Studies on postmortem changes and disposal of animal carcass

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By

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CERTIFICATE-I

This is to certify that the thesis entitled **"Studies on postmortem changes and disposal of animal carcass"** submitted in partial fulfilment of the requirements for the award of the degree of **Doctorate in Philosophy (Veterinary Pathology)** to the Orissa University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by **Supriya Das** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged.

CHAIRMAN

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CERTIFICATE-II

This is to certify that the thesis entitled **"Studies on postmortem changes and disposal of animal carcass"** submitted by **Supriya Das** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **Doctorate in Philosophy (Veterinary Pathology)** has been approved/disapproved by the student's advisory committee and the external examiner.

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Place :

Date : **(Supriya Das)**

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LIST OF ABBREVIATIONS

%	:	Per cent (age)
@	:	at the rate of
ALP	:	Alkaline phosphatase
ALT	:	Alanine aminotransferase
As	:	Arsenic
AST	:	Aspartate aminotransferase
Bp	:	Base Pair
BUN	:	Blood urea nitrogen
C:N	:	Carbon:Nitrogen
Ca	:	Calcium
Cd	:	Cadmium
cDNA	:	Complementary deoxy ribonucleic acid
Cr	:	Chromium
Cu	:	Copper
DLC	:	Differential leucocyte count (s)
DNA	:	Deoxyribose nucleic acid
dNTP	:	Deoxy nucleotide triphosphate
EC	:	Electrical cunductivity
EDTA	:	Ethylene diamine tetra acetic acid
Fig	:	Figure
fl	:	Femtolitre
g	:	Gram
GAPDH	:	Glyceraldehyde -3- phosphate dehydrogenase
GGT	:	Gamma glutamyl transpeptidase
GLB	:	Globulin

Gr	:	Group (s)
H&E	:	Haematoxylin and eosin
Hb	:	Haemoglobin
Hg	:	Mercury
hrs	:	Hour
i.e.	:	That is
IU	:	International unit
K	:	Potassium
kA	:	King Armstrong
Kg	:	Killogram
kg/ ha	:	kilogram / hector
LDH	:	Lactate dehydagenase
MCH	:	Mean Corpuscular Hemoglobin
MCHC	:	Mean Corpuscular Heamo concentration
MCV	:	Mean Corpuscular Volume
Mg	:	Magnesium
Mn	:	Manganese
mRNA	:	Messenger ribose nucleic acid
N	:	Nitrogen
NFW	:	Nucleus free water
ng	:	Nano gram
Ni	:	Nickel
OC	:	Organic carbon
OD	:	Optical density
P	:	Phosphorous
P.M	:	Post Mortem
Pb	:	Lead

PBS	:	Phosphate buffer saline
PCR	:	Polymerase chain reaction
PCT	:	Proximal convoluted tubules
PCV	:	packed cell volume
pH	:	pouvoir hydrogène
PMI	:	Postmortem interval
qRT-PCR	:	quantitative Real time Polymerase chain reaction
RBCs	:	Red blood cells
RNA	:	Ribose nucleic acid
RNase	:	Ribonuclease
rpm	:	Revolutions per minute
S	:	Sulphur
S.E.	:	Standard error
sec	:	Second
TEC	:	Total Erythrocyte
TGL	:	Triglyceride
TLC	:	Total leukocyte count
tRNA	:	Transfer RNA
TSD	:	Time since death
U	:	Unit(s)
UV	:	Ultra-violet
vs	:	Versus
WBC	:	White blood cell
wk	:	Week(s)
Zn	:	Zinc
βactin	:	Beta Actin
µl	:	Microlitre

ABSTRACT

Post-mortem changes were studied for accurate determination of post-mortem interval or time of death. Tissue enzymatic study of liver & heart revealed gradual increase in AST & ALT values at 6 & 12 hrs. Serum enzymes like AST, ALT, LDH and alkaline phosphatase increased with increase in time after death upto 24 hrs of post death. Haematological parameters like TEC & TLC decreased with increase in time since death within 24 hrs. pH of blood, decreased with increase in time since death upto 24 hrs from 7.42 ±0.04 to 6.12 ±0.07. Morphological changes of leukocytes like pyknosis at 6 hrs, nuclear & cytoplasmic vacuolation at 12 hrs, fragmentation & disintegration of nucleus at 24 hrs except lymphocytes. Histopathological examination of liver specimens collected at 12-hours of slaughter showed condensation of nucleus along with increased granularity of cytoplasm in the hepatocytes and increased sinusoidal space. At 24-hours of slaughter the liver showed advanced autolytic changes, perinuclear halo in the hepatocytes, disruption of chords and increase sinusoidal space and complete disruption of nucleus in hepatocytes with invasion of saprophytes. The heart specimens at 12-hours of slaughter showed autolytic changes, pyknotic nuclei, increased granularity of the cytoplasm in myofibrils. At 24-hours, the heart specimens showed advanced autolytic changes with disintegration of nuclei, loss of polarity of myofibrils along with their detachment. At 12 hours kidney showed, cellular swelling with increase granularity of cytoplasm of tubular epithelium and disruption of tubular epithelium at many places. At 24 hours disintegration and dissolution of nuclei of some glomerular epithelial cells with collapse of glomeruli. In molecular study it was observed in semi-quantitative PCR expression of β-actin was slowly down regulated till 6 hrs of post-sacrification and it could not be in detectable limit from 12 hrs till 96 hrs in liver tissue but in brain tissue the RNA was intact and degrades slowly after 3 hrs of post death. As real time q-PCR is more sensitive so the result was further validated with real time q- PCR. There was gradual decrease in expression of b-actin gene in liver. So by real-time PCR the time of death can be predicted upto 12 hrs in liver whereas as in brain there is no gradual degradation observed. So, liver tissue found to be more effective to co-relate the time of death with RNA degradation till 12 hrs. This molecular study is first time applied for estimation of time of death which is novelty of the work, besides other parameters are also unique for application in other forensic animals as well as in wild life death cases. Studies were also conducted on disposal of carcasses by burial and composting methods for a suitable method of disposal of carcass. Simple, innovative, economical and biologically safe compost unit for poultry carcass disposal was developed. Result of finished compost in our study showed C:N ratio of 12:1 which can be well utilised as a soil biofertilizer.

CHAPTER I

INTRODUCTION

After death, a sequence of changes i.e. a series of chemical as well as physical reactions naturally occur in the dead body (carcass). These changes are called as the postmortem changes. The rate of postmortem changes is affected by various external as well as internal factors. A proper understanding on postmortem changes or artifacts is very important which limit the possibilities of misdiagnosis during necropsy.

Postmortem interval (PMI) is the time that has elapsed since the animal has died. Post mortem interval estimation is very important in human as well as in animal forensic death investigations (Brooks, 2016). In case of animals there are very few data regarding PMI are available from actual case study. Therefore veterinary pathologist and forensic workers depends on the human data to evaluate the PMI in animal cases. Estimation of PMI is a very important subject in case of postmortem examination. For forensic pathologist estimation of exact time of death become a major problem as they have to answer questions about PMI in the court of law continues to be a major problem for the forensic pathologist and its determination. Detailed knowledge on the process of postmortem and the factors which affect them helps in estimation of time of death.

The gross changes in the dead body like rigor mortis, algor mortis, post mortem hypostasis, postmortem staining, postmortem imbibition of bile, postmortem softening, postmortem rupture, autolysis, putrefaction are traditionally used for determination of PMI. These traditional methods of estimating the PMI depending on gross changes in the body after death give a rough approximation of time of death. To increase the accuracy in estimation of time of death intense study on both physical and chemical changes should be evaluated together along with study on changes in molecular level.

Despite of various research on this aspect, accuracy in estimation of time of death cannot be significantly improved and there is not a single reliable method for estimating time of death accurately till now. Advanced techniques like molecular methods (PCR, electrophoresis etc.), forensic entomology, biochemical estimation,

histopathological interpretation, hemogram etc. can be employed. As evaluation of accurate time of death is a very difficult task because it is influenced by a lot of environmental factors so we cannot use a single method for it. As very limited research has been done in these aspects especially in veterinary field till now, more studies are needed in this area basing on different new and advanced methods. Such that we can correlate them to estimate the PMI which should be nearly accurate.

Waste management and associated environmental issues are one of the most critical problems currently facing the livestock industry. Carcass disposal remains one of the major problems faced by the livestock and poultry farmers. The dead animals as well as the poultry, should be disposed in a safe and environmental friendly way Safe and environmental friendly disposal of livestock carcasses, is an essential consideration as proper disposal of carcass is necessary to avoid risk of disease transmission to other animals as well to humans (in case of zoonotic diseases), control scavenging animals and vermin and also to avoid contamination of ground and surface water. It is now recognized that mortality disposal is a continuous and growing challenge for livestock producers. In developing country like India associated cost relating carcass disposal is also another essential consideration. Many different methods are used throughout the world to dispose of these carcasses. But in Indian condition mostly burial method is used in spite of more costly methods like rendering, alkaline hydrolysis, incineration, landfill etc. However, in burial method there is risk of ground water consideration so now a days it is restricted in many areas.

Keeping consideration about all these above discussed problems another method that is composting can be used which provides an economically and biologically safe means of carcass disposal. It is a process of converting carcasses resulting with other waste of livestock farm like poultry litter, paddy straw into an odourless, humus-like material which can be used as a soil amendment. Composting is defined as the controlled decomposition of organic materials which is an inexpensive and environmentally-friendly way to dispose of livestock carcasses (Shearer, 2006). Carcasses in a compost are having low C:N ratio (excess N), high moisture and devoid of porosity whereas paddy straw as co-composting material have, :N ratio, relatively low moisture and having sufficient porosity. Hence carbon rich co-composting materials & nitrogen rich carcass contribute for balancing required C:N

ratio for compost. Again porosity requires for aerobic decomposition which is provided by co-composting materials which accelerate decomposition. As in composting during bacterial degradation heat is generated nearly about 130^0-150^0F and that much heat is sufficient to kill the harmful microorganisms, parasites, larva or even the spores. According to Millner (2003) when heated at temperature of 55^0 C for three consecutive days over 99% pathogens as well as parasites are killed. Another important point of compost is that there is no offensive odour as the gases and liquids (odorous) diffuse into dry and aerobic co-composting materials and there microorganisms ingest them and degraded into simple organic compounds and ultimately to CO_2 and water which are not harmful (Keener *et al.,* 2000). Composting is a simple and economic process so no special equipment or techniques are required and can be easily accepted by the livestock farmer.

Taking into consideration all these above hypotheses, current study is undertaken with the following objectives:

1. Accurate determination of postmortem interval or time of death through studies on post-mortem changes like haematological and enzymatic alterations of blood and tissue changes at microscopic & molecular level.
2. Comparison of decomposition by burial and composting methods of disposal of animal carcass.
3. Evaluation of fertility of soil (at disposal site of burial method) and composting materials.

CHAPTER II

REVIEW OF LITERATURE

A series of irreversible and progressive changes naturally occur after death in the dead body or carcass which is called as the post-mortem change. A proper understanding on post-mortem change is very important which limit the risk of misdiagnosis at the time of necropsy as well as to determine the post-mortem interval or time of death.

As soon as an animal dies certain changes occur which are known as post-mortem changes (Vegad J.L., 2012). It is important to know these changes because every animal that dies shows some of these changes. One should be able to differentiate post-mortem changes from the lesions found in disease. These changes are influenced by many factors like environmental temperature, size of the animal, external insulation species of animal, nutritional status of animal etc. There are different post-mortem changes like autolysis, putrefaction, rigor mortis, post-mortem clotting of blood, imbibition of haemoglobin, hypostatic congestion, pseudo melanosis, imbibition of bile, post-mortem emphysema etc. Brooks (2016) described that, immediately after death a sequence of chemical as well as physical changes occur in the dead body which are progressive as well as irreversible. To prevent misinterpretation of postmortem changes as lesions caused by diseases proper understanding of postmortem changes is very much important for a pathologist.

