airtight containers for further analysis. Dry matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the samples in muffle furnace at 525°C for 8 hours and Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude fiber is determined by acid digestion and alkali digestion of pomegranate fruit waste. Crude protein (CP) was calculated as N × 6.25. Moisture, crude protein, ether extract, total ash (TA) and crude fiber (CF) contents of pomegranate fruit waste were determined according to the procedures of the AOAC (2000). The fibre fraction of pomegranate fruit waste was determined according to procedure of Van Soestand Robertson(1976).

For estimation of polyphenolic compound, the PFW extracts were prepared and estimated as per the method explained by Makkar *et al*. (1993).The measurement of total phenolic (TPs) content was conducted according to the modified Folin–Ciocalteu colorimetric method. Total phenolics were estimated by adding 0.1 ml of PFW extract with the following *viz.* 0.9 ml of distilled water, 0.5 ml of 1N Folin Ciocalteu reagent and 2.5 ml of 20 % sodium carbonate solution. The contents were mixed and incubated for 40 minutes at room temperature. The optical density was measured at 725 nm in a UV spectrophotometer. The concentration was calculated with tannic acid equivalent standard curve.The standard curve was drawn from different concentrations of standard tannic acid solution (0.1mg/ml)

ranging from 0 to 0.3 ml by adopting the same procedure as that for plant extracts.

For non-tannin phenolics estimation, 100 mg of poly vinyl poly pyrrolidone was taken in test tube and to it one ml of distilled water along with 1 ml of PFW extract was added. Then it was centrifuged at 3000 rpm for 10 minutes and the supernatant was collected. The supernatant contained non tannin phenolics. Total tannin phenolics were calculated by subtracting non tannin phenolics from the total phenolics.

The condensed tannin present in the PFW extracts were estimated as per the method of Porter *et al*. (1986) where in 0.5 ml of PFW extract was taken in test tube and to it was added 3ml of butanolHCl and 0.1 ml of ferric reagent. The test tubes were vortex mixed to ensure proper mixing. The mouth of the tubes was covered with glass marble and contents boiled for 60 minutes. The tubes were cooled to room temperature and optical density was read at 550 nm using spectrophotometer. Condensed tannin as leucocyanidine equivalent was calculated by using the following formula.

$$\text{Percentage of condensed tannin} = \frac{\text{Absorbance at 550 nm x 78.26 x dilution factor}}{\text{Percentage of dry matter}}$$

The hydrolysable tannin was calculated by subtracting condensed tannins from total tannins (Singh *et al*., 2005).

Total flavonoid content was measured by the Aluminum chloride ($AlCl_3$) colorimetric assay (Jia *et al*., 1999). An aliquot of 1 ml PFW extract was added to 10 ml volumetric flask containing 4 ml of double distilled water. Then 0.3 ml of 5 % $NaNO_2$was added to the flask and after 5 minutes, 0.3 ml $AlCl_3$(10 %) was also added. At 6^{th} minute, 2 ml NaOH (1 M) was added and the total volume was made up to 10 ml with double distilled water. The solution was mixed completely and the absorbance level was measured at 510 nm against reagent blank. Six different concentrations of quercetin solutions (20–100 mg/l) were prepared and used for calibration of standard curve.All data were statistically analysed as per the Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The results of proximate composition of PFW showed that 12.72% DM, 4.80 % CP, 12.26 % CF, 3.43% TA, 1.47 % EE, 65.3 % NFE, 28.21 % NDF, 23.30 % ADF, 4.91 % Hemicellulose,13.79 % Cellulose and 5.14 % Lignin. These results were in conformity to the findings of Mirzaei-Aghsaghali*et al.*(2011),who reported the DM, CP, EE, NDF, ADF and NFC content of pomegranate were 13.01, 3.60, 0.61, 20.80, 15.10 and 69.57 %, respectively. Further the present result of chemical composition was consistent with earlier report by Kushwaha*et al.* (2013) and Rowayshed*et al.*(2013). The proximate composition of PFW did not vary much with that analysed in present study as well as earlier reports. Hence pomegranate fruit waste can be used as an alternative resource for ruminants.

Further the polyphenolic content of PFW were 2.57 % total phenolic compound, 2.43 % total tannin content, 0.13 % non-tannin phenolics, 1.75 % condensed tannin, 0.69 % hydrolysable tannin and 2.05 % total flavonoid content.The present findings of polyphenolic compound of PFW were almost consistent with earlier reports by Yunfeng and Changjiang (2005). Also the total phenolic compound of PFW were also similar with those found by Kushwaha*et al.* (2013). However,Besharati (2015) reported the total phenolic (1.8 %) and total tannin (0.8 %) content of pomegranate waste which were considerables lower than the present study.

In a livestock production system, pomegranate fruit waste found to be appropriate for use in animal feeds due to the presence of various micronutrients. Addition of supplements such as ionophores, antibiotics, defaunating agents and methane inhibitors to ruminants improve their fermentation, which subsequently improves the milk and meat efficiency (Abarghuei *et al*., 2013). Dkhil (2013) demonstrated that the methanolic extract of Pomegranate peel (PP) has natural antioxidant capacity and it also has anthelmintic activity. Because of its high nutritional value and antioxidant capacity, PP is a health-promoting food source for animals, especially ruminants. Shabtay *et al.* (2008) demonstrated that dietary supplementation of pomegranate peels promoted increase in feed intake with a tendency to increase weight gain in bull calves.

Ruminants, have the unique capacity to utilize fibre, because of the presence of rumen microbes. This means that cereals can largely be replaced by pomegranate by-products (Mirzaei-Aghsaghali*et al*., 2011). Shabtay*et al.* (2008) concluded that different pomegranate components may have different nutritive effects and influence milk production in different ways. Therefore, based on present study it is concluded that pomegranate fruit waste is nutrient rich feed source which can be used for feeding to dairy cattle to increase the production and performance of animal.

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Prevalence of extended spectrum beta-lactamase-producing *Escherichia coli* in chicken meat

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ABSTRACT

Extended spectrum β-lactamase (ESBL) producing *Escherichia coli* (*E. coli*) have emerged as a global threat to both human and veterinary medicine and the prevalence has increased throughout the world. The objective of the present study was to determine the prevalence of ESBL-producing *E. coli* in chicken meat collected from retail shops in Chennai, India. Around thirty one *E. coli* strains were isolated and screened for ESBL production by double disc diffusion method Further, ESBL-positive isolates were analyzed for the presence of CTX-M, TEM, and SHV genes. Sixteen *E. coli* isolates (51.6%) were found to be ESBL positive in the initial screening. All the 16 isolates were found to harbour one or more ESBL genes and 10 isolates (62.5 %) were found to harbour all the three genes. The results of this study clearly indicated the emergence of ESBL-producing *E. coli* in chicken and the need to curb the nontherapeutic usage of antimicrobials in poultry production.

Key Words: *Escherichia coli* – Poultry – ESBL – Beta-lactamase - Resistance

INTRODUCTION

Antibiotics of the β-lactam group including penicillins, cepahlosporins, monobactams and carbapenems are used commonly in livestock and poultry owing to their high efficacy and broad spectrum of activity. Due to continuous use of these antibiotics in veterinary practice, resistance has been reported (Kong *et al,* 2010). The most common mechanism by which the microorganisms such as *Escherchia coli* acquire resistance to these β -lactam antibiotics is by the production of β-lactamase.

Development of oxyimino cephalosporins (third generation cephalosporins) in early 1980s was a major breakthrough to combat β-lactamase-mediated bacterial resistance (Paterson and Bonomo, 2005). However, resistance to these cephalosporins emerged soon in *Klebsiella pneumoniae*, *K. ozaenae* and *Serratia marcescens* and it was first reported in 1983 (Rawat and Nair, 2010). The resistance is due to the production of enzymes by microorganisms which are evolved as mutations outside the active site of β-lactamase and they are referred to as extended-spectrum β-lactamase (ESBL) because of their increased spectrum of activity, especially against oxyimino

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cephalosporins. ESBLs can hydrolyze and mediate resistance to all β-lactam antibiotics including penicillins, cephalosporins and monobactams (e.g., aztreonam) but do not affect cephamycins or carbapenems and they are inhibited by β-lactamase inhibitors like clavulanic acid (Rawat and Nair, 2010). Production of ESBL is one of the most common methods of acquiring resistance to third and fourth generation cephalosporins among Enterobacteriaceae such as *E. coli* and *Klebsiella*. Chicken and other animal produce are frequently incriminated in the spread of resistance to human pathogens. However, there is paucity of information regarding the prevalence of ESBL-producing *E. coli* in chicken meat in India. Hence, the present work was undertaken with the aim to study the prevalence of ESBL–producing *E. coli* in chicken meat collected from retail outlets of Chennai.

MATERIALS AND METHODS

Sample Collection

A total of 105 chicken meat samples were collected from the retail outlets in Chennai, Tamilnadu, India from May 2016 to July 2016. About 5 grams of single meat samples were collected from each bird following Food Safety and Standards Authority of India guidelines. Fresh meat samples were collected immediately after slaughter in sterile plastic envelopes. They were stored at 4° C until processed.