During death investigations postmortem interval (PMI) or time of death is most important subject which helps to identify both criminal and victim by eliminating the suspects and connecting the deceased with the individuals who are missing at that time. (Catts, 1990; Geberth, 1996). According to Goff M.L. (2009), as death proceeds a series of changes occur in the dead body which in a definite in the body appearance before onset of recognizable (gross) decompositional changes. These changes are rigor mortis, algor mortis, livor mortis, mummification, putrefaction, greenish discolouration, saponification which are traditionally used in PMI estimation. These changes have traditionally been used in estimations of the PMI. Dokdoz *et al.* (2001) described that in forensic medicine and law PMI estimation is one of the important problem. To estimate PMI both chemical as well as physical postmortem changes

should be evaluated together. According to Perper (2006) and Swift (2010) accuracy in PMI determination has not significantly improved despite of many decades of research on this topic and for exact estimation of time of death not a single method can be reliable used. Sinha M *et al.* (2012) stated that the PMI is the time that has elapsed since a person has died. For forensic cases the significance of PMI is very important. Munro and Munro (2013) suggested that to the contemporary vety. Forensic medicine estimation of PMI is a very important challenge. They also reported that in animal cases estimation of time of death may be very useful in forensic death cases in some important issues such as insurance fraud, neglect or abuse, in cases where there is both simultaneous death of animal and human, harvest of game animals with relation to hunting season. Kumar B (2015) concluded that estimation of TSD (time elapsed since death) helps in investigation of mysterious and complex cases such that the truth can be revealed for the justice. Determination of time since death is extremely difficult in general and 100 % accuracy is practically almost impossible. But time of death can be determined by intense and careful study of different chemical, biological parameters and macroscopic, microscopic lesions which may be nearly accurate. According to Brooks (2016) determination of postmortem interval is very important in both human and animal death investigations. There are very limited data available on postmortem interval in animal cases. So veterinary pathologist have to relay on human data during estimation of PMI in animal death cases Furthermore, knowledge of the post-mortem processes and the factors that affect them will aid in the estimation of the post-mortem interval (PMI). According to Lia *et al.* (2016) estimation of PMI or time of death is the most important and also frequently asked question in forensic investigation. For forensic pathologist it is the fundamental task in legal cases. Determination of time of death helps in verification of witness statement which limits the number of suspects and possible abilities in criminal cases. In the other hand inappropriate estimation of time of death may create confusion and complicate the investigation. Das (2017) suggested in medicolegal investigation estimation of time since death is the most important real time problem. To help the police investigation after conducting the autopsy the forensic pathologist have to answer how much time elapsed since death. Estimation of PMI accurately helps to reveal many unfolded medicolegal mysteries. Although it is not possible to estimate accurate time of death by help of any postmortem findings some reasonable approximation of PMI is always needed.

2.1 PARAMETERS OF POST MORTEM CHANGES TO DETERMINE POSTMORTEMINTERVAL

2.1.1 Tissue enzymatic changes

A study on enzyme histochemistry of liver collected from 22 dead bodies (2-12 hrs postmortem) was done by Santos-Martin and Mello de Oliveira (1995) to determine sensitivity of enzyme activities to the postmortem autolysis process. According to the G 6PA, PHLYA a, PHLYA t are the possible elements to determine to determine time of death in forensic cases.

A study on organ enzyme concentration in heart and liver with relation to time of death was carried out by Gandhi and Patnaik (1997). They estimate the enzyme activity by three steps i.e sonication, centrifugation and enzyme assay. According to their result there was rising and declining trends which followed by the same or reverse pattern in the time interval between 4-38 hrs post death. The graphic behaviour of these enzymes show reverse of expected pattern and also repeated reverseand expected pattern which can be called as a dissociated paradox in postmortem bio-chemical clock.

Ramakrishnan *et al.* (1980) reported that ALT and AST are having much clinical importance. Normally the concentration of serum ALT and AST are low but due to tissue damage they released into serum with rising their concentration. As after death there is extensive tissue damage occur so their concentration increases.

2.1.2 Gross and microscopic alteration

To determine time of death study on time bound histochemical as well as histological degenerative changes in various tissue and organs may be a good solution. (Nemilow, 1928: Anderson, 1946: Florey, 1962: Fatteh, 1973).

Throughout the world forensic pathologist are trying to determine time of death by observing degenerative changes in the tissues at different time interval but they cannot find a conclusion (Berenbaum, 1956: Chatterjee and Goyal, 1977: Chowdhari and Chatterjee, 1974).

An experimental study on liver tissue of goat was carried out by Chowdhury *et al.* (1970) to know the correlation of histological changes with PMI. According to their result at 6 hrs of death liver tissues showed focal area of autolysis with increase granularity of cytoplasm. Then beyond 12 hrs in liver cell cytoplasm the basophilic character was replaced by eosinophilic colour. There was also disintegration and rarefaction in the nuclei. There was merging of cells outline after 24 hours of death with some nuclear pyknosis.

According to Rodriguez and Bass (1983) decomposition have 4 stages i.e fresh, bloat, decay and dry. Fresh stage is the stage after death until bloating begins (4-36 days. Bloated stage is the stage starts with onset of bloating to resolution of bloating (3-19 additional days). Decay stage starts with resolution of bloating to drying of carcass (6-183 additional days). After that dry stage starts till there is no carrion insect activity (13-27 additional days).

On PM histopathological study of kidney (at 30^0C), Tandon (1985) found that after 12 hrs of death there was cloudy swelling in PCT and DCT, then after 24 hrs diffuse cloudy swelling was observed in the cells of renal tubules, glomeruli and blood vessels. Then there was more diffuse and intense changes occur after 30 hrs. Beyond 48 hrs severe autolysis was seen in kidney tissue. After 72 hrs there was bacterial infiltration and liquefaction of kidney. As temperature increased to 40^0 C the changes became more diffuse at 24 hrs and after 6 hrs there was advanced autolytic changes such that only vogue outlines of glomeruli, blood vessels and tubules could be observed.

Francois *et al.* (1988) did PMI estimation experimentally with help of many biochemical component of pectoral muscle of hen. He found that there was significant correlation of creatinine concentration and AST activity with time passed since death. There was negative correlation of creatinine and NPN (%) with time passed since death and positive correlation with AST activity

Andersan and VanLaerhoven (1996) described that in final decay stage there was only cartilage, bones and few dry skin left with carcass which have no odour.

Clark *et al.* (1997) said that after death due to decomposition there was production of gases in the abdomen and also in other body parts. The composition of this gases vary with body to body but hydrogen sulphide is the significant component. Hydrogen sulphide being a small molecule readily diffuses from the body, with reaction to haemoglobin it forms sulfohaemoglobin..

DiMaio (2001) defined livor mortis that it is the purple discolouration of soft tissue of body which occur due to postmortem gravity dependent pooling of blood. It may be seen internally in thoracic and abdominal viscera mostly observed in lungs or externally in skin and mucous membrane. In humans within 30 min to 2 hrs of death it develops. It must be differentiated from haemorrhage. In haemorrhage there is leakage of blood to internal or external spaces or to connective tissue from blood vessels but in case of livor mortis there was pooling of blood into the dilated vascular channels. He also stated that postmortem rigidity of muscle is called as rigor mortis .This rigidity of muscle increases till all the ATP is consumed. Then this rigidity of muscle or rigor mortis persists till there is decomposition of muscle fibres i.e actin and myosin.

According to Nashelksy and McFelley (2003) livor mortis starts within 1 hour of death and complete livor mortis is observed 2-4 hour of death. The PM blood is still liquid during this time and blood squeeze out the area (Blanching) on pressing the skin and when pressure removed blood will return. This blanching occur till 9-12 hrs, then the livor motis become fixed. That means there will no discolouration even after pressure.

Saukko and Knight (2004) observed that after death throughout the body in muscle fibres of skeletal and cardiac muscle there are a series of bio-chemical reaction occur. Between actin and myosin fibres there is formation of cross bridge by consuming ATP which is the molecular source of contraction. As after death the production of ATP stops so there is no remaining ATP for decoupling of actin-myosin fibre. As a result relaxation of this bond cannot occur. So there is no relaxation and muscle fibres remain in that contracted stage which is called as rigor mortis. According to them onset of rigor mortis starts in 2-6 hrs of death and remain upto 36 hrs. After that it resolve slowly. This duration of rigor mortis depends on many

external and internal factors like environmental temperature, body temperature, cause of death, antemortem activity etc.

According to Lew and Matshes (2005) lividity of dead body helps in forensic cases. They suggested that if lividity does not follow the gravity dependent pattern then the pathologist must consider after lividity became fixed whether the body may have been moved. They also described that there was artefactual changes in colour or texture of mucous membranes and also delicate skin surfaces due to postmortem drying . Immediately after death this desiccation process starts and in case of moist mucous membrane it progress rapidly. In the eyes of human it became more prominent which results in a horizontal band of rad to brown-black discolouration of sclera and there is failure in closing of eye lids. This condition is called as tache noir. As animals have lager corneas and smaller exposed scera this condition cannot be observed prominently.

Erlandsson and Munro (2007) stated that in case of animals lividity cannot observed prominently even after shaving of hairs like humans, but it may be visible on pinnae of ear in case of dogs.

Munro (2008) reported that there was green discolouration of abdominal skin after 24 to 30 hours of death due to denaturation of Hb to biliverdin which react with HCN which releases during PM putrefaction. In dogs there is greenish discolouration on the ventral abdomen and there may have no colour change in case of malnourished dogs and cats. This green discoloration is often prominent on the ventral abdomen in dogs, but malnourished dogs and cats may have no obvious colour change.

Using immunohistochemistry Wehner *et al.* (2009) tried to estimate time of death. Their result showed that upto 4 days calcitonin can be stained, upto 6 days glucagon can be stained, thyroglobulin upto 5 days, insulin 12 days. But calcitonin and thyroglobulin cannot be stained beyond beyond 12 hours of death, after 14 days glucagon cannot be stained and beyond 29 days insulin cannot be stained.

Dettmeyer (2011) described histologically there are saprophytic bacteria, gas bubble, loss of cellular architecture and cellular staining quality within 1-3 days after death in tissues.

On analysis of protein profile of six organs after death i.e. heart, liver, brain, pancreas, kidney, brain of post death on SDS-PAGE Sinha *et al.* (2012) found that all protein were degraded except Hb subunit (Alpha & Beta) and Albumin after 10 days. The protein concentration per weight of tissue sample gradually decreased with increase in time after death. They also found that the Hb subunit Beta and albumin were degraded at last than other body proteins. In relation to degradation of proteins with time passed since death out of all the tissues kidney and liver showed most consistent profile. Hence upto 10 days after death for determination of PMI protein profile of vital body organs may be a useful method. Advantage of this approach over others is that by using this method PMI can be estimated for a long interval i.e. upto 10 days. For determining PMI this method can be used as reliably as a molecular index.

Martins *et al.* (2015) found that those who have more muscle mass typically show rigormortis more typically. So male humans have more prominent rigor mortis than females.In infants and very old person rigor mortis is very faint.

Brooks (2016) described about algor mortis i.e. the cooling of the body after death. After death there is loosing of body heat to the external environment with result in decrease in internal body temperature. But the extent of cooling of body in case of animals differ than humans.

Ortmann *et al.* (2017) reported that the helps in rough approximation of estimation of PMI. After staining of pancreatic tissue for glucagon and insulin ; thyroid gland tissue for calcitonin and thyroglobulin they found that thyroglobulin and calcitonin showed negative staining So in forensic pathology immunohistochemistry may act as an useful method in determination of PMI. .

In a study on postmortem hepatic changes Kushwaha *et al.,* found that at 30^0 C at the biging of 24 hrs there was mild autolytic and the changes became more prominent between 36-48 hrs. But there was loss of lobular architecture in hepatic tissue with loss of all cellular detail beyond 72 hrs. With increase in time of death and the temperature rate of microscopic changes increases.

2.1.3 Molecular changes

Nunno (1998) reported that researchers have investigated the effect of time passed since death on DNA degradation using flow cytometry PM eukaryotic cells upto 36 hrs of post death.

The analyst can evaluate the DNA content using a DNA specific fluorescent dye and analysing the amount of fluorescence emitted by a cell. The amount of DNA in cell can be assessed by this measurement when the fragment size from about 1-150 kilobases which helps in quantification of DNA and finally with PMI estimation. (Yan, 2000: Ferris, 2004).

Bauer *et al.* (2003) reported that degradation of nucleic acids after death has been suggested as an elegant alternative to classical methods for time of death estimation. Through the quantitative analysis of mRNA degradation by multiplex-qPCR in combination with laser-induced fluorescence capillary electrophoresis they attempted to establish the PMI. According to their result a significant correlation between PMI and RNA degradation in stored refrigerated human blood and brain samples for up to 5 days.

It has been reported an absence of significant correlation between PMI and mRNA degradation in brain tissue of humans (Preece and Cairns, 2003: Heinrich, 2007).

The analysis of time-dependent degradation of nucleic acids i.e. RNA and DNA became a focus of attention in forensic medicine with the advances of molecular biology (Bauer *et al.,* 2003: Liu *et al.*, 2007).

Green *et al.* (2005) reported that RT-qPCR based methods have become the standard method for forensic sample DNA quantification over the last ten years.

According to Huggett *et al.* (2005), Dheda *et al.* (2004) Vennemann and Koppelkamm (2010) five commonly used reference genes were chosen i.e. Hprt, Cyp2E1 Actb, , Ppia and Gapdh as widely accepted stable expressed genes.

Bauer (2007) suggested that by studying the RNA decay the estimation of the PMI may be possible since loss of RNA transcripts or RNA degradation after death seems to be rapid and time-dependent.

Liu *et al.* (2007) stated that for exact determination of PMI needs the evaluation of the parameters that change constantly with time passed since death. This definition fits well in post mortem degradation of nucleic acids.

Previous studies by Heinric, 2007 and Partemi *et al.*, 2010 revealed that on gene expression in human brain, cardiac, and skeletal muscle (iliopsoas) after death for estimatation of time of death did not find any correlation between gene transcription decay and PMI.

In a study on quantitative post-mortem DNA degradation Itani *et al.* (2011) concluded that when the dead body was kept in cooler temperature for estimating the post-mortem interval by quantifying the DNA, liver seemed to be the most useful investigated organs.

According to Bishop (2015) the results of the pig studies show that to estimate PMI measurable changes in tooth pulp after can be used. This change is greatly depend upon the temperature. They have shown that a window of time within which death occurred can be generated with a 95% confidence using individual amplicon pairs. Still more studies and statistical analysis will be required before this means of estimating PMI is implemented in forensic investigations. According to their result it is a very efficient method of PMI estimation. This result from the pig case can be implemented in human casers. When fully developed, without specialized knowledge of insect fauna, this means of estimating PMI can be used for samples collected anywhere in the world and it is a cost effective method as compared to other methods, and may be used for estimations PMI ,

Šaňková and Račanská (2016) stated that in forensic practice estimation of time of death, i.e. the post-mortem interval (PMI), is one of the most problematic issues. Accurate estimatio of the PMI is a very complicated task till today even for an experienced forensic pathologist. Many physical changes i.e. algor mortis, livor mortis and rigor mortis can be observed already during the first hours after death. But

with these physical changes accurate determination of PMI is not possible because these changes are variable with different factors like constitution of the body, temperature of surrounding environment, drug abuse, cause of the death, location of the body etc. Assessment of the parameters which constantly changes after death but independently on ambient factors is required for accurate estimation of PMI. In the field of molecular biology according to current research, it appears that a PM degradation of nucleic acids (both RNA and DNA) will correspond to this definition.

2.1.4 Haematological changes

Pentilla and Laiho (1981) studied on 123 medico legal autopsies cases with PMI ranging from 1.7 hours to 270.4 hrs which were kept at 4^{0} C. The haematocrit value of the blood found to be increased with time passed since death. Red cells were quite rapidly transformed to crumbled disc, echinocyte and spherocyte from a discoid configuration. But burst cells or no debris configurations were seen. After death there is rapid deterioration of the staining properties and marked morphological changes in many WBCs occurred rapidly. To the effects of autolysis lymphocyte seemed to be the most resistant and the Basophils the least resistant. As time passed after death morphologically alteration in platelets and aggregation were seen in each dead bodies. The present quantitative results and morphological observations suggests that various cells of the PM blood seem to be quite resistant to PM autolytic effects. When the dead body is kept at reduced temperature many cells apparently remain viabile for longer periods of time in the PM blood.

In their study Plat *et al.* (1989) found that when post-mortem duration is greater than 12 hour, the cells of cerebrospinal fluid become vacuolated and cannot be identified.

Babapulle *et al.* (1993) studied on correlation between time of death and PM cellular changes. They showed that PM cellular changes mainly nuclear changes in the WBC of non-refrigerated dead bodies could be compared with the time passed from death. According to them neutrophils degenerated rapidly; lymphocytes did so slowly; the Monocytes and Eosinophils degenerated in between. In PM blood total of identifiable leucocytes nearly decreases to 0 to within 84 hrs, identifiable Monocytes and Eosinophils were first disappear within 60 hrs, then neutrophils after 66 hrs and

finally lymphocytes. After 84 hrs of death lymphocytes disappeared. They also reported that the cells were normal in the first 6 hrs, then normal and abnormal between 6 and 72 hrs & beyond 72 hrs all blood cells became abnormal even difficult to identify them.

Wyler *et al.* (1994) reported that in cerebrospinal fluid the PM cell count can be correlated to the time since death and it can be described.

Henssge *et al.* (1995) stated that after death due to post-mortem putrefaction and autolysis blood cells lose their normal morphology and then became unidentifiable just like other tissue cells in the body. This changing process from normal morphology to unidentification period can be used as a useful aid for estimating PMI.

Dokgoz *et al.* (2001) did a comparison of morphological changes in WBCs after death and also in invitro storage blood for calculating PMI. Blood smears which were obtained from 10 non-refrigerated cadavers (experimental group) and from 40 hospital patients (control group) have been evaluated that identifiable degenerative changes were first observed in eosinophils at 6 hrs after death then became unidentifiable after 72hrs of death. In case of neutrophils identifiable degenerative changes were first observed at 6 hrs after death then became unidentifiable beyond 96hrs of death. The neutrophils, eosinophils and monocytes showed pyknosis of nucleus at 6 hrs, cytoplasmic and nuclear vacuolation at 12 hrs, nuclear fragmentation at 18 hrs then beyond 48 hrs there was disintegration of the cells. For lymphocytes identifiable degenerative changes were first observed at 24 hrs of death and were still identifiable beyond 120 hrs of death. In case lymphocytes at 24 hrs nucleus became swollen , cytoplasmic and cellular membrane became indistinct, at 36 hrs there was pyknosis of nucleus, at 72 hrs nuclear fragmentation was observed then at 96 hrs there was complete disintegration o lymphocytes occur.

In study a Kumar *et al.* (2015) concluded that in most of the PM cases the shape of RBC became dimorphic with peripheral area became pale within 12 - 24 hours post death. The normal central pallor lost in 18-36 hrs. Then after 24- 48 hrs there was lysis of most RBCs. These changes may be helpful in estimation of PMI which is a very simple procedure and interpretation is very easy.

Kundu (2017) studied in PM blood hemogram which can be act as an aid to determine PMI. For the experiment 84 cases were randomly selected ranging from 0-48 hrs post death with age group of 2.5 - 93 years. He found that haematocrit as well as haemoglobin values were not correlated with PMI. But TLC and TEC had strong correlation with time passed since death but they were inversely correlated. Even total no of Platelet strongly correlated with PMI.

2.1.5 Biochemical changes

Warren (1938) reported that in two cases when the defibrinated blood was kept at room temperature the blood sugar level decreased with time passed. There was of blood sugar in 26 hrs from 460 to 16 mg/dl in one case and also in 24 hrs 35 mins from 300 to 113 mg/dl in another case. When comparison was done between PM and AM blood the result showed that there was decrease in blood sugar with time passed since death but he could not give any formula for the rate of fall of blood sugar levels.

Hill (1941) found that after death in right auricle of normal animal, sugar concentration of rapidly raised which became 400 mg per cent within 2 hrs and became 720 mg per cent after 4 hours. But if the blood was collected from the left ventricle, it decreased to 28.7 mg per cent from 93.5 mg percent after 5 hrs of death. Fasting patients or animals or having severe liver damage did not show any PM rise of dextrose. According to him when blood was taken from normal humans at 37.5°C the rate of glycolysis was 12.8 mg% per hr. So he reported in hypoglycaemia suspected blood should be taken within 2 hrs of death.

Within first 24 hrs of death the pH of post-mortem blood decreases to 6.0 from 7.45. This decrease plasma pH is significant because fibrinolysin enzymes may activate which prevent blood clotting which results in in increase in fluidity. (noncoagulation) of the blood (Mole, 1948, Takeichi, 1986 and Takeichi, 1984).

Enticknap (1950) reported that after death amylase levels showed a biphasic rise or double peaks. Amylase concentration increased steeply from 100 units to 350 units within 6 hrs of death after that it decreased to 150 units after 30 hrs and again became a peak.

Hall (1958) evaluated that after death there is rapid increase in SGOT in intracardiac blood but in femoral blood he found little elevation from clinically fit patients. SGOT in their blood after 40 hrs become 350 units and then after 50hrs.

Jaffe (1962) used 31 cases who were free from electrolyte imbalance/ uraemia then correlated the vitreous potassium level to the time passed since death. According to his result there was consistent increase in the concentration of potassium after death and which continued for 125 hrs after death. According to him there was no significant difference between refrigerated bodies and those kept at room temperature.

Schleyer (1963) reported that the PM ammonia concentration in plasma increases rapidly after 8 hrs of death.

Mukherjee (1975) classified theatre death changes that could occur in the body i.e. chemical, physical, histological, bacteriological etc. which changed progressively . He further concluded that the at the time of death values of such parameters may not be known, also may differ from time to time, place to place, person to person. He also discussed some other PM changes like entomology of cadaver, post-mortem staining, changes in ocular and cerebrospinal fluid, ocular signs, changes in bone marrow cells, biochemical changes in the gastric contents, blood, hairs, condition of rectum and bladder, emptying time of intestine.

Schoning and Strafuss (1980) studied PMI by analysing blood, cerebrospinal fluid and vitreous humour of 60 euthanised dogs after 3, 6, 12, 24 and 48 hours which were maintained at 4, 20 and 37°C. The chloride value of cerebrospinal fluid decreased but phosphorus and potassium concentration slightly increased. Blood creatinine, and phosphorus potassium concentration raised whereas chloride and sodium concentration declined after 6 hrs of death. Blood carbon dioxide concentration declined to one fourth of concentration which was at the time of ante-mortem. The concentration of Glucose decreases to less than 50% after 3 hrs at any temperatures. The creatinine concentration in PM plasma was inconsistent.

By chemical analysis of vitreous humor Henry and Smith (1980) estimated the time of death. Their result showed that there was a linear rise of potassium in vitreous with of 12-100 hours of post death. This rate of increase of vitreous potassium was

fairly independent of environmental factors. But ammonia and magnesium in vitreous humor had no reliability.

In a study Mason *et al.* (1980) found that there was increase in PM potassium in vitreous humor with time passed since death. But there was no linear increase in rise.