Isolation and Identification of *E. coli*

One gram of minced meat samples was inoculated into 9 ml of sterile nutrient broth and incubated overnight at 37° C. The nutrient broth samples which showed growth as indicated by the turbidity of the growth medium were then inoculated into MacConkey agar plates and Eosin Methylene Blue agar plates for selective isolation of *E. coli*. The isolated strains were confirmed by the following biochemical tests: Oxidase, TSI, Urease, Motility, Catalase, Indole, Simmons Citrate, Methyl Red and Voges Proskauer (Thanigaivel and Anandhan, 2015).

Phenotypic Screening for ESBL

Isolated organisms were screened for extended-spectrum β-lactamase production by double disc diffusion method as per CLSI guidelines (CLSI, 2016). For this purpose, following four antibiotic disc were used: cefotaxime (30 µg), ceftazidime (30 µg), cefotaxime-clavulanic acid (30/10 µg) and ceftazidime-clavulanic acid (30 µg/10 µg). Bacterial inoculums were prepared from single colonies and the turbidity of the inoculum was adjusted to the 0.5 McFarland standard. Using a sterile cotton swab, the organisms were inoculated into Mueller Hinton agar plates by lawn culture. Using a sterile forceps, antibiotic discs were placed on Mueller Hinton agar plates inoculated with the *E. coli* isolates organism. Plates were incubated at 37°C for 18 h. After incubation, the zone of inhibition around disc was measured. The zone of cephalosporin and the respective cephalosporin/clavulanic acid combination were compared and a difference of ≥ 5 mm was considered as positive for phenotypic confirmatory test for ESBL production.

Detection of ESBL genes by PCR

The *E. coli* isolates were inoculated into 5 ml nutrient broth and incubated

at 37°C overnight. After overnight incubation, bacterial cells were harvested by centrifugation at 5000 x g for 10 min. The supernatant was discarded and cell pellets were used for DNA extraction. DNA was extracted and purified using Qiagen DNA extraction kit as recommended by the manufacturer. The primers used for bla TEM were 5' AAAATTCTTGAAGACG 3' and 3' TTACCAATGCTTAATCA 5'; bla SHV were 5' GGGTTATTCTTATTTGTCGCT 3' and 3' TAGCGTTGCCAGTGCTCG 5' and for bla CTX-M were 5'TTTGCGATGTGCAGTACCAGTAA3' and 3'CGATATCGTTGGTGGTGCCATA 5'. The amplicon size of bla TEM, bla SHV and bla CTX-M were 1080 bp, 929 bp and 544 bp, respectively (Bora *et al*., 2014).

Amplification of DNA was performed in a final volume of 25 µl PCR mixtures containing 12.5 µl Master mix, 1 µl forward primer, 1 µl reverse primer, 3 µl template DNA, 7.5 µl nuclease-free water with cycling parameters comprising initial denaturation at 94°C for 3 min followed by 35 cycles each of denaturation at 94°C for 30s, annealing at 54°C for 30s, amplification at 72°C for 30 s and final extension at 72°C for 10 min, for the amplification of bla TEM. For bla SHV and bla CTX-M, amplifications conditions for thermal cycling remained the same except for the annealing temperature of 53°C.

Ten µl of the amplified PCR product was electrophoresed on a 1.5% (CTX) and 0.8 % (TEM and SHV) agarose gel in 1X Tris-acetic acid-EDTA buffer (TAE) containing ethidium bromide (0.5 µg/ml) and stained DNA bands are visualized using gel documentation unit. The PCR amplification for bla CTX, bla SHV and bla TEM genes were shown in Fig. 2 and the product sizes were of 544, 929, 1080 bp, respectively.

RESULTS AND DISCUSSION

Thirty one isolates of *E. coli* were recovered from the chicken meat samples and were screened for ESBL production by double disc diffusion method . Of the total *E. coli* isolates, 16 *E. coli* isolates (51.6%) were ESBL producers and the overall prevalence of ESBL-producing *E. coli* in chicken meat was 15.2% and it was comparatively less than the other reports. Hussain *et al* (2017) reported a prevalence rate of 46% in chicken meat collected from retail chain outlets in Karnataka, Telangana, Andhra Pradesh and Maharastra States of India in 2015.

In order to determine the presence of ESBL genes, DNA were extracted from all the 16 isolates and analyzed by PCR (Fig 1.). All the isolates showed presence of one or more ESBL genes. Nearly 93.75% (15 isolates) of the isolates harboured CTX-M genes and 68.75% and 87.5% of the isolates were found to harbour other ESBL genes, TEM and SHV, respectively. More than one resistance gene belonging to different ESBL families was found in 87.5% of isolates (14 isolates) and 62.5% (10 isolates) of the isolates were found to harbour all the three ESBL genes. The most prevalent combination of ESBL genes were CTX/SHV (75%) followed by TEM/SHV (68.75%) and CTX/TEM (62.5%).

In our study, the prevalence of ESBL (15.2%) in chicken meat was lower

compared to reports from other countries. Overdevest *et al* (2011) reported that 79.8% of retail chicken meat samples in Netherlands contained ESBL genes. Egea *et al* (2012) reported high prevalence of ESBL genes (93.3%) in chicken meat in Spain. In France, higher prevalence of 91.7% ESBL-positive isolates (44 samples positive out of 48 samples) in chicken meat samples were reported in 2016 (Casella *et al*., 2017).

There were only few reports on the prevalence of ESBL-producing *E. coli* in chicken meat in India. Kar *et al* (2015) conducted study on prevalence of ESBL-positive *E.coli* from food producing animals from India. They have isolated 252 *E. coli* isolates from poultry faecal samples and in which 16 isolates were reported as ESBL-producing *E. coli* (6.3%). In a study conducted in Hyderabad, 6.7% of *E. coli* isolates from raw chicken were ESBL producers (Rasheed *et al.,* 2014). Brower *et al* (2017) conducted a study on occurrence of ESBL-producing *E. coli* isolates from cloacal samples collected from poultry farms in Punjab, India. They have reported a higher prevalence of ESBL-producing *E. coli* (87%) in broiler farms.

The findings of our study clearly indicated the emergence of ESBL-producing *E. coli* in chicken. Though the incidence of ESBL-producing *E. coli* was comparatively lesser, it is still a matter of public health concern. The ESBL-producing strains are resistant to all penicillins, cephalosporins and aztreonam and can exhibit cross-resistance to trimethoprim / sulfamethoxazole and quinolones (Lenart-Boron, 2017). These genes are plasmid coded and are easily transferable to other bacterial strains. They can also be transferred to the human through food chain and might become potential health risk. Emergence of resistance to third generation cephalosporins is considered to be most serious since this group of antibiotics are categorized by WHO as highest priority critically important antibiotics currently used to treat serious infections in human medicine (WHO, 2017).

The most important factor responsible for the emergence of drug resistant microbes is the increased and indiscriminate use of antimicrobial agents. Hence, there is a need to emphasize the rational use of antibiotics and to strictly restrict the non-therapeutic and inadvertent use of "reserve antimicrobials" in food producing animals to preserve the effectiveness of the drug in the treatment of multidrug resistant infections. In addition, regular surveillance studies covering large geographical area have to be done to assess the real status of ESBL-producing *E. coli* in India.

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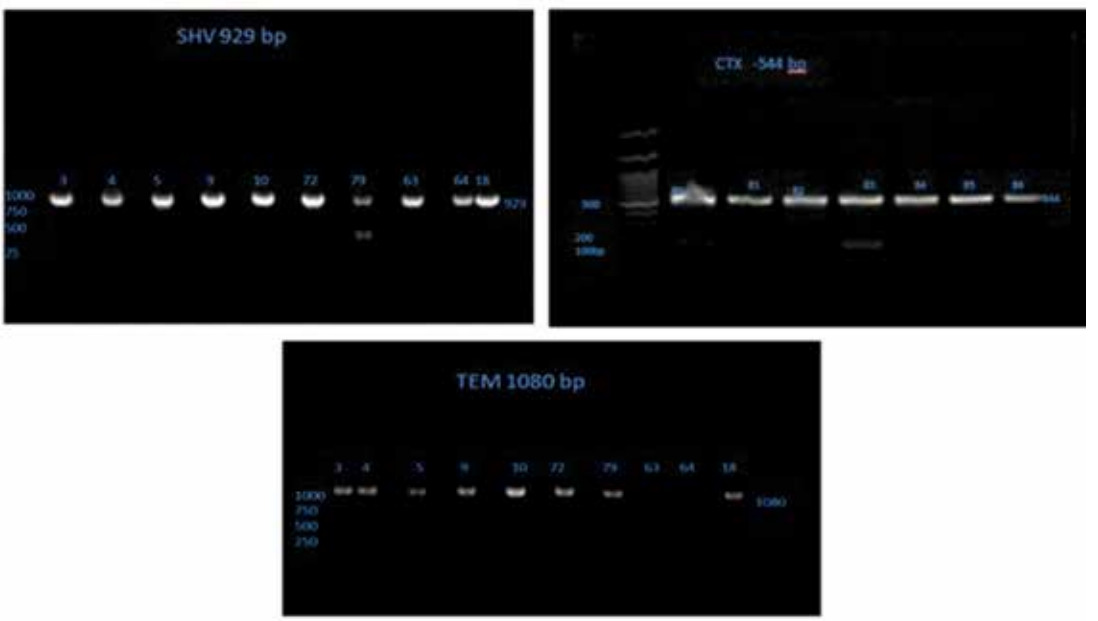
Fig. 1 Gel pictures of amplified PCR products of SHV gene (929 bps), CTX-M gene (544 bps) and TEM gene (1080 bps)



Prevalence of Ovine theileriosis in Northern Karnataka

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ABSTRACT

An epidemiological survey on ovine theileriosis was conducted in six districts of northern Karnataka, India. On examination of 525 (209 clinical and 316 healthy/tick infested) Giemsa stained thin blood smears of sheep, 107 (51.20%) and 129 (40.82%) sheep smear samples from clinical and healthy/tick infested flocks were found positive for *Theileria* organisms respectively, with an overall prevalence of 44.95% (236/525). The parasitemia ranged between 0.8 to 1.2 and 0.2 to 0.4 per cent in clinical and apparently healthy/tick infested sheep, respectively. The haemogram of *Theileria* infected sheep revealed severe anaemia with haemoglobin levels ranging between 2.3 to 4.6 g / dl. However, no significant ($P< 0.05$) difference was found between the age, gender, breed and district wise prevalence. The present study indicated that ovine theileriosis is an endemic disease in this study area and aids in understanding and implementation of measures to control ovine theileriosis in northern Karnataka.

Key Words: Stained blood smear, Prevalence, Theileriosis, Sheep, North Karnataka.

INTRODUCTION

Small ruminants especially sheep contribute to the livelihood of the rural population in most of the developing countries. As per 19th Livestock Census (2012), India accounts for 65.06 million sheep. Karnataka stands second position in sheep population with 9.58 million (Shiva Kumara *et al*., 2017). The global loss due to ticks and tick borne diseases (TTBDs) was estimated to be between US$ 13.9 and 18.7 billion annually (de Castro, 1997), whereas in India, the estimated loss due to tick borne disease was around US$ 498.7 million per annum (Minjauw and Mcleod, 2003).

Ovine theileriosis is an important hemoprotozoan disease of sheep in tropical and subtropical regions (Altay *et al*., 2007) that leads to economic losses in these animals. The disease is also important due

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to its significance in the international trade of animals and animal products (Uilenberg, 2001). In India *T. hirci, T. ovis* and *T. luwenshuni* are the most prevalent species reported in small ruminants (Sisodia, 1981; Kaufmann, 1996; Mamatha *et al*., 2017).

Diagnosis of theileriosis is based on the clinical findings and microscopic observation (MO) of thin blood smears stained with Giemsa in acute cases (Aktas *et al*., 2005). In the present study, an epidemiologic survey of *Theileria* infection in sheep in six districts of northern part of Karnataka by blood smear study was undertaken.

MATERIALS AND METHODS

In the present study, a total of 525 (209 clinical and 316 healthy/tick infested) sheep blood samples were collected in EDTA vaccutainers from both apparently healthy/ tick infested sheep and clinically suspected (pyrexia 105–107°F, tick infestation, inappetance, weakness and anaemia) sheep from flocks located in six districts covering five different agro climatic zones of northern part of Karnataka during the period from August 2017 to October 2018 (Table 1). Thin blood smears were prepared immediately after the blood collection, air-dried, fixed in methanol for one minute and stained with 10 per cent Giemsa staining solution (1:10) for 20 minutes and subsequently examined microscopically under oil immersion. The *Theileria* organisms were identified as per Yin *et al.* (2007), Lefevre *et al.* (2010) and Soulsby (2012). Parasitemia was assessed by counting the number of infected red blood cells by examination of 200 microscopic fields (approximately 1,00,000 RBCs) (Jalali *et al.,* 2014) and expressed as percentage. Haemoglobin level was determined in an automated haematology analyzer (Erba, Germany). Age, gender, breed and district wise prevalence of theileriosis in sheep was recorded and statistical analysis of data were carried out by Chi-square test using graph pad prism software, version 5.01.

Table1: Details of blood samples collected from sheep from different Agro climatic Zones of Northern Karnataka

Sl. no.	Districts	Agro climatic zones	No. of samples collected	Latitude	Longitude	Elevation (m)	Average annual temperature (C^0)	Average annual precipitation (mm)	Mean relative humidity (%)
1	Yadgir	North eastern dry zone	45	16.76 N	77.14 E	389	27.8	711	16 to >99
2	Kalaburgi	North eastern transition zone, North eastern dry zone	105	17.40 N	76.64 E	454	27.2	777	19 to >99
3	Raichur	Northern dry zone, North eastern dry zone	75	16.21 N	77.34 E	407	27.7	713	21.5 to >90
4	Belagavi	Northern dry zone, Northern transition zone and Hilly zone	150	15.84 N	74.51 E	753	24.2	1200	18.3 to 86.2
5	Vijayapur	Northern dry zone	75	16.82 N	75.72 E	601	26.6	553	17.7 to >99
6	Bagalkot	Northern dry zone	75	16.17 N	75.65 E	537	25.8	683	17.3 to >99

RESULTS AND DISCUSSION

On examination of 525 blood smears 107 (51.20%) and 129 (40.82%) sheep samples from clinical and healthy/tick infested flocks were found positive for *Theileria* organisms respectively, with an overall prevalence of 44.95 (236/525) per cent (Table 2). The highest rate of infection was observed in Raichur (58.66%) followed by Yadgir (48.88%), Kalburgi (46.66%), Vijayapur (44%), Bagalkot (40%) and Belagavi (38.66%). Based on statistical analysis, there was no significant difference between different districts in sheep ($P< 0.05$) (Table 2).

Table 2: Prevalence of theileriosis in sheep from six districts of north Karnataka

Sl. No.	Districts	No. of samples collected			No. positive (% positive)		
		Clinical cases	Healthy/Tick infested flocks	Total	Clinical cases	Healthy/Tick infested flocks	Total
1	Yadagir	15	30	45	9 (60)	13 (43.33)	22 (48.88)
2	Kalaburgi	50	55	105	28 (56)	21 (38.18)	49 (46.66)
3	Raichur	30	45	75	17 (56.66)	27 (60)	44 (58.66)
4	Belagavi	48	102	150	23 (47.92)	35 (34.31)	58 (38.66)
5	Vijayapur	31	44	75	14 (45.16)	19 (43.18)	33 (44)
6	Bagalkot	35	40	75	16 (45.71)	14 (35)	30 (40)
Total		**209**	**316**	**525**	**107 (51.20)**	**129 (40.82)**	**236 (44.95)**

The statistical difference between district wise prevalence in sheep was found to be non significant ($p<0.05$).

In the present study, the highest prevalence of *Theileria* spp., (44.95%) were recorded in all six districts of north Karnataka, however 56.1 per cent (799/1424) infection was reported in sheep from Mandya district from six sheep farms (Prabhakar and Hiregoudar, 1977); 69.7 and 46 per cent in sheep from Iran (Jalali *et al.,* 2014; Yaghfoori *et al.,* 2013); 58.59% in small ruminants from China (Li *et al.,* 2014) and these findings are in contrast to the lower prevalence rates reported elsewhere viz., 19.35% of prevalence was recorded in sheep from Turkey (Altay *et al.,* 2005); 11.9, 18.6 and 22.27% in sheep from Iran (Razmi *et al.,* 2006; Razmi and Yaghfoori, 2013; Bami *et al.,* 2009). The differences in the prevalence of theileriosis in different geographical areas could be due to varied climatic conditions that affect both intensity of tick infestation and parasitemia ratio and severity of disease (Zaeemi *et al.,* 2011).

The climatic conditions that affect the intensity of ticks that feed on hosts are effective on parasitemia ratio and severity of disease. In this study, dry zones (Yadgir, Kalaburgi, Raichur, Vijayapur and Bagalkot) with mean annual temperature (25.8 to 27.8 C^0) and latitude (16.17 to 17.40 N) have relatively suitable conditions for development of ixodid ticks that lead to high prevalence of theileriosis in sheep (Table 1) rather than cold regions such as Belagavi with mean annual temperature (24.2 C^0) and latitude (15.84 N). These findings were in accordance with the findings of Zaeemi *et al.* (2011). The variability in the prevalence

among districts was due to close relationship between incidence of theileriosis, activity period and differences in climate which probably had an effect on distribution of vector especially hard ticks.