On analysing McLanghlin and McLanghlin (1987) compared the vitreous humor collected from swine and cattle just after death and compared with serum samples collected from them. Serum and fresh incubated vitreous humour samples were analysed for concentration of Ca, Mg, Na, k, CI and P. In the incubated vitreous humor samples the stability varied for Ca, Mg, CI ,Na, k, and P valus whereas the concentration were more stable when samples were incubated at for shorter incubation periods and lower temperature. Potassium and phosphorus value raised temperature of incubation of the samples and with the time.

Blocks *et al.* (1988) studied the reliability and accuracy of the vitreous potassium concentration as an indicator of the post-mortem interval in cattle. Sampling not more than 1 ml of vitreous humour from the centre of corpus vitreous was found to improve both accuracy and reliability. In the study, they found significant correlation between post-mortem interval and the vitreous potassium concentration.

In a study Gallois-Montbrun (1988) reported that, NPN (non-protein nitrogen) and aspartic amino transferase activity, total soluble protein and creatinine level had significant correlation with the PMI.

Sharma and Gahlot (1997) have described that there are several physical changes occurring as time passed after death. They stated that it was not possible to estimate exact time of death and physical changes could only give an approximate value. So scientists now interested in PM chemical change that could provide an nearly accurate value of PMI.

Donaldson *et al.* (2013) reported after death there is very severe biochemical changes occur in all tissues of body as after death there is absence of circulating

oxygen, cellular degradation, cessation of anabolic production of metabolites, altered enzymatic reactions. These biochemical changes can act as chemical markers which helps for accurate determination of PMI.

In a study done by Donalson and Lamont (2013) concentration of six metabolites (lactic acid, hypoxanthine, uric acid, ammonia, NADH and formic acid) and blood pH changes in the PM blood were studied within 96 hrs of post death in blood taken from animal corpses i.e. rat and pig and blood collected from humans and rats stored in vitro. The pH and the concentration of all six metabolites changed after death but the rate and extent of change varied. Blood pH in corpses fell to 5.1 from 7.4. The level of ammonia, NADH, formic acid, hypoxanthine raised as time increases so these metabolites can be used as potential markers for time of death estimation. The level of lactate raised, then remained at an elevated level. But changes in the levels were different in the rat compared to the pig and human.

2.2 Carcass disposal

Carcass disposal is a growing and continuous for livestock farmers. There are many different methods used throughout the world to dispose of these carcasses. But in Indian condition mostly burial method is used in spite of more costly methods like rendering, alkaline hydrolysis, incineration, landfill etc. However, in burial method there is risk of ground water consideration so now a days it is restricted in many areas. Composting can be used which provides an economically and biologically safe means of carcass disposal. It is a process of converting carcasses resulting with other waste of livestock farm like poultry litter, paddy straw into an odourless, humus-like material which can be used as a soil amendment.

2.2.1 Burial method of disposal of carcass

Burial method is mostly used as it is generally easiest to organize, quicker, environmentally cleaner, cheaper, and often the most convenient means of disposing of large numbers of livestock (AUSVET Disposal plan, 1996).

By microbial analysis Davies and Wray (1996) reported that buried carcass of cattle which was experimentally infected with the pathogenic micro-organisms i.e. S. enteritidis, S. typhimurium, Clostridium perfringens and Bacillus cereus caused

severe contamination in the soil as well as nearby drainage within 1 week of burial of carcass. Again, the organisms which were used experimentally were isolated from the burial site for next 15 wks. Burial method is not satisfactory for disposal of anthrax infected animals which can infect most domestic animals.

Anthrax spores can move to the soil surface after a soil disruption like ploughing where as spores may reach the surface without any mechanical disturbance (Turnbull 2001).

Shearer (2006) stated that pit burial is the commonly used method of disposal in poultry and swine carcasses Pit burial is a popular method as it is an easy method to prepare and use. The areas where the water label is deep and the soil is non-porous are best for pit burial. Because of ground water contamination problem, burial pits should be carefully regulated and inspected. Individual burial is usually allowed but rules vary from state to state and from county to county. It is necessary to consult local officials for advice since there are often regulations as to the number of pounds of animal carcass per acre per year that may be buried. Large animals, such as cows and horses, are usually buried in a trench approximately 7 ft × 9 ft deep. Since a mature cow requires approximately 14 ft^2 of trench space and death rates may be as high as 5% or more but large herds may require alternate methods of carcass disposal.

2.2.2 Composting method of disposal of carcass

Turnbull and Snoeyenbos (1973) suggested that ammonia production may result in Salmonella inactivation in addition to the increased temperatures that occur in stacked litter,

Murphy *et al.* (1988) stated, chicken carcasses have been primarily treated using bin composting which is considered an in-vessel system but manures and other wastes have been composted using all varieties of techniques

Carcass composting began in the poultry industry during the late 1980s, where dead chickens were fully biodegraded in only 30 days (Murphy and Handwerker 1988).

In a field survey Blake *et al.* (1992) conducted in 12 mini-composters were monitored for the presence of pathogenic microbes. Throughout the survey, temperatures in all heaps were above the 55 ^{0}C threshold then *Campylobacter jejuni* Salmonella and *L. monocytogenes* were not detected in any of the samples.

United States Environmental Protection Agency (EPA) regulations state that for Class A designation, which means that pathogens are below detectable levels, static aerated compost heaps containing biosolids should be maintained at temperatures of 55^{0}C for 3 days (EPA, 1994).

Senne *et al*. (1994) evaluated survival of egg drop syndrome-76 virus and highly pathogenic avian influenza virus during composting. They used tissues from chickens infected with highly pathogenic avian influenza virus and tissues from chickens infected with egg drop syndrome76 virus distributed among the chicken carcasses. In a compost mixture, they included a ratio of 1part straw, 1-part carcasses, and 2 parts manure. They tested tissues at 10days of composting then turned the pile, again retested tissues 10 days later. After 10 days of composting the avian influenza virus was not detected. The egg drop syndrome-76 virus could be recovered at 10 days of composting but not after 20 days.

Composting is a method that can be used effectively to treat animal manures as well as carcasses. It is defined by Glanville and Trampel (1997) as a method where "biological degradation of organic materials occur under aerobic condition resulting into products consisting of water, ammonia, heat, carbon dioxide, a humus-like material consisting of, non-biodegradable inorganics and organic compounds that are resistant to rapid biodegradation, microorganisms."

Odorous liquids and gases diffuse into drier and more aerobic co-composting materials where they are ingested by microorganisms and then degraded into simpler organic compounds and ultimately to water and CO_2 (Keener *et al.,* 2000).

Poultry carcasses and waste require treatment for later disposal on the environment. They can be applied as organic fertilizer after by reducing the producer's costs and contributes to chemical, physicochemical, physical and biological soil properties with improving soil fertility (Luchese *et al.,* 2001).

The composting of poultry carcasses is important, as bacteria breakdown the carcass, except feathers and bones (Sander *et al.,* 2002). This method of disposal provides a better option for poultry waste treatment as compare to burial and rendering and also techniques that are expensive respectively.

Properly composted wastes are effective in the destruction of pathogens, viruses, weed seeds, and nematodes (Misra *et al.,* 2003).

Colorado (2003) reported that before composting carbonaceous bulking materials and grinding whole carcasses has been reported to decrease decomposition times by 30 to 60% and to decrease the amount of carbonaceous materials needed by a factor of approximately 16.

In his study Rynk (2003) found that carcass degradation time was decreased to 75 days for large carcasses that had been simultaneously ground and mixed with a carbon source and subsequently processed in a rotating drum composter. This method required about one-fourth of the composting material needed for bin or windrow composting.

Millner (2003) estimated that over 99% of parasites pathogens are killed when heated to 55°C for 3 consecutive days.

Gonzalez and Sanchez (2005) suggested that static heaps could successfully be used to compost straw, hen manure, and poultry mortalities.

Sherman (2005) stated that though composting may be conducted using a variety of substrates, there are guidelines for ensuring successful composting. One major parameter of compost that should be monitored is the carbon to nitrogen (C:N) ratio. It is stated that the C:N ratio of 20:1 – 40:1 is acceptable for composting, while 25:1 – 30:1 is preferred. If the right balance of these materials is not achieved, composting may be hindered, as microbes use carbon for energy and growth, while nitrogen stores are used for protein synthesis and reproduction. Another important parameter of composting is the moisture content of composting materials. Moisture contents in the range of 40 – 65% are acceptable, while 50 – 60% is preferred.

Poultry carcasses are commonly treated through burial, incineration and rendering, Rendering is r expensive and burial and incineration are detrimental to air and water quality. Composting poultry wastes is an inexpensive process in comparison to rendering, and functions to improve soil conditions. (Gonzalez and Sanchez, 2005)

In their study Cochran and Carney, (2006) found that other factors that will influence the composting process are pH and heap size. The optimal range for pH in composting is 6.5 – 8.0. Numerous microorganisms involved in composting allow for continuation of the process without disruption due to pH fluctuations.

Normally swine mortality compost is turned only once or twice. Turning speeds carcass decomposition, but research has shown that turning is not essential if the Carbon material used to cover the carcasses is sufficiently permeable for oxygen diffusion into the pile (Glanville *et al.,* 2006).

Harper *et al.* (2008) found that carcasses within a compost matrix are characterized by low C:N ratios (excess N), high moisture and no porosity, whereas the plant material surrounding them have relatively high C:N ratios, low moisture, and sufficient porosity.

According to Wiedemann (2008) composting is a good alternative method for disposing of daily mortalities, egg waste, spent hens. Under controlled systems and the correct management, good results can be achieved which is a safe. The nutrient rich soil amendment produced which can be reuse on-farm or sale to other farmers. Compost piles must be turned a minimum of three times and reach temperatures of 55°C for 3 consecutive days after each turning to pasteurise the material. The compost process must go for a minimum of six weeks.

Moruza (2009) reported that the carbon:nitrogen ratio (C:N) also affects the rate of biological activity. Carbon:nitrogen ratios of 15:1 to 35:1 are acceptable. If the C:N ratio is less than 25:1, organisms cannot utilize all of the nitrogen available, and nitrogen is then lost as ammonia. This results in an unpleasant odour, possible air pollution, and loss of potential fertilizer value. When the C:N ratio exceeds 30:1, the rate of composting decreases. Inorganic nitrogen such as urea or ammonium nitrate

can be mixed with the carbonaceous material to lower the C:N ratio to 30:1, or below. Temperature is a good indicator of biological activity in the compost pile, and is easily measured. Moisture content, oxygen availability, and microbial activity all influence temperature. Two or three days after wastes are mixed and placed in piles, thermophilic microbes should begin to dominate. These organisms prefer a temperature of 100 degrees F to 150 degrees F. Compost will be highly variable in nutrient content depending upon the amount and compassion of the manure and straw used, the age of the compost, and storage and handling.

Collins (2009) said that composting is a controlled biological decomposition process that converts organic matter to a stable, humus like product called compost. Composting offers a convenient and environmentally acceptable method of disposal of normal poultry flock mortality. The carbon:nitrogen ratio (C:N) greatly affects the rate of biological activity. Carbon:nitrogen ratios of 15:1 to 35:1 are acceptable and temperature is a good indicator of microbiological decomposition. Disease and insect problems are minimal, and ground or surface water contamination as a direct result of composting are practically nil. The composting process stabilizes ingredients to a useful organic fertilizer that will not attract flies, dogs or rodents. Compost will be highly variable in nutrient content depending upon the amount and quality of the manure and straw used, the age of the compost, and storage and handling.

Kiehl (2010) described that for the efficient compost process to oc, some parameters need to be monitored. Temperature, which correlates with physicochemical and microbiological parameters from composting, is a simple and inexpensive means for this purpose.

Seekins (2011) described temperature of 130^0 F in compost will result in pathogen and weed seed kill and will reduce significantly the vector attraction. Generally, temperatures in the static pile may be over 130°F (55°C) at the end of the 21 day period. Longer compost periods are recommended for a stable final product.