Table 3: Age, gender and breed wise prevalence of theileriosis in sheep

Age (months to years)	Total No. of animals examined	Total No. of animals positive	Per cent Positive
6 months to 1 year	104	44	42.30
1 year to 3.5 years	421	195	46.31
Total	525	239	45.52
Gender			
Male	228	95	41.66
Female	297	144	48.48
Total	525	239	45.52
Breed			
Kenguri	124	58	46.77
Shahapuri	64	25	39.06
Deccani	125	59	47.2
Bellary	83	43	51.80
Non-descript (ND)	129	54	41.86
Total	525	239	45.52

The statistical difference between age, gender and breed wise prevalence in sheep was found to be non significant ($p<0.05$).

The *Theileria* organisms observed in the red blood cells of sheep during this study were highly pleomorphic. The nail forms (28%) were predominant followed by rod forms (16%), comma forms (14%), round forms (11%), dot forms (10%), parachute forms (8%) and other forms (13%) (Plate 1). Different forms of *Theileria* organisms observed in the present study were also reported in sheep and goats from China (Guo *et al.,* 2002; Yin *et al.,* 2007); sheep from Turkey (Aktas *et al.,* 2005); sheep from Iran (Razmi *et al.,* 2006).

A higher prevalence of theileriosis was noticed in the age group of one to 3.5 years of age (46.31%) followed by six months to one year of age (42.30%); Females were showing more positive (48.48%) when compared to males (41.66%) and Bellary sheep breeds were showing more positive percentage (51.80%) followed by other breeds (Table 3). However, no significant ($p< 0.05$) difference was found between age, gender, breed and district wise prevalence.

During this study, symptoms like high fever (105–107 °F), inappetance, weakness and anaemia with heavy tick infestation were noticed in clinically affected sheep.

The level of parasitemia in the present study ranged between 0.8 to 1.2 and 0.2 to 0.4 per cent in clinical and apparently healthy/tick infested animals respectively, which were in contrast to the lower parasitemia levels reported elsewhere viz., parasitemia of 0.01 to 0.1 and 0.00001%

recorded in sheep from Turkey (Aktas *et al.,* 2005; Altay *et al.,* 2005). This could be attributed to the stage of the disease at which the blood smears were made because, high parasitemia will be seen in acute/ clinical stage whereas, low parasitemia is a characteristic feature of carrier or chronic stage of the disease (Yin *et al.,* 2008).

The haemogram of *Theileria* infected animals revealed severe anaemia with haemoglobin levels ranging between 2.3 to 4.6 g / dl. The change in the haematological values could probably be due to development of intravascular hemolysis or destruction of red cell by intra-erythrocytic stages of *Theileria* spp. (Barnett, 1978).

The present study indicated that ovine theileriosis is an endemic disease in six districts of northern part of Karnataka.

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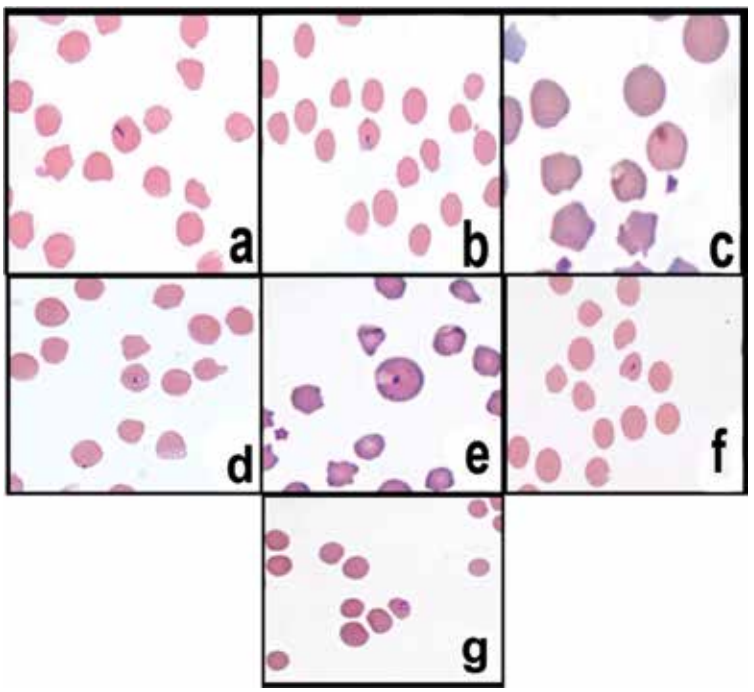

Plate 1: Different morphological forms of *Theileria* organisms in Giemsa stained blood smears

a: Rod form b: Nail form c: Dot form d: Round form

e: Parachute form f: Comma form g : Other forms

Estrus response and conception rate in non-descript goats synchronized with TRIU C and $PGF_2\alpha$

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ABSTRACT

In the present study thirty parous, healthy does were selected from Goat Breeding unit, Post Graduate Research Institute in Animal Sciences, Kattupakkam, Kanchipuram district, Tamil Nadu and randomly divided into three groups of ten each. Group I does were treated with 125 microgram of $PGF_{2\alpha}$ intramuscularly and Group II does were kept with intravaginal progesterone device TRIU C for seven days and an intramuscular injection of 50 microgram of $PGF_{2\alpha}$ on the sixth day of treatment and ten does served as control. The mean ± SE estrus onset interval in control and treatment groups were 207±55.35, 55 ± 0.50 and 36.40 ± 0.40 hours respectively and significant difference was observed between treatment groups. The mean ± SE duration of estrus and estrus response in the groups were non- significant. The present study concluded that the use of progesterone device (TRIU C and $PGF_{2\alpha}$) enhanced the estrus response, shortened the duration for onset of estrus, high estrus intensity score and well pronounced estrus behavior as compared to $PGF_2\alpha$ alone treated goats. The conception rate was higher in group II (60 %) as compare to other groups even though it was non-significant. The present study concluded that the progesterone device (TRIU C and $PGF_{2\alpha}$) treated group has shown better estrus response, higher estrus intensity score and improved conception rate in non-descript goats.

Key words: Estrus synchronization, Goats, $PGF_{2\alpha,}$ Progesterone device, TRIU C

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INTRODUCTION

Estrus synchronization (ES) plays a key role in fixed time breeding, Artificial Insemination (AI), Multiple Ovulation-Embryo Transfer (MOET), Laparoscopic Ovum Pick-up (LOPU) for oocyte or embryo collection and Embryo Transfer (ET) in farm animals (Rahman *et al.,* 2008). ES in livestock focuses on the manipulation of either the luteal or the follicular phase of the estrous cycle. In does and ewes, the opportunity for control of estrous cycle is greater during the luteal phase, which is of longer duration and more responsive to manipulation by using hormones. A considerable amount of information was produced and published on ES in sheep, however meager in goats. Progesterone or synthetic progestin can be administered by using an intra vaginal-releasing device or by feeding. Intravaginal sponges have been the treatment of choice for ES in small ruminants. Intravaginal sponges with progestogens are effective at lower dose levels than natural progesterone (Wildeus 1999). A Controlled Internal Drug Releasing device (CIDR) is another form of implant contains 0.3 mg of progesterone and it can inserted in to the vagina of doe for 8 to 17 days followed by the injection of $PGF_2\alpha$ 24 h before withdrawal. CIDR implants can be used in association with GnRH and prostaglandin $F_2\alpha$ to induce estrus (Robin *et al*., 1994). However, reports on intra-vaginal devices and its synchronization efficacy and conception rate in goats are inadequate in nature. Hence, the present study has been designed with objectives to study the efficacy of intra-vaginal progesterone implant (TRIU C) and $PGF_2\alpha$ in synchronization of estrus and conception rate in non-descript goats.

MATERIALS AND METHODS

Thirty parous, healthy does maintained at Goat Breeding Unit, Post Graduate Research Institute in Animal Sciences, TANUVAS, Kattupakkam, Kanchipuram district, Tamil Nadu weighing between 20-30 kg were selected for the study. All the goats were maintained under semi-intensive system and uniform feeding and managmental conditions. Transrectal ultrasonography using B-mode, Real time scanner (Mindray Biomedicals Ltd, China) equipped with 7.5 MHz linear transducer was carried out to eliminate pregnancy and to assess the ovarian status of non-pregnant does. Animals were kept off-feed for 12 h prior to ultrasonographic scanning. Goats with evidence of CL in the ovary were divided into three groups of ten each. Group I does were treated with 125 µg of PGF2α (Pragma, Intas pharmaceuticals, India) and Group II does were subjected to Intravaginal insertion of progesterone device (TRIU C) for seven days (Fig 1 and 2) and I/M injection of 50µg of $PGF_{2\alpha}$ on sixth day of treatment.

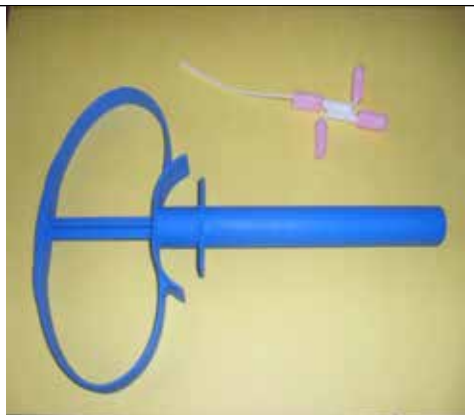

Figure 1. TRIU C (Intra vaginal Progesterone device) with applicator

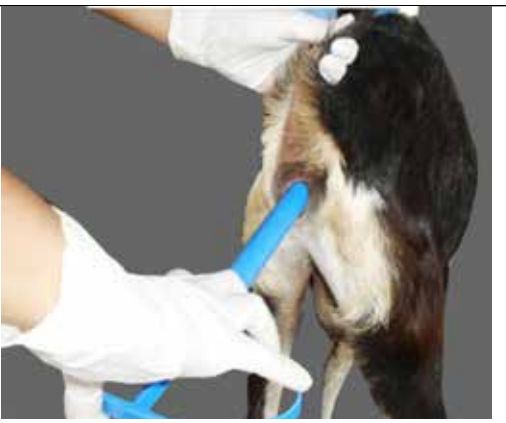

Figure 2. Intra-vaginal insertion of TRIU C (Progesterone device) by using applicator

The remaining ten does were kept as untreated control. Experimental does were observed for onset of estrus, duration of estrus, estrus behavior and intensity of estrus based on behavioral, physiological signs and vaginal exfoliative cytology as per the study conducted by Selvaraju (1994). The experimental does were allowed for natural service after exhibition of estrus. The pregnancy was confirmed on 30 – 35 days of post breeding by trans-rectal ultrasonography using a real time B-mode scanner equipped with 7.5 MHz linear array transducer. The data collected were subjected to statistical analysis as per Snedecor and Cohran (1989).

RESULTS

The estrus response in control, group I and group II was 80, 70 and 100 per cent, respectively. In group II, all the does treated with TRIU C exhibited estrus (Table. 1). There was a significant difference in the estrus onset interval between group I and group II. The group II does treated with TRIU C showed earlier onset of estrus when compared to group I.

Table 1. Estrus response, onset and duration of estrus in does in control and treatment groups

Groups	Estrus response (%)	Estrus onset interval (h) Mean ± SE	Duration of Estrus (h) Mean ± SE
Control (n =10)	80 (8)	207 ± 55.35	46.62 ± 0.67
Group I ($PGF_{2\alpha}$) (n=10)	70 (7)	55.50 ± 0.50^{a}	47.85 ± 1.48
Group II TRIU-C®) (n =10)	100 (10)	36.40 ± 0.40^{b}	44.00 ± 1.85

Means with different superscript differed significantly - $P< 0.01$
Figures in parenthesis indicate number of animals

The intensity scores of behavioral signs exhibited in the control, group I and group II were 5.75, 6.85 and 8.80, respectively. The intensity scores of physiological signs exhibited in the control, group I and group II were 6.62, 6.57 and 8.30, respectively.

The conception rate in the control, group I and group II was 50, 57 and 60 per cent, respectively. In the present study, the conception rate was higher in TRIU C group when compared to other groups even though the difference was non- significant.

DISCUSSION

Exogenous administration of $PGF_2\alpha$ analogue elucidated 87.5 per cent estrus response at a dose of 125µg. The variation in estrus response observed in group II could be due to the difference in the age of the corpus luteum at the start of the experiment which could have resulted in refractoriness to $PGF_2\alpha$ (Greyling and Van Niekerk 1986). All the does treated with the intravaginal progesterone device TRIU C responded to the estrus synchronization treatment and exhibited estrus signs. This is in agreement with the findings of Oliveria *et al* (2001) in Saanen goats; Romano (2004) in Nubian goats who also observed similar response elucidated by the progesterone device.

The onset interval of 55.70 ± 15.46 h recorded in $PGF_2\alpha$ treated group is in accordance with Karikari *et al* (2009) who similar results in West African Dwarf does and further, Khanum *et al* (2006) reported that 19 out of 20 animals treated with 125µg of $PGF_2\alpha$ analogue exhibited estrus after 56-72 h. In the present study, the variation in the estrus onset interval with $PGF_2\alpha$ treatment may be related to the size or maturity of the largest non-atretic ovarian follicle during the mid-cycle that continue to mature and ovulate after the corpus luteum regression by prostaglandin in cattle (Scramuzzi *et al*., 1980). The estrus onset interval of 36.40 ± 0.40 h was observed in the progesterone treated group was similar to the findings of Selvaraju (1994) who reported 32-42, 32-54 and 34-48 h with a mean of 36.71 ± 0.80, 38.71± 1.71 and 38.17±1.36 h in Tellichery goats treated with FGA, MAP and CIDR and 600 IU of PMSG intramuscularly on the day of progesterone withdrawal.

The shorter duration of estrus interval in group II observed in the study could be due to the effect of TRIU C on follicular dynamics resulting in early initiation of new follicular wave and subsequent reduction in progesterone level followed by administration of 50µg of luteolytic dose of $PGF_2\alpha$ analogue prior to withdrawal of implant followed in this study. The difference in duration of estrus in control and both the treatment group was found to be non-significant, however, small variation was observed in the Group I and Group II. The individual variability inherent in the events following estradiol peak during estrus which is beyond the capacity of synchronization may be responsible for the differences in the estrus duration with prostaglandin treatment (Frietas *et al*., 1996b).

The estrus intensity score was maximum in group II which clearly reflected the effect of progesterone in psychic expression of estrus. In the present study the intensity score was maximum in TRIU C treated group which is similar

to the results observed by Selvaraju (1994). This could be due to the effect of progesterone in modulating the follicular wave (Menchaca and Rubianes 2002) and increased quantity of estrogen secreted by the follicle (Armstrong *et al*., 1982).

Greyling and Van Niekerk (1986) reported mean conception rate of 58 per cent with prostaglandin treatment and Romano (2004) obtained 63 per cent with CIDR which concurred with the present finding. Therefore, the present study concluded that the use of progesterone device enhances the estrus response, shorter duration for onset of estrus, high estrus intensity score and well pronounced estrus behavior and improved conception rate as compared to $PGF_2\alpha$ treated goats. Hence, the present study concluded that the progesterone device may be used to synchronize estrus in non-descript goats for better estrus response and conception rate.

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Development and quality evaluation of probiotic shrikhand

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ABSTRACT

Probiotic shrikhand was prepared by incorporating probiotic *Bacillus coagulans* culture along with curd starter culture. The probiotic and control shrikhand samples were examined for the physico-chemical, microbiological and sensory properties.There was no significant difference in fat, ash and protein content between control and probiotic shrikhand. Sensory quality of the probiotic shrikhand was similar to that of control. There existed a significant difference in acidity and total solids content between control and probiotic shrikhand. Probiotic count could be maintained within the minimum recommended level during 15 days of refrigerated storage.

Key words: Shrikhand , Functional shrikhand , Probiotic shrikhand, *Bacillus coagulans.*

INTRODUCTION

Fermented dairy products have long been an important component of healthy diet. Lactic acid bacteria are commonly used as starters in the industrial production of dahi, Shrikhand, mishtidoi, lassi and yoghurt. Shrikhand is an indigenous fermented and sweetened milk product having a typical pleasant sweet-sour taste. It is prepared by blending Chakka, a semi-solid mass obtained after draining whey from dahi with sugar and other ingredients like fruit pulp, nut, flavour, spices and colour to achieve the finished product of desired composition, consistency and sensory attributes. Shrikhand has a typical semi-solid consistency with a characteristic smoothness, firmness and softness that makes it suitable for direct consumption. Probiotics are "live microorganisms which when administered in adequate amounts confer health benefits to the host" (FAO/ WHO 2001). *Bacillus coagulans* is a beneficial probiotic that helps to promote digestive health, reduces diarrhoea caused by antibiotics, encourages a healthy immune system, normalizes nutrient absorption in the body and helps to balance gut micro flora. It produces lactic acid inside the digestive tract which inhibit the growth of harmful bacteria.

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MATERIALS AND METHODS

Fresh whole milk required for the study was collected from the University Dairy Plant, College of Veterinary and Animal Sciences, Mannuthy. Freeze dried mixed dahi culture (NCDC-352) was procured from National Collection of Dairy Cultures, National Dairy Research Institute, Karnal. *Bacillus coagulans* culture was procured from Unique biotech Ltd. Hyderabad, India.

Dahi was prepared from fresh clean milk which was heated to a temperature of 71°C for 10 minutes with constant stirring. It was then inoculated with curd culture (NCDC 352) at 1 per cent level and incubated at 30^0C for 8 hours. It was then stirred and hung in a muslin cloth for 6 to 8 hours, to drain off whey in order to obtain chakka. Chakka was mixed with food grade cane sugar (20%) to get shrikhand. Probiotic shrikhand (T) was prepared from whole cow milk by incorporating *Bacillus coagulans* culture. Control shrikhand samples were prepared without the addition of probiotic culture.