Bonhotal *et al*. (2012) observed that internal compost pile temperatures affect the rate of decomposition as well as the destruction of pathogenic bacteria, fungi, and some seeds. The most efficient temperature range for composting is between 104°F and 140°F (40°C and 60°C).

Composting a carcass does not require removal of the animal off-farm legally, which decreases the risk of biosecurity. Composting is an easy process as only the basic understanding of compost temperature, pH balance and time is all which needed to succeed in case of carcass composting.

Almost any animal carcass can be composted, whether it died via captive bolt guns, bullets, barbiturates or other means. Exceptions include animals infected with diseases caused by spongiform pathogens, such as scrapie and bovine spongiform encephalopathy. Sawdust, wood shavings, wood chips or a combination these is necessary to support the bacteria that help minimize odours. The material must be dry in order to absorb the decomposing fluids (Carcass Disposal Option: Composting 2013).

Ritz and Worley (2005) recorded that temperature of the range of 130 - 150 degrees F inside the compost pile indicate that the composter environment is suitable for composting and the carcass is decomposing well. During breaking down the organic material of in the compost pile the high temperature produced by microorganisms enhance the growth and reproduction of thermophilic bacteria that are especially good at digesting organic material. Ratio of 3:1 litter to mortality by volume ratio is desired for proper composting. The heat produced by the microorganisms contributes to their own growth and increase the decomposition process and helps to kill pathogenic microorganisms present inside the compost. When oxygen becomes decreases, the temperature of the compost will begin to fall. When the compost temperature drops below 130 degrees F, the compost can be turned. Moving the compost material aerates the mixture and revives the microorganisms so another heat cycle can occur, leading to a more complete breakdown of the compost. They also reported that the average values which available nutrient content of dead bird compost are: Total Nitrogen (N): 44 lbs/ton Phosphorus (P_2O_5): 65 lbs/ton Potassium (K_2O): 48 lbs/ton.

Composting is bio-secure as composting allows immediate year-round disposal of carcasses so that disease is not spread. There is no entry of off-farm vehicles that can bring disease in the farm from other operations and the high temperatures in the compost pile can kill pathogens, environmentally sound as properly functioning compost pile gives off no odour and does not harm or affect

groundwater. Composting transform a waste into a beneficial fertilizer and soil amendment, resulting in on-farm nutrient recycling, cost effective Composting has low to moderate start up costs and minimal operating costs and easy to accomplish Composting requires only good management and minimal training. It utilizes readily available organic materials (Morse, 2009).

Since weed seeds are usually destroyed at 62°C (144°F), thermophilic temperatures inactivate weed seeds, which may be present if the animals ingested weeds (Looper 2002).

Harper *et al.* (2002), Keener and Elwell (2000), and Langston *et al.* (2002) indicated that the rate of the decomposition process at thermophilic temperatures ranging from 40°C /105°F to 71°C /160°F is much faster than that of a mesophilic range of 10°C /50°F to 40°C /105°F.

As thermophilic temperatures eliminate pathogens more effectively than mesophilic or ambient process, cold weather does not seriously affect composting process when the piles are adequately sized and properly loaded (Kashmanian and Rynk, 1996).

Langston *et al.* (2002) indicated that a pH of 6.5-8.0 is optimal for dead swine composting.

CHAPTER III

MATERIALS AND METHODS

The present study was undertaken in the Department of Veterinary Pathology, C. V. Sc. & A.H., O.U.A.T, Bhubaneswar, Odisha.

3.1 STUDIES ON POSTMORTEM CHANGES FOR DETERMINATION OF PMI

Experimental design on various parameters of post mortem changes to determine postmortem interval is depicted in Table.1

Table 1. Experimental Design for studies on postmortem changes

Parameters of PM Change for PMI	Animals	No of Animals	Source	Sample	Time/No of Samples
Histopathology	Goat	20	Slaughter House	Liver	0hr-20 12hr- 20 24 hr-20
Molecular studies	Goat	3	Slaughter House	Brain	0hr-3 3hr-3 6hr-3 12hr-3 24hr-3 48hr-3 96hr-3
				Liver	0hr-3 3hr-3 6hr-3 12hr-3 24hr-3 48hr-3 96hr-3
Hematological	Goat	12	Postmortem case	Blood	0hr-12 6hr- 12 12 hr-12 18-hr-12 24hr-12
Serum Biochemical	Goat	6	Postmortem case	Serum	0hr-6 3hr-6 6hr- 6 12 hr-6 24hr-6

3.1.1 Tissue enzymatic study

Collection of material

Tissue samples like liver and heart were collected from 20 goats immediately after slaughter in slaughter house at an in interval of 6 hrs upto 12 hrs. Immediately after slaughter with help of sterile B.P. Then immediately 20 g of liver and heart sample were washed with sterile normal saline were processed for estimation of ALT & AST enzymes (at 0 hr) and designated as 0 hour sample which was equivalent to antemortem sample. The left heart and liver tissue were put in separate sterile beaker each (Fig. 1 & Fig. 2) and then kept in normal room temperature.

In same process sampling is done at 6 hour and 12 hour

Procedure

According to Gandhi and Patnaik (1997) the enzyme estimation (ALT & AST) of heart and liver samples was done in three step i.e. sonication, centrifugation and enzyme assay in air conditioned laboratory.

i) **Sonication:** 1 g of heart and liver sample was weighed on electronic balance for estimation of ALT and AST. In glass meat extractor the weighed liver and heart sample were minced with adding PBS of pH 7.2 @ 2 ml/g of sample. During mincing by filling chilled water in piston of meat extractor the temperature of 4-8^{0}C was maintained. After mincing the mixture was transferred into a cleaned glass beaker and then this beaker was kept in ice pack containing beaker such that 4^{0}C temperature was maintained during sonication. In ultrasonic processer sonication was done at 100 mA for 10 min.

ii) **Centrifugation:** The sonicated sample was transferred in to a test tube. In refrigerated centrifuge machine it was centrifuged a 4^{0}C and 10,000 rpm for 20 mins. Then the supernatant fluid was drawn and transferred in to a small pyrex sample vial (screw capped). Estimation of ALT & AST was done with that supernatant fluid i.e. tissue homogenate.

iii) **Enzyme assay:** Then with this homogenate of liver and heart sample In spectrophotometer concentration of ALT & AST was measured in spectrophotometer with kits supplied CPC Biomedicals Pvt. Ltd.

3.1.2 Histopathological studies

Collection of material

The liver, heart and kidney tissues were collected from 20 goats immediately after slaughter in slaughter house at an in interval of 6 hrs upto 24 hrs. Just after slaughter collection of sample was done with sterile BP blade (500 g of each organ tissue). At that time sampling of each tissue was done for 0 hr sample which was equivalent to antemortem sample and kept in 10% formalin in tightly capped jars. The liver, heart and kidney samples which were left after 0 hr sampling were kept in sterile beaker separately (Fig. 1 & Fg. 2) and remained as such in room temperature.

Sampling at 12 hrs and 24 hrs was done in same above discussed process.

Procedure

The representative portions of different organs with or without gross lesions were fixed in 10% formal saline solution and were processed by routine histological techniques. For that, first the collected tissues were trimmed into 1-2 mm thick sections and kept in 10% formal saline solution to be fixed properly. The fixed tissues were washed overnight washing in running tap water. Then dehydration of samples was done in ascending grades of alcohol i.e., 70%, 80 %, 90 %, absolute alcohol . After dehydration clearing was done with xylene. Then the tissues were embedded in paraffin maintained at a temperature of 58-60 degree Celsius in an incubator and paraffin tissue blocks were prepared with help of brass moulds. Tissue sections were cut at 4-5 µm thickness with help of microtome and stained by routine Hematoxylin and Eosin method (Anderson and Gordon, 1996). After staining the stained slides were mounted DPX mountant and with cover slip. For histopathological examination the prepared slides were examined under microscope.

3.1.3 Molecular studies

Collection of Material

Whole liver and brain samples were collected from 3 goats immediately after slaughter in slaughter house and immediately 1 mg of liver and brain sample were

taken with help of sterile BP blade and kept in Eppendorf of 1.5 ml containing RNA later and immediately kept in refrigerator at 5^0C. Then after 24 hours it was transferred to refrigerator of -20^0C. Then the remaining portion of brain and liver were kept in separate sterile beaker and covered with aluminum foil to prevent evaporation in an incubator at 37^0C (Fig. 3). Sampling was repeated at 3 hrs, 6 hrs, 12 hrs, 24 hrs & 48 hrs after slaughter in the same process.

Procedure

Sample collection and primers design

Aseptically samples were collected in RNA later of Sigma- Aldrich, St. Louis, MO, USA). Then for further use samples were preserved in refrigerator at -20^0 C. The beta actin primers BA1/BA2 (BA1-GAG AAG CTG TGC TAC GTC GC and BA2 – CCA GAC AGC ACT GTG TTG GC) ordered from Bioserve, USA (Collins *et al.,* 1995) were used in this study.

Extraction of RNA and c- DNA synthesis

Using TRIS reagent of Sigma-Aldrich, St. Louis, MO, USA, RNA extraction was done. These extraction was done according to the manufacturer's instruction. And the purified RNA was stored in -20^0C until further use. Using Nano drop ND-1000 spectrophotometer (by Thermo Scientific) the absorbance was measured (at 260 nm) and nucleic acid concentration in sample was quantified. By measuring the ratio of OD 260 nm/ OD 280 nm the purity of all the samples were checked. For c- DNA synthesis only those samples were taken which have ratio of 1.8-2.0. Then the RNA was treated with DNase 1 supplied by Fermentas, USA such that the chances of genomic contamination became minimum. After treatment inaction was done according to the manufacture instruction before reverse transcription. Briefly, 1 µg of RNA was added to a master mix containing 1 µL of DNase I (1U/µL), DNase I buffer (10X) and RNase inhibitor (1U/µL) each in a total reaction volume of 10 µL. Then for 30 min incubation was done at 37^0C. Then addition of 1 µL of EDTA (Fermentas, USA) was done and again incubated for 10 min at 65^0C such that DNase I enzyme was removed. The DNA free RNA was then incubated at 70^0C for 5 minutes with 1 µL of 20X RHP by Fermentas (Random Hexamer Primer, Fermentas), then cooling was done for 10 minutes at 25^0C. Then addition of dNTPs, RNase inhibitor b

Fermentas and Moloney Murine Leukemia Virus-Reverse Transcriptase by Sigma were done. After mixing the mixture was incubated in the thermocycler (Master cycler gradient Ependrof, USA at 25^0C for 5 minutes, then 1 hr at 42^0C and 2 minutes at 95^0C. cDNA was diluted 10 times with nuclease free water and 2 µL of this was taken as a template for quantitative PCR. Dilution of c DNA was done with nuclease free water 10 times then for quantification PCR 2 µL of this was taken as a template.

Semi-quantitative PCR analysis

Amplification of β- actin gene was amplified with the primers having the sequence BA1-GAG AAG CTG TGC TGC TAC GTC GC and BA2- CCA GAC AGC ACT GTG TTG GC. PCR was done with the steps i.e. initial denaturation for 2 min at 95^0C, followed by denaturation for 45 second at 95^0C, then annealing for 45 second at 55^0C, extension for 40 cycles for 1 minute 30 second at 72^0C and a final extension for 10 minutes at 72^0C. Then in 1 % agarose gels analysis of PCR products were done.