1. Chemical analysis

Control and treatment groups of shrikhand were analyzed for titratable acidity, fat, total solids, ash and protein.The titratable acidity, fat, protein, total solids and ash content of shrikhand samples were determined according to the procedure described by the FSSAI (2016).

2. Microbiological quality of shrikhand

The coliform count, yeast and mould count of shrikhand samples were determined according to the procedure described by BIS (1981).

3. Sensory evaluation

The fresh shrikhand samples were evaluated for their sensory characteristics such as color and appearance, flavor, body and texture and overall acceptability as per the method recommended by BIS (2003).

RESULTS AND DISCUSSION

Acidity

The mean titratable acidity values of control and probiotic shrikhand were 1.02±0.004 and 1.42±0.019 (Table 1). There was a significant difference in titratable acidity ($P<0.05$) between control and probiotic shrikhand. The control samples had significantly ($P<0.05$) lower acidity than the probiotic samples. Similar findings were reported by earlier researchers (Jagdishbhai 2013, Swapna*et al*., 2013). According to FSSAI (2017), maximum permitted titratable acidity in shrikhand is 1.4 per cent lactic acid.

In the present study incorporation of probiotic cultures had increased the acidity of probiotic shrikhand.

Fat

The mean fat per cent of control and probiotic shrikhand were 9.15±0.075 and 9.13±0.072 (Table 1). There was no significant difference ($P>0.05$) in fat per cent between control and probiotic shrikhand.

Phate (2004) studied the quality of shrikhand prepared from cow milk using

probiotic culture. He reported that the fat per cent was 9.75.

Total solids

The mean total solids per cent of control and probiotic shrikhand were 55.97±0.622 and 58.09±0.088 (Table 1). There was a significant difference ($P<0.01$) in total solids content between control and probiotic shrikhand. Similar observations were made by Phate (2004). He reported that the total solids content of probiotic shrikhand prepared from cow milk was 59.52 percent.

Sivasankari *et al.* (2017) had reported that the total solids content of control shrikhand was 54.88 per cent whereas, the total solids content of probiotic shrikhand was 56.62 per cent

Ash

The mean ash per cent of control and probiotic shrikhand were 0.92±0.014 and 0.91±0.022 (Table 1).There was no significant difference ($P>0.05$) in ash content between control and probiotic shrikhand. According to FSSAI (2017) total ash content in shrikhand should be not more than 0.9 per cent on dry matter basis.

Protein

The mean protein per cent of control and probiotic shrikhand were 9.07±0.021 and 8.97±0.034(Table 1). There was no significant difference ($P>0.05$) in protein content between control and probiotic shrikhand. Jagdishbhai (2013) had also reported no significant difference in protein content between control and probiotic shrikhand samples.

Gupta and Sharma (2015) studied the qualities of probiotic shrikhand. They had reported that the protein content was 8.6 per cent.

Microbiological analysis

Coliform count

Coliforms were present on the 1st day and 5th day of storage. The mean coliform counts of control shrikhand (C) were 1.13±0.09 and 0.55±0.25 log cfu/g on 1st and 5th day respectively. In probiotic shrikhand the mean coliform counts were 1.18±0.08 and 0.93±0.20 log cfu/g respectively(Table 2). Coliforms were absent on 10th and 15th day of storage. There was no significant difference in coliform count between control and probiotic shrikhand. According to FSSAI (2016), coliform count of shrikhand should not be more than 10 cfu/g. The prepared samples met with legal standard.

Yeast and Mold count

The mean yeast and mould count of control shrikhand (C) were 1.05±0.06, 1.36±0.038, 1.50±0.02, and 1.58±0.02 log cfu/g for the 1st, 5th, 10th and 15th days of storage respectively. The corresponding values for probiotic shrikhand (T) were 1.06±0.17, 1.42±0.04, 1.47±0.04 and 1.56±0.03 log cfu/g(Table 2). There was no significant difference in yeast and mould count between control and probiotic shrikhand samples during storage. Jagdishbhai (2013) conducted a study on the microbial quality of probiotic shrikhand. They have reported a significant increase in the yeast and mold count of shrikhand samples during storage at refrigerated temperature.

Probiotic count

The mean values of probiotic count during 1st, 5th, 10th and 15th day were 7.11±0.03, 7.04±0.18, 7.02±0.04 and 7.00±0.17 log cfu/grespectively(Table 2). The probiotic count decreased during storage days. At the end of storage for 15 days, the probiotic count was higher than 6.000 log cfu/g which is the minimum requirement for probiotic products.

In a study conducted by Adriano et al. (2007), the probiotic count of shrikhand on 0 day ranged from 7.445 to 9.428 log cfu/g. The probiotic count on 28th day ranged from 6.709 to 8.963 log cfu/g.

Sensory evaluation

The mean sensory scores for flavour, colour and appearance, body and texture, container and overall acceptability of control were 48.83±0.17, 29.00±0.26, 14.33±0.21, 4.33±0.21 and 96.50±0.50 respectively. The corresponding scores for probiotic shrikhand were 47.33±0.67, 28.83±0.31, 14.17±0.31, 4.33±0.21 and 94.67±0.84 respectively (Table 3). No significant difference in sensory scores was observed between control and probiotic shrikhand. Jagdishbhai (2013) had also reported that no significant difference existed in sensory scores between control and probiotic shrikhand.

Shinde*et.al.* (2017) reported that sensory quality of probiotic shrikhand was more acceptable than control shrikhand.

CONCLUSION

From the above results it can be concluded that, acceptable quality probiotic shrikhand can be prepared by using *Bacillus coagulans* as a probiotic culture. A significant increase in acidity and total solids was observed in probiotic shrikhand. Sensory quality of the probiotic shrikhand was comparable to control. Probiotic count could be maintained within the acceptable limit during storage.

ACKNOWLEDGEMENTS

The authors acknowledge Kerala Veterinary and Animal Sciences University for providing infrastructure facilities and Unique biotech, Hyderabad for providing probiotic cultures for the conduct of research work.

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FLOW CHART FOR PREPARING PROBIOTIC SHRIKHAND

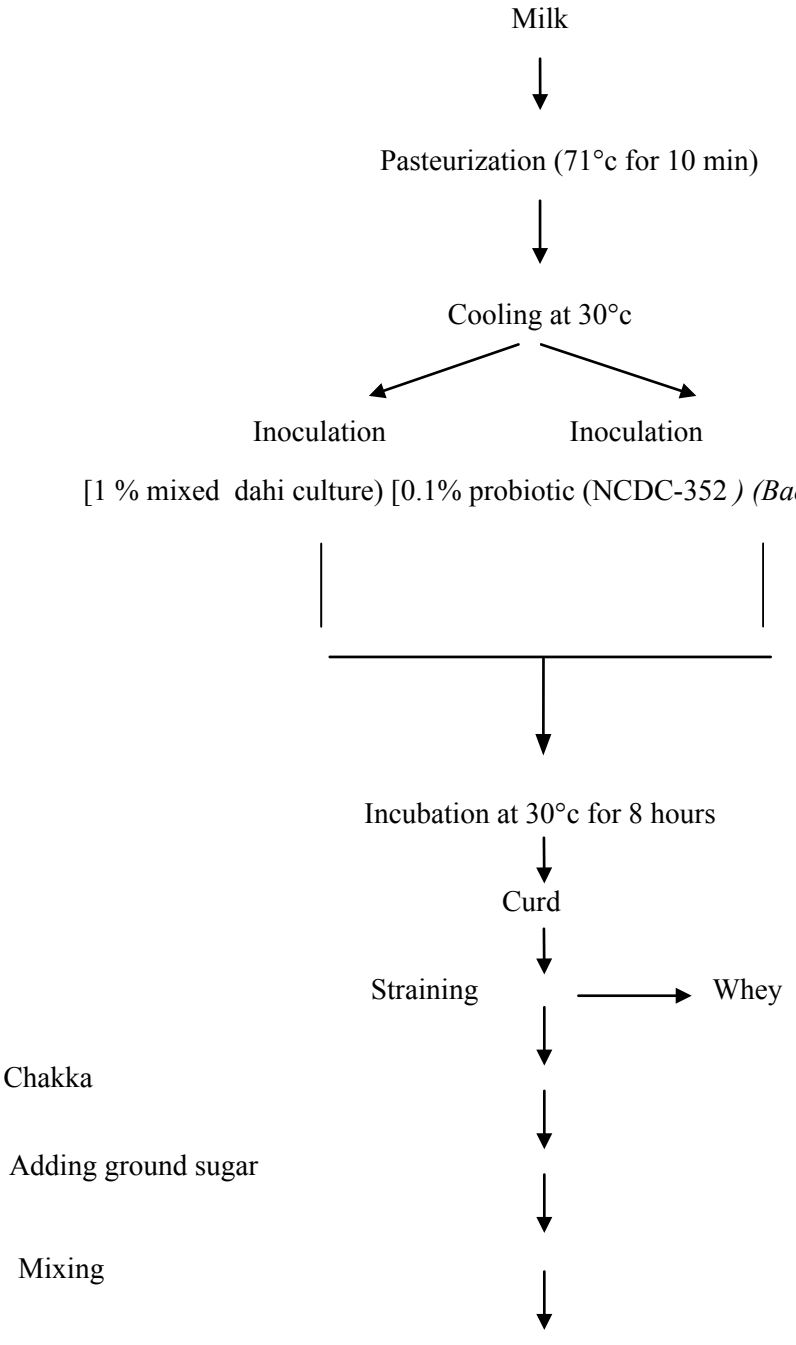

Table.1 Results of physico-chemical analysis of shrikhand

Parameters	Control shrikhand (Mean±SE)	Probiotic shrikhand (Mean±SE)	t-value
Acidity	1.02±0.004	1.42±0.019	-25.033*
Fat	9.15±0.075	9.13±0.072	0.191^{ns}
Total solids	55.97±0.622	58.09±0.088	-19.28**
Ash	0.92±0.014	0.91±0.022	0.508^{ns}
Protein	9.07±0.021	8.97±0.034	2.918^{ns}