Quantitative PCR (qPCR) analysis

Using a set by Light Cycler 96 SW 1.1 supplied by Roche, Germany RT- q PCR was carried out. Preparation of a final reaction mixture of 10 µL was done with 0.5 µL or 5 pmole of each forward and reverse primers, 10 µL consisting of 5 µL of 2 × Fast Start Essential DNA Green Master by Roche Germany, 3 µL of PCR grade H_2O which was provided along with the kit. The q PCR process included the following steps i.e. a predenaturation step for 10 minutes at 95^0C and 40 cycles for amplification (denaturation for 10 sec at 95^0C, annealing for 10 sec at 59^0C, extension for 20 sec at 72^0C) followed by the melt curve analysis for 10 sec at 95^0C, for 60 sec at 65^0C, for 1 sec at 97^0C then cooling for 30 sec at $37\ ^0$ C. The Cq value for each sample was obtained and plotted against different time of post- sacrification.

3.1.4 Hematological studies

Collection of Material

For haematological study blood samples collected from 12 dead goats of apparently healthy herd immediately after death in accidental cases i.e. 0 hr which was equivalent to antemortem sample followed by series of collection at an interval of

6hrs upto 24hrs after death. Postmortem blood sample were collected by piercing jugular vein, femoral vein, Common Carotid artery and on dissection from chambers of Heart with the help of 5ml disposable syringe. About 5 ml of blood samples were collected and kept into an EDTA vial. To avoid hemolysis, the needle was removed from the syringe before transferring blood to the vial containing the anticoagulant. Additionally, the blood and the anticoagulant were mixed adequately by inverting the vial several times. The blood samples were processed shortly after collection. Then the blood was put for estimation of hematological parameters.

Procedure

Blood samples were examined to estimate Haemoglobin, Packed cell volume, TLC (Total Leukocyte Count), TEC (Total Erythrocyte Count), DLC (Differential Leukocyte count). pH of blood samples also estimated by Ph meter immediately after collection.

Estimation of Hb (gm/dl), TLC (10^3 / µL), TEC (10^6 / µL, DLC (%) were done as per the methods suggested by Coles (1986).

3.1.5 Serum enzymatic studies

Collection of material

Collection of blood samples were done from 12 dead goats of apparently healthy herd immediately after death in accidental cases i.e. 0 hr sampling which was equivalent to antemortem sample, followed by series of collection at an interval of 6 hrs upto 24hrs after death. Postmortem blood sample were collected by piercing jugular vein, femoral vein, Common Carotid artery and on dissection from chambers of Heart with the help of 5ml disposable syringe. Blood samples collected were kept in a slanting manner in the room temperature for 3-4 hours. Then it was centrifuged, serum was separated, labeled and kept in -20^0 C for further analysis.

Procedure

Serum samples were analyzed for estimating LDH, AST, ALT, ASP in autoanalyzer by using kits by CPC Biomedicals Pvt. Ltd.

3.2 DISPOSAL OF CARCASS

Experimental design of disposal of carcass by burial and composting method is depicted in Table. 2

Table 2. Experimental design for carcass disposal

Method of carcass disposal	Animals	No of Animals per bin/pit	Size of bean or pit used (length × Breath × height)	Materials used	Total time of decomposition	Sample	Days of sampling
Burial	Calf	1	3 ft × 3ft × 4 ft	Lime and Soil	12 months	Soil from burial site	0 day 4months 8 months 12 months
Compost-ing	Poultry	45	10 ft × 8 ft × 6 ft	Used poultry litter (paddy husk)	45 days	Compost	45 days

3.2.1 Disposal of carcass by burial method

For the disposal by burial method a burial pit was dug as deep as needed to cover the carcass completely and prevent scavenging by other animals (Fig. 4). One calf buried in a burial pit after detailed post-mortem examination. The size of the burial pit or trench was of 3ft wide, 3 ft length and 4ft deep ($3 \times 3 \times 4ft^3$). The carcass was transferred to the pit (Fig. 5). Layers of lime was applied below and above the carcass to help accelerate decomposition of the waste. Then it was covered with of soil which was not compacted. Surface of the pit was protected by fencing.

Sampling of soil

Sampling of the soil (about 50 g) under the burial pit was done to test the change in soil constituents at 0 day, 4months, 8 months & 12 months (Fig. 6).

3.2.2 Disposal of carcass by composting

Preparation of compost unit

i) Carcass for compost

Poultry birds those were presented to Deptt. of Vety. Pathology, C. V. Sc. & A. H , OUAT for post mortem examination were collected for composting.

ii) Co-composting materials

The co-composting material used were the used poultry litter collected from college poultry farm.

iii) Preparing static composting bean

The bean prepared on impermeable surface made up of concrete to prevent leaching (here we used the concrete roof of a soak pit) (Fig. 7). All the sides of the bean were closed by wire mesh to prevent scavenges or vermin (Fig. 8). The size of the bean prepared was 10 × 8 × 6 ft^3 (length × Breath × height). This was an innovative and economic compost been developed near the Postmortem hall of the department.

Procedure of composting

To supply the main ingredients (i.e. carbon, nitrogen, oxygen and water) in appropriate amount, carcass (dead birds), used poultry litter materials and water were used in correct ratio (1:2:0.25).

a) Litter material was placed on concrete roof of 1 ft height which absorbed the liquids and also provided a source of bacteria which helps in composting process.
b) Then an additional 6-inch layer of used litter material, was placed (it should not be too wet).
c) A single layer of birds were placed on co-compost material separately one by one (Fig. 9). Care was taken that none the carcass touched the side wall of bean and not closer than 6inches from the sidewalls of the bin which prevent seeping of fluids from the walls of the bin and temperature at the sides was also very less for decomposition.

d) Water was sprayed or poured on the carcasses so that they became wet but the co-composting materials under these carcasses was not too wet. According to the moisture content of the co-composting materials the amount of water to be poured was decided.

e) Then 6 inch of litter materials was placed on the carcasses such that the carcasses were covered completely (Fig. 10).

f) The above steps were repeated until the compost pile became 4-5 feet high. The thickness of final covering of litter materials was 10-12 inches because this extra thickness helps to maintain temperature and act as an insulation to the compost.

g) Then daily monitoring of the temperature was done with a long stemmed thermometer to ensure the rise in temperature. The temperature raised upto 130 to 150^0F within 48 hour (Fig. 11). When the temperature declined after 15 days then the pile was turned to areate the pile to hasten microbial degradation and to redistribute excess moisture (Fig. 12). The same process was repeated till there is complete degradation of the carcass.

h) After 45 days the decomposition was over when the carcass were completely mixed with co-composting materials (Fig. 13).

Collection of samples for testing compost

After proper mixing of the compost 50 g of compost sample was taken for the nutrient analysis at Department of Soil Science, College of Agriculture, OUAT (Fig. 14 to 17).

3.2.3 Testing of soil and compost

Table 3. Methods used for nutrient analysis of soil samples

Sl. No.	Nutrients	Methods used
1	Available Phosphorous	Olsen method (1954)
2	Available nitrogen	Modified Kjeldhal method (Jackson, 1993)
3	Organic Carbon	Walkley and Black rapid titration (Page *et al.*, 1982)
4	Available Potassium	Flame-Photometer using neutral normal ammonium acetate extracts (Jackson, 1962)
5	EC(Electrical Conductivity)	Conductivity Bridge Method
6	pH	Blackmans pH meter (Piper, 1966) with soil:water ratio of 1: 2.5

3.3 STATISTICAL ANALYSIS

The average value of hematological and biochemical parameters of the post mortem blood were compared with average values of samples taken at 0 hour (which was equivalent to antemortem samples) by one way ANOVA using SPSS 22 software and expressed as mean ± SE. The mean ± SE with $P \leq 0.05$ was considered as statistically significant.

CHAPTER IV

RESULTS

Present study was dealt with studies on post-mortem changes like haematological and enzymatic alterations of blood and tissue changes at microscopic and molecular level for accurate determination of post-mortem interval or time of death. Studies were also conducted on disposal of carcasses by burial and composting methods.

4.1 STUDIES ON POSTMORTEM CHANGES FOR DETERMINATION OF PMI

4.1.1 Tissue enzymatic study

The values (mean ±SE) of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentration in the liver and heart samples collected from 20 goats at 0-hour, 6-hours and 12-hours of slaughter are presented in the Table 4.

Table 4. Mean ±SE value of AST and ALT changes in liver and heart after death (P≤0.05)

Enzymes	0 hour	6 hours	12 hours
AST (IU/L) (Liver)	$67.65^{c} \pm 2.77$	$103.35^{b} \pm 3.49$	$131.20^{a} \pm 4.70$
ALT(IU/L) (Liver)	$269.10^{c} \pm 3.61$	$301.72^{b} \pm 4.59$	$336.25^{a} \pm 4.49$
AST (IU/L) (Heart)	$151.28^{c} \pm 3.44$	$174.99^{b} \pm 3.58$	$209.45^{a} \pm 5.47$
ALT(IU/L) (Heart)	$231.72^{c} \pm 2.00$	$268.05^{b} \pm 3.39$	$308.79^{a} \pm 4.45$

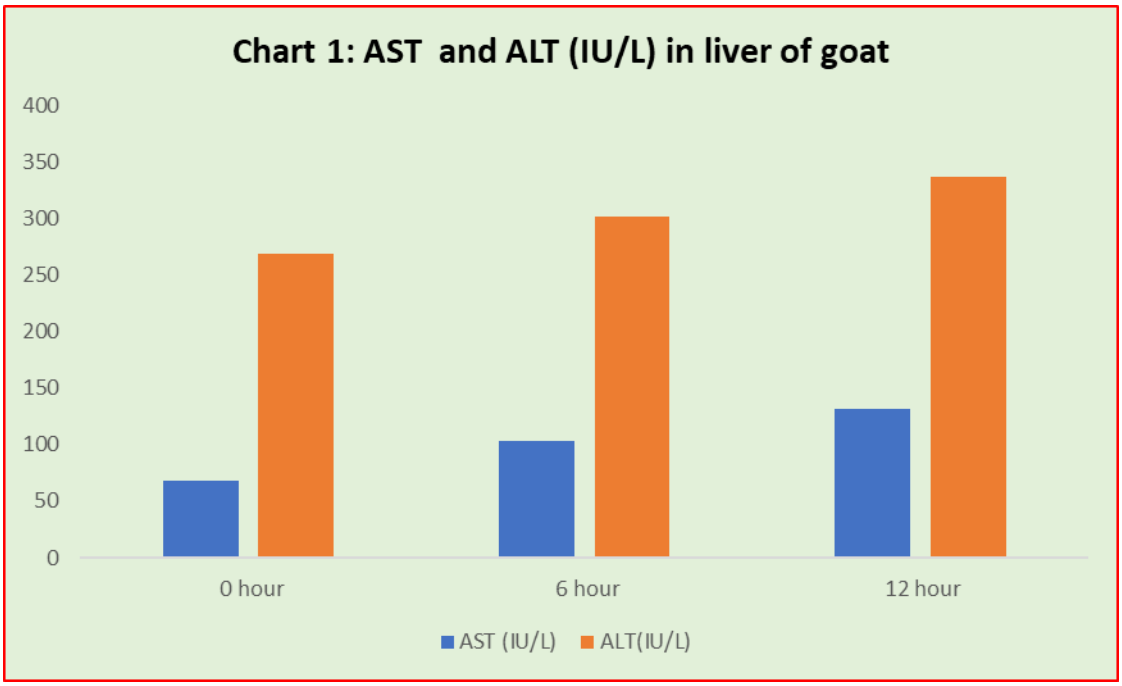
Aspartate aminotransferase (AST) concentration in liver

The mean values of AST level in the liver at 0-hour, 6-hours and 12 hours of slaughter were 67. 65±2.77, 103.35±3.49, 131.20±4.70 (Table 4, Chart 1) indicating significant increase at 6 hrs and 12 hrs (P≤0.05).

Alanine aminotransferase (ALT) concentration in liver

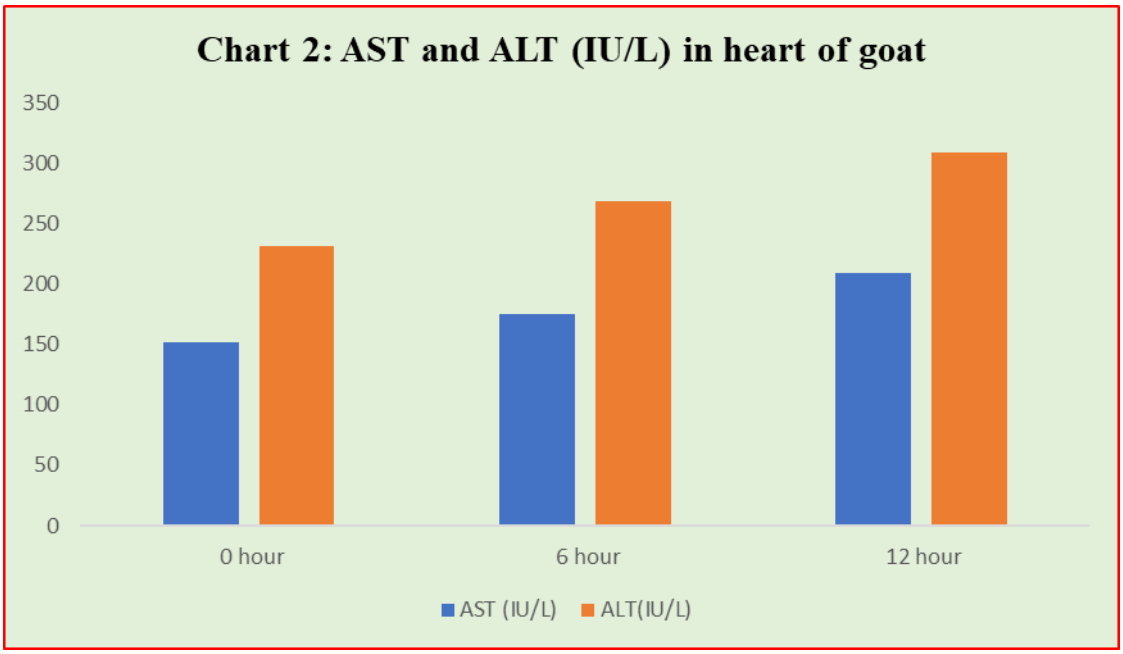
The mean values of ALT levels in the liver at 0-hour, 6-hours and 12 hours of slaughter were$269.10^{c} \pm 3.61$, $301.72^{b} \pm 4.59$ and $336.25^{a} \pm 4.49$ (Table 4, Chart 1)

showing significant increase from 0 hr to 12 hr (P≤0.05). The ALT concentration in the liver increased significantly at 6-hours as well as in 12 hours of slaughter (P≤0.05) than 0hr with a significant difference between the groups.



Aspartate aminotransferase (AST) concentration in heart

The mean values of AST level in the heart were151.28^{c}±3.44, 174.99^{b}±3.58 and 209.45^{a}±5.47 IU/L at 0-hour, 6 hour and 12 hours of slaughter (Table 4, Chart 2). The AST concentration in the heart increased significantly at 6-hours and 12 hours of slaughter than 0-hour (P≤0.05) and there is also significant difference between the hours.



The mean values of ALT level in the heart were 231.72^{c}±2.00, 268.05^{b}±3.39 and 308.79^{a}±4.45IU/L at 0-hour, 6 hours and 12 hours of slaughter showing significant increase at 6hrs and 12 hours than 0hr ($P \leq 0.05$) and there is also significant difference between the hours (Table 4, Chart 2).

4.1.2 Histopathological studies

Histopathologically, the liver specimens collected at 12-hours of slaughter showed condensation of nucleus along with increased granularity of cytoplasm in the hepatocytes (Fig.18 to19). At 24-hours of slaughter the liver showed disintegration of nucleus in some hepatocytes with disruption of chords and increase sinusoidal space (Fig. 20). In some places there was complete disintegration of nucleus of hepatocytes with many invading saprophytes (Fig.21). The heart specimens at 12-hours of slaughter showed heart showing pyknotic nuclei, loss of granularity of cytoplasm in myofibrils (Fig. 22). At 24-hours, the heart specimens showed loss of granularity polarity, detachment of myofibrils and pyknosis, disintegration of nucleus (Fig. 23). There was partial liquefaction in myofibrils at some places. At 12 hours kidney showed, cellular swelling with increase granularity of cytoplasm of tubular epithelium and disruption of tubular epithelium at many places (Fig. 24 to 25). There were swollen glomeruliin some places but architecture was maintained. At 24 hours disintegration and dissolution of nuclei of some glomerular epithelial cells with collapse of glomeruli (Fig. 26-to27). The architecture was disturbed and disruption of epithelium was prominent at some places.

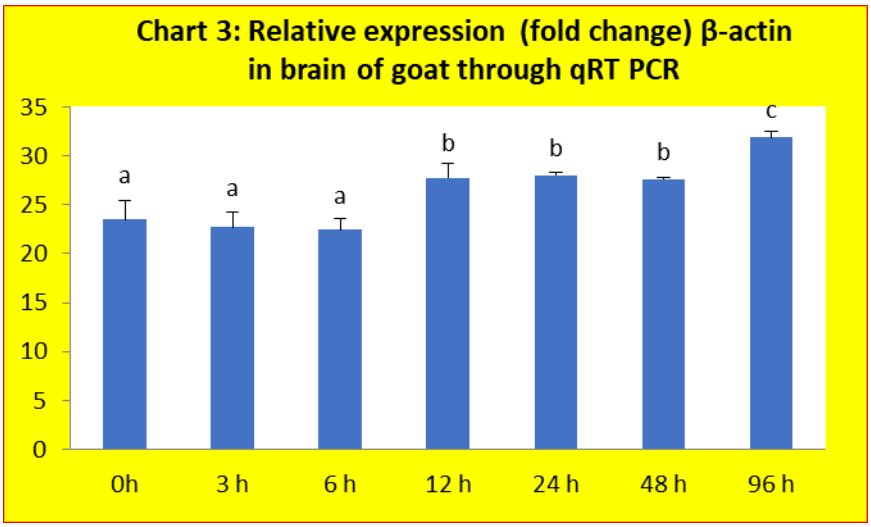
4.1.3 Molecular study

PCR amplification of β-actin was carried out with brain and liver tissues collected at different time intervals after sacrificing the goats using semi quantitative PCR. The expression of β-actin was slowly down regulated till 6 h of post-sacrification as compared to 0 h, and it could not be in detectable limit from 12 h till 96 h in liver tissue (Fig. 28). In brain tissue, the expression of β-actin was detected at all time periods. The highest level of expression was detected in 0 h which was gradually decreased in subsequent time periods having lowest expression at 96 h of post- sacrification (Fig. 29).

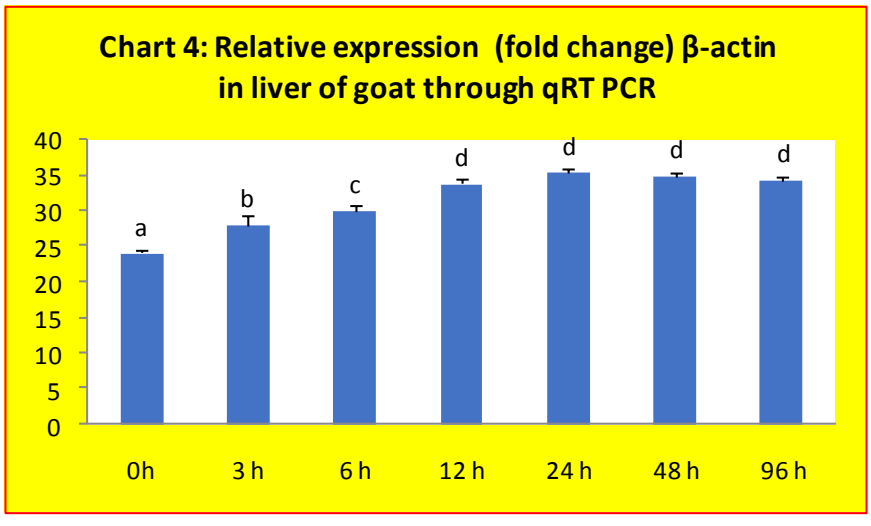
Real time q-PCR

The result was further validated using real-time PCR which is more sensitive as compared to semi-quantitative PCR. In liver tissue a significant decrease in expression was noticed from 0 hour to12 hours rand attends constant expression till 96 hrs. In brain tissue the expression was constant in 0 hrs, 3 hrs and 6 hrs of post- sacrification. As the RNA of dead animal started degrading after 6 hrs, the expression decreased at later time points with the lowest expression was detected at 96 hrs (Chart 3).

Brain	0 hr	3 hrs	6 hrs	12 hrs	24 hrs	48 hrs	96 hrs
Avg	23.49	22.74	22.47	27.77	28.02	27.61	31.9
SE	1.9	1.48	1.09	1.45	0.33	0.23	0.66



Liver	0 hr	3 hrs	6 hrs	12 hrs	24 hrs	48 hrs	96 hrs
Avg	24.09	28.05	30.03	33.84	35.4	34.95	34.35
SE	0.4	1.24	0.64	0.75	0.43	0.32	0.34



It has been suggested that estimation of time of death by studying postmortem degradation of nucleic acid act as an elegant alternative to the common classical methods. Indeed, besides the endogenous andexogenous ribonucleases, RNA degrades more rapidly after death because of chemical and thermal conditions.

In goat, general PCR results depicts that the RNA of liver tissue degraded more rapidly as compared to brain tissue after death. The amount of RNA in liver can be detectable upto 6 hrs after death whereas in brain tissue the RNA was intact and degrades slowly after 3 hrs of post death (Chart 4). In real-time PCR the time of death can be predicted upto 12 hrs whereas as in brain there is no gradual degradation observed. So, liver tissue found to be more effective to co-relate the time of death with RNA degradation till 12 hrs.

4.1.4 Hematological studies

For haematological study total 60post-mortem blood samples were taken in different time interval after death from 12 postmortem cases. Different haematological parameters like Hb, TEC, TLC, PCV as well as Ph of blood samples were studied. The average value of all haematological parameters at different time interval in post-mortem blood of goat were compared with 0 hour of death which is equivalent to antemortem blood.

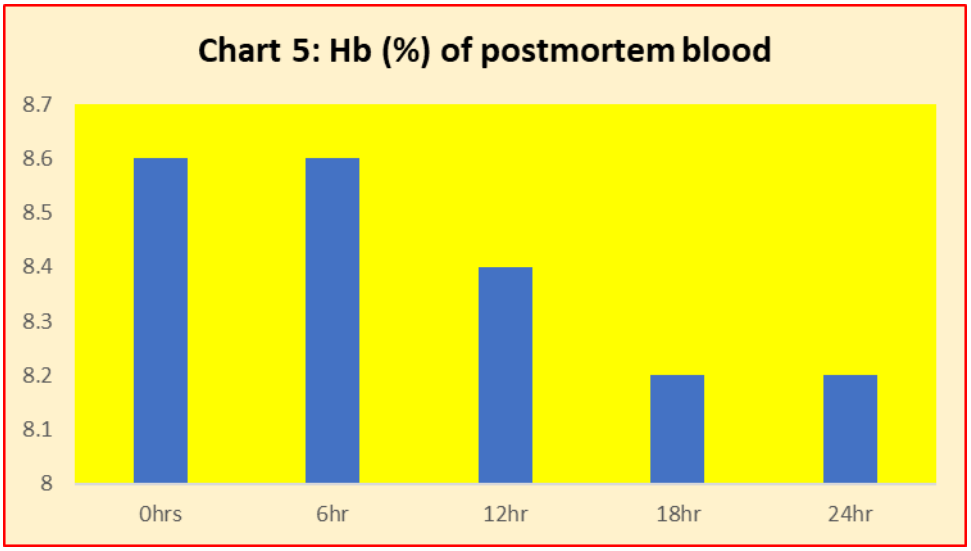
The average values of all haematologic parameters at different time interval (after death) are presented in table no.2. The mean±SE at different time interval considered as statistically significant with $P \leq 0.05$.