** Significant at 0.05 level*
^{ns}*Non significant*
*** Significant at 0.01 level*

Table. 2 Results of Microbiological analysis of shrikhand

Microbiological analysis	Sample	1st day (Mean±SE)	5th day (Mean±SE)	10th day (Mean±SE)	15th day (Mean±SE)
Coliform count	C	1.13±0.09	0.55±0.25	0	0
	T	1.18±0.08	0.93±0.20	0	0
	t value	-0.418^{ns}	-1.206^{ns}	0^{ns}	0^{ns}
Yeast and Mould count	C	1.05±0.050	1.36±0.038	1.50±0.02	1.58±0.02
	T	1.06±0.17	1.42±0.04	1.47±0.04	1.56±0.03
	t value	-0.075^{ns}	-1.118^{ns}	0.681^{ns}	0.620^{ns}
Probiotic count	T	7.11±0.03	7.04±0.18	7.02±0.04	7.00±0.17
	F-value	0.136^{ns}			

^{ns}*Non significant*

Table 3. Sensory evaluation (Mean ± S.E) of shrikhand

Sample	Flavour	Colour and appearance	Body and texture	Container	Total
C	48.83±0.17	29.00±0.26	14.33±0.21	4.33±0.21	96.50±0.50
T	47.33±0.67	28.83±0.31	14.17±0.31	4.33±0.21	94.67±0.84
t value	2.18*	0.42^{ns}	0.45^{ns}	0.00^{ns}	

^{ns}*Non significant*

Short Communication

Microanatomical studies of the trachea in pigeon

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ABSTRACT

Trachea was collected from two adult male and female pigeons of Tirunelveli district. The aim of the study was to find out the microanatomical details of trachea in granivore birds. Trachea was lined by a pseudostratified ciliated columnar epithelium. Lamina propria was made up of loose connective tissue and contained alveolar mucous glands. The submucosa contained elastic fibres and was found related to perichondrium of cartilaginous rings. The cartilaginous rings were flattened in cross section. Overlapping of cartilaginous rings was prominent. Thin tunica adventitia covered the cartilaginous rings. Trachealis muscle was absent.

Key words: Microanatomy, trachea, Partridge

Respiratory organs of birds differ from those of mammals in many features, which are associated partly with the requirements of flight and partly with voice production. Tracheal cartilages formed complete rings in birds, which overlapped and interlocked with adjacent rings (Dellmann and Eurell, 1998). Literature available on microanatomical studies on the trachea in pigeon is limited. To bridge this gap, the present study was under taken.

MATERIALS AND METHODS

The trachea was collected from two apparently healthy six weeks old adult male and female pigeons of Tirunelveli district and was studied for their histological details. The trachea was cut across into small pieces and was processed conventionally. Paraffin sections of 4 to 5 µm thickness were taken and stained using Haematoxylin and Eosin (Luna, 1968). Micrometrical parameters like height of the lining epithelium and width of the lamina propria, cartilaginous rings and tunica adventitia were recorded using image size recording system in digiscope with imaging system.

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RESULTS AND DISCUSSION

Histological section through the wall of the trachea showed the following layers from inner to the outer surface: the mucosa, submucosa with cartilage rings and adventitia.

The mucosa was lined by pseudo stratified ciliated columnar epithelium (Fig.

1). Similar observations were also reported in Japanese quails by Rajathi *et al.* (2009) and in male turkeys by Al-Mussawy *et al.* (2012). In contrast to the above findings, non ciliated cells were also found in the mucosal epithelium in Japanese quail (Pourlis *et al.,* 2018). The mean height of the pseudo stratified ciliated columnar epithelial cells ranged from 9.18 to 12.20 µm. The basal cells were smaller and had round nuclei, while the ciliated columnar cells showed oval or elongated nuclei.

The width of lamina propria was 220 to 316 µm. It showed loose connective tissue with mucous glands. The alveolar mucous glands were lined by elongated cells with wide basal part and slightly narrowed apical part. The nucleus of the mucous glands was oval and placed towards the basal part of the cells. (Fig 2). The apical part showed foamy cytoplasm. Lamina propria also contained collagen and elastic fibres, blood vessels and nerve fibres. Similar observations were made in chicken by Aughey and Fyre (2001) and in Japanese quail by Rajathi *et al.* (2009). Lamina propria also contained diffuse lymphocytes.

The cartilaginous rings were flattened in cross section with a mean width of 115 µm. Overlapping of the cartilaginous rings were found in many areas with separate perichondrium. Similar findings were observed in male turkey (Al-Mussawy *et al.,* 2012). The cartilaginous rings were made up of hyaline cartilage (Fig. 3) as reported by Dellmann and Eurell (1998) in chicken. The tracheal cartilage showed perichondrium with flattened nuclei, lacunae containing chondrocyte and clear intercellular matrix. The chondrocytes are oval in shape with dark flattened to round nucleus (Fig. 3). Externally there was thin adventitia of 4.19 to 11.32 µm width. It was made up of connective tissue with numerous blood vessels and some adipocytes. Trachealis muscle seen in the case of mammals was absent as reported by Dellmann and Eurell (1998).

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Figures

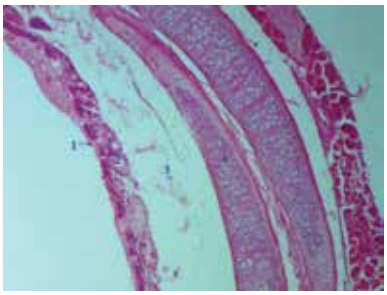

Fig. 1: Cross section of the trachea showing Mucosa

1. Pseudostratified columnar epithelium 2. Alveolar mucous gland
3. Submucosa containing blood vessel 4. Tracheal cartilage H & E x 100

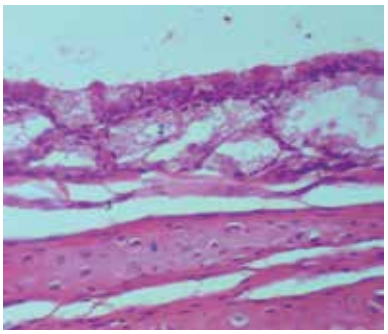

Fig. 2: Cross section of the trachea showing Submucosa

1. Pseudostratified columnar epithelium 2. Alveolar mucous gland
3. Submucosa with connective tissue 4. Tracheal cartilage H & E x 400

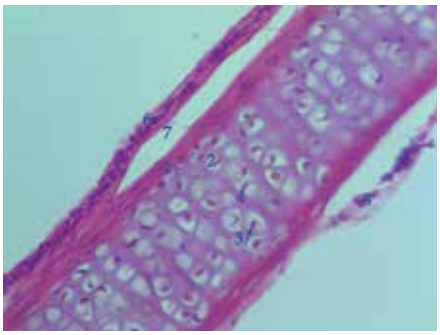

Fig. 3: Cross section of the tracheal cartilage

1. Perichondrium 2. Lacunae 3. Chondrocyte 4. Intercellular matrix
5.Adventitia 6. Mucosa 7. Submucosa H & E x 400

Histomorphology of the pancreas in myna

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ABSTRACT

Histological study was performed in the pancreas of two adult male mynas. Tissue pieces from different lobes of pancreas were fixed and processed in routine manner. Four to Five micron thick sections were stained using Haematoxylin and Eosin staining technique. The present study observed that the histological study of the pancreas in Myna consisted of exocrine acinar portion with ducts and endocrine diffuse portion with no connective tissue separation between them. The pancreas was covered by a thin connective tissue capsule which consisted of collagen, elastic and reticular fibres. The parenchyma was divided into lobes and lobules by connective tissue septa from the capsule. The exocrine acini were round to oval in shape. The acinar cells were columnar to polygonal in shape with apical eosinophilic large granules and basally dark cytoplasm with oval nucleus. The duct system started with intercalated, intralobular, interlobular, intralobar and large ducts which were lined by simple squamous, simple cuboidal, simple columnar, stratified cuboidal and stratified columnar cells, respectively. Centroacinar cells were absent. The endocrine portion consisted of lightly stained cells with different shapes. The nucleus of these cells was also compact in some cells and vacuolated in some cells. Numerous sinusoids were found between the acini.

Key words: Histology, Pancreas, Islets, Myna

Bird's pancreas is composed of two or three lobes with diffused islets. It is located in the right side of the abdominal cavity between the two loops of duodenum. Avian pancreas also consisted of exocrine and endocrine portion like mammals. The exocrine acinar portion is responsible for the production of digestive enzymes and the endocrine portion is responsible for the production of hormones like insulin, glucagon etc. Literature on the digestive organs of myna is scanty. Hence, this research work was undertaken to study the histology of pancreas in myna. This will form the basis for correlating the digestive functions of the birds.

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MATERIALS AND METHODS

The pancreas was collected from two apparently healthy predator killed adult male mynas in Tirunelveli district and was studied for their histological details. The pancreas from all the lobes was cut across into small pieces and was processed

conventionally. Paraffin sections of 4 to 5 µm thickness were taken and stained using Haematoxylin and Eosin (Luna, 1968). Histological images were recorded using image size recording system in digiscope with imaging system.

RESULTS AND DISCUSSION

The pancreas of myna as like other birds was located between the two duodenal loops in the right abdominal cavity. Three lobes were found between the two loops viz, dorsal lobe, ventral lobe and a small lobe near the spleen. This was contrary with the results of Saadatfar and Asadian (2009) who reported that the splenic lobe of the pancreas was absent in myna while in the case of pigeon, the pancreas was composed of four lobes namely dorsal lobe, ventral lobe, small lobe and splenic lobe.

Histologically, the pancreas in the present study was covered with a thin capsule. This was in agreement with the findings of Mobini (2011) in geese, Faris (2012) and Mobini (2013) in pigeon while in turkeys the capsule was thick (Mobini, 2009). The capsule was made up collagen elastic and reticular fibres which is similar to the results of Mobini (2011) in geese, Faris (2012) and Mobini (2013) in pigeon. The septa from the capsule entered into the parenchyma of the pancreas and was divided into lobes and lobules (Fig.3). The connective tissue fibres surrounded each acinus and there was no prominent connective tissue fibres separating the endocrine and exocrine part of the pancreas in the present study (Fig. 3). These interlobular connective tissue fibres contained blood vessels like artery, vein and sinus including nerve fibres as bundles. This was in accordance with the results of Mobini (2011) in geese.

The parenchyma of the pancreas in the present study consisted of exocrine and endocrine portions. The exocrine portion was serous in nature and compound tubuloacinar gland. It also consisted of rounded secretory acini and had duct system which was similar to the findings of Mobini (2011) in geese. The acini were lined by columnar to polygonal shaped acinar cells. The acinar cells were columnar in geese (Mobini, 2011). The acinar cells conatined apically eosinophilic large granules and basally dark cytoplasm with oval nucleus (Fig. 3). Numerous sinusoids were found between the acini (Fig. 2).

The duct system of myna started with intercalated lined by simple squamous, cells, intralobular lined by simple cuboidal cells and interlobular lined by simple columnar, intralobar and large ducts lined by stratified columnar cells (Fig. 1). This was in accordance with the results of Mobini (2011) in geese, Faris (2012) and Mobini (2013) in pigeon. Goblet cells and glands in the duct system were absent in the present study but was found in the pancreatic duct of goose (Mobini, 2011). Centroacinar cells were absent in the present study. Beheiry and Karkit (2018) found centroacinar cells in the pancreas of geese.

Endocrine portion or pancreatic islets consisted of lightly stained cells with different shapes (Fig. 3). The nucleus of these cells was also compact in some cells and vacuolated in some cells. Numerous sinusoids were found between the acini. The islets consisted of large alpha and small

beta islets. This was in concurrence with the reports of Mobini (2011) in geese, Faris (2012) and Mobini (2013) in pigeon but in the case of pancreas in duck, mixed islets were found (Das et al., 2003). Alpha islets consisted of alpha and beta cells and beta islets consisted of beta and delta cells.

CONCLUSION

Histological structure of pancreas in myna was similar to that of geese, turkey, pigeon except capsule thickness, presence of centroacinar cells and goblet cells and gland in the ductular epithelium.

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FIGURES

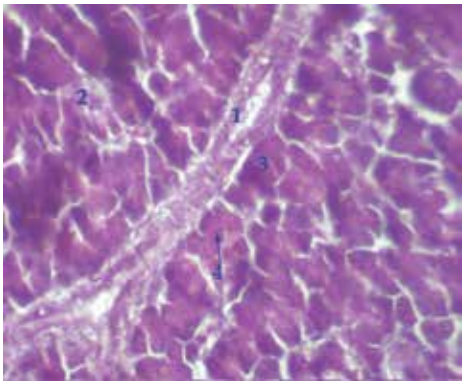

Fig. 1: Cross section of dorsal lobe of pancreas

1 – Intralobular duct 2 - endocrine portion – light islets

3 - exocrine portion 4 – Eosinophilic granules H & E x 400

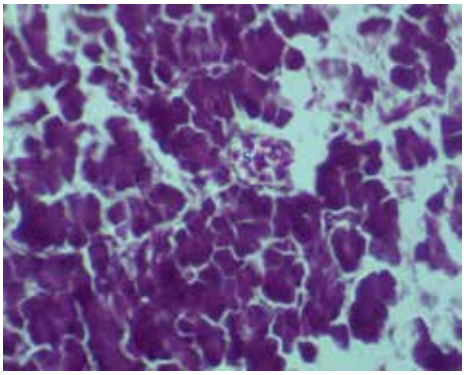

Fig. 2: Cross section of splenic lobe of pancreas showing exocrine acini (1) and blood sinusoids (S) H & E x 400

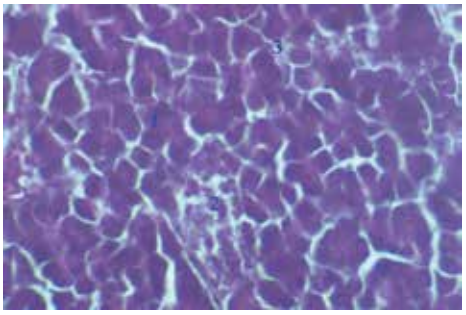

Fig. 3: Cross section of ventral lobe of pancreas showing exocrine (1) and endocrine portion – dark islets (2) and interlobular connective tissue septa (3) H & E x 400

INSTRUCTIONS TO AUTHORS

Scope of the Journal

"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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All manuscripts should be typed on one side of the A4 paper, double-spaced throughout, with margins of at least 25mm all around. All contributions will be subjected to editorial revision.

Major headings are centered, all capitals except for scientific names and the full length papers should consist of Abstract, Introduction, Materials and Methods, Results and Discussion, Acknowledgement (optional) and References. First subheadings begin at the left margin and the text that follows a first subheading should be in a new paragraph.

Full length papers should normally not exceed 3000 words in length including tables and illustrations i.e. approximately five journal pages and should contain the following section, each written concisely:

A **Title** page containing (a) the title of the paper in capital letters in exception for scientific names, (b) the names of authors in full with initials at the beginning, (c) the authors' department and complete postal address. Superscript numbers should be used to link authors with

other institution. Provide maximum of five key words for full length paper and three for short communication for subject indexing. The author wise contribution should also be mentioned in nutshell.

An **Abstract** will be printed at the beginning of the paper. Abstract should not be more than 150 words emphasizing objectives, experimental procedure, results and conclusions. Use complete sentences and limit the use of abbreviations. It should be in a form suitable for abstracting journals to use.

A brief **introduction** with specific emphasis on the necessity for such a kind of research may be given.

Materials and methods section may refer to previous description of methods whenever possible. This section should include experimental designs and methods of statistical analysis.

Results and Discussion may contain subheading if appropriate. This part should be brief and to the point, without repetition of results.

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References section should contain only essential references which should be listed alphabetically and written as indicated below. In the text, give the author's name followed by the year in parenthesis: Suresh (2009). If there are two authors, use 'and': Suresh and Mani (2015); but if cited within parenthesis: (Suresh and Mani, 2015). When reference is made to a work by three or more authors, the first name followed by et.al. should be used: Rama et.al.(2015); but if cited within parenthesis: (Rama et.al., 2015). Reference to unpublished data and personal communications should not appear in the list but should be cited in the text only (e.g. Amutha T, 2015. Unpublished data).

Journal articles and abstracts

Bardbury, J.M., Mc Carthy, J.D and Metwali, A.Z. (1990). Micro immunofluorescence for the serological diagnosis of avian Mycoplasma infection. *Avian Pathology*, **19**:213-222.

Raja, S., Rani, A., Ravi, M and Kumar. K. (2007). Histopathology of CPV infection. Page no. 120-122….Venue…Date…Place…

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.

Electronic publications

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