Table 5. Mean ±SE value of different Haematological Parameters of post-mortem blood in different time interval. (P≤0.05)

Haematological Parameter	0 hour	6 hours	12hours	18 hours	24 hours
Hb(g/dl)	10.62± 0.37	10.62±0.37	10.43± 0.37	10.23± 0.39	9.96±0.36
PCV (%)	30.50^{a}±0.95	29.75^{a}±0.99	26.75^{b}±1.12	22.50^{c}±1.17	17.42^{d}±0.48
TEC(10^{5} µl)	11.44^{a}±0.57	11.09^{a}±0.50	8.32^{b}± 0.41	5.00^{c}± 0.27	2.83^{d}± 0.25
TLC((10^{4} µl)	9.64^{a}±0.59	9.44^{a}±0.57	8.6^{a}±0.57	7.02^{b}± 0.51	5.17^{b}± 0.44
pH	7.42^{a}±0.04	7.27^{b}±0.05	6.99^{c}±0.07	6.55^{d}±0.12	6.12^{e}±0.07

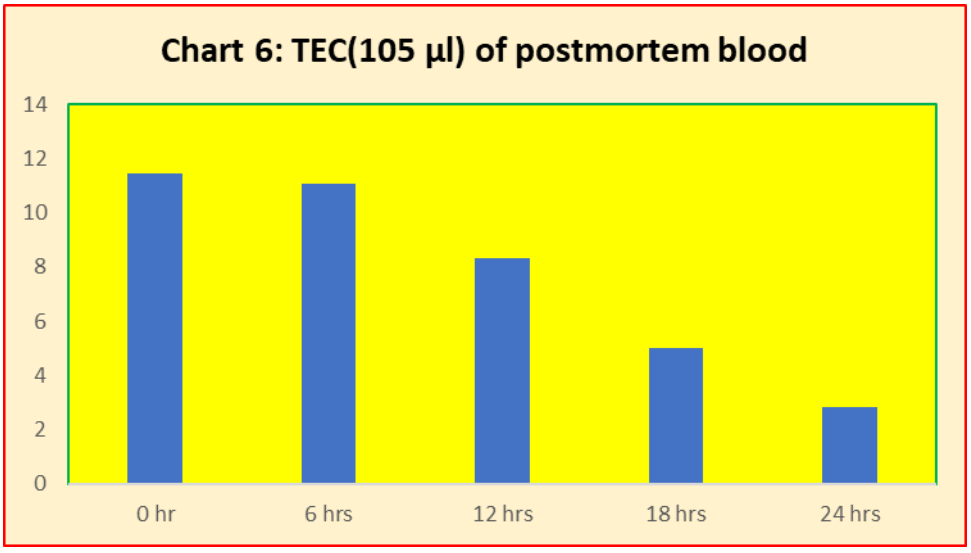
Haemoglobin

Haemoglobin (Hb%) values in , 6 hours, 12 hours, 18 hours and 24 hours post-mortem were 10.62±0.37, 10.62±0.37, 10.43±0.37, 10.23±0.39 and 9.96±0.36 indicating no much difference of Hb at different time interval as compared to 0hr which is equivalent to antemortem blood of death(Table5, Chart 5).



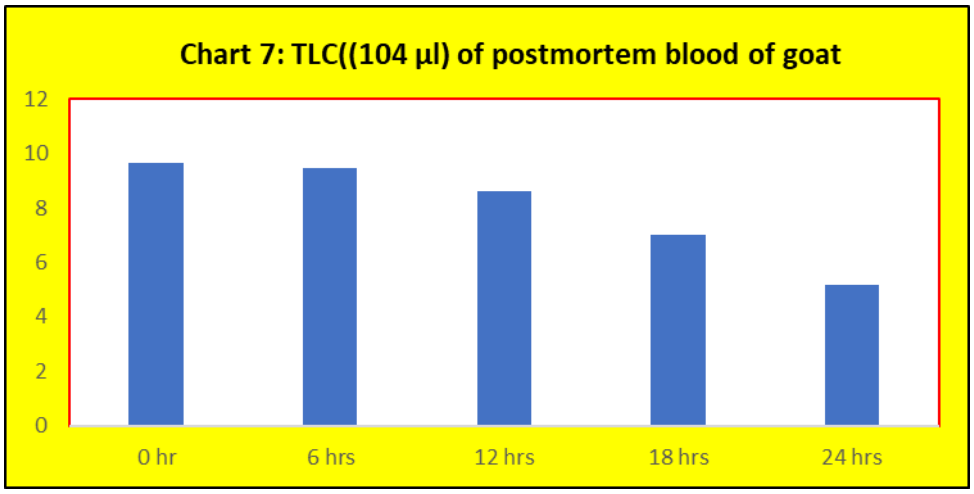
Total erythrocyte count (TEC)

TEC (10^5 μl) values in 0 hrs, 6 hrs, 12 hrs, 18hrs & 24 hrs of post-mortem were 11.44 ±0.57, 11.09 ± 0.50, 8.32 ± 0.41, 5.00 ± 0.27 and 2.83± 0.25 (Table 5, Chart 6) indicating decrease in TEC with increase in time after death. In this TEC different time interval were compared with 0hour of death which is equivalent to antemortem blood. Here there is no significant difference between 0 hour and 6 hour of post-mortem but there is significant difference after wards i.e.18 hour & 24 hour.



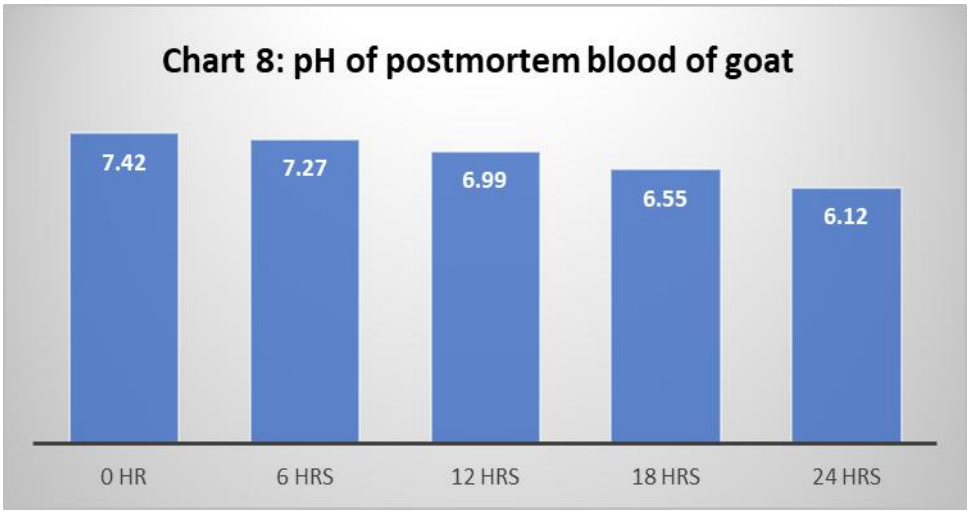
Total leukocyte count (TLC)

TLC (10^4 *µl*) values in 0 hour, 6 hours, 12 hours, 18 hours, 24 hours post-mortem were 9.64 ±0.59, 9.44 ±0.57, 8.6 ±0.57, 7.02 ± 0.51 and 5.17 ± 0.44 (Table 5, Chart 7) indicating there is significant decrease in TEC with increase in time after death (P≤0.05). In this TLC different time interval were compared with 0 hour of death which is equivalent to antemortem blood. Here there is no significant difference between 0 hour 6 hour and 12 hour of post-mortem but there is significant difference afterward i.e.18 hour & 24 hour.



pH

pH values in 0 hrs, 6 hrs, 12 hrs, 18 hrs & 24 hrs of post-mortem were 7.42 ±0.04, 7.27 ±0.05, 6.99 ±0.07, 6.55 ±0.12 and 6.12 ±0.07 (Table 5, Chart 8) indicating there is significant decrease in pH with increase in time (6 hours, 12 hours, 18 hours, 24 hours post-mortem after death) than 0hr with a significant difference between the groups (P≤0.05). In this pH different time interval were compared with 0 hour of death which is equivalent to antemortem blood.



Morphological changes of Leukocytes

There were marked morphological changes occur in the leukocytes of the post-mortem blood collected at different time since death (0 hr, 6 hrs, 12hrs, 18 hrs and 24 hrs).

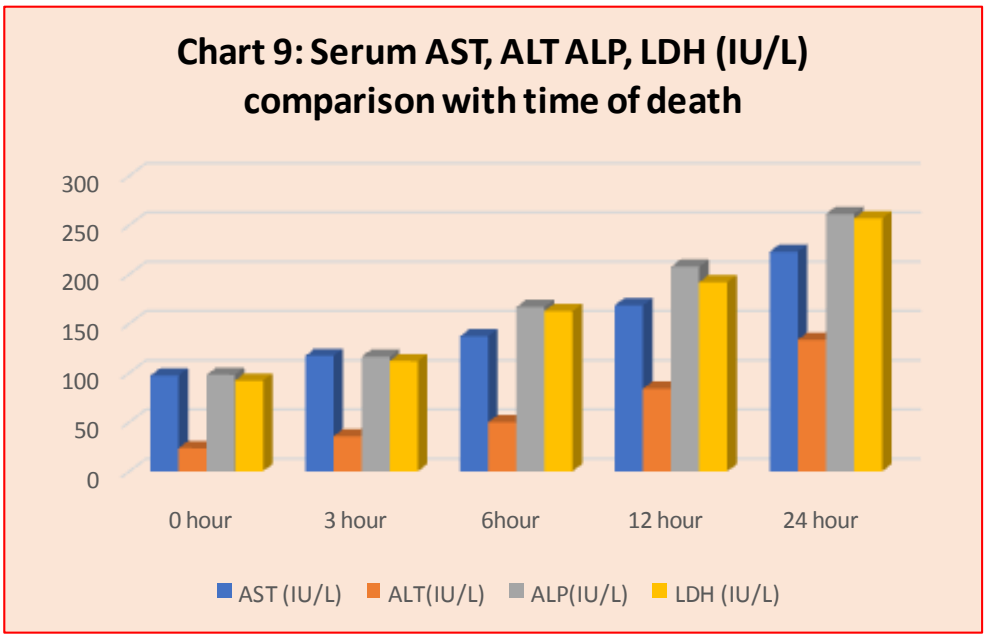
Identifiable degenerations of neutrophils, eosinophils and monocytes were first noticed at 6 hrs of death. At 6 hrs there was pyknosis of nucleus of neutrophils, eosinophils and also in monocytes. There was cytoplasmic and nuclear vacuolation in neutrophils as well as in eosinophils and monocytesat 12 hrs (Fig. 30 to 31). At 18 hrs there was nuclear fragmentation of neutrophils, eosinophils and monocytes (Fig.32 to 33). Beyond 24 hrs, disintegration and fragmentation of nucleus in these cells were evident. But lymphocytes were quite stable than other leukocytes (neutrophils, eosinophils and monocytes) and the first identifiable degeneration was noticed in lymphocytes at 24 hrs post death.

4.1.5 Serum enzymatic study

Serum samples of total 30 were analyzed for different serum enzymes i.e., ALT, AST, LDH, Alkaline phosphatase from 6 post-mortem cases. Serum samples were taken in different time interval (0 hour, 3 hours, 6 hours, 12 hours and 24 hours) after death. The analysis was done as per instruction of the equipment. The average values of all biochemical parameters at different time interval (after death) are presented in(Table no.6 and Chart 9). The values of mean ±SE in different time interval considered as statistically significant ($P \leq 0.05$).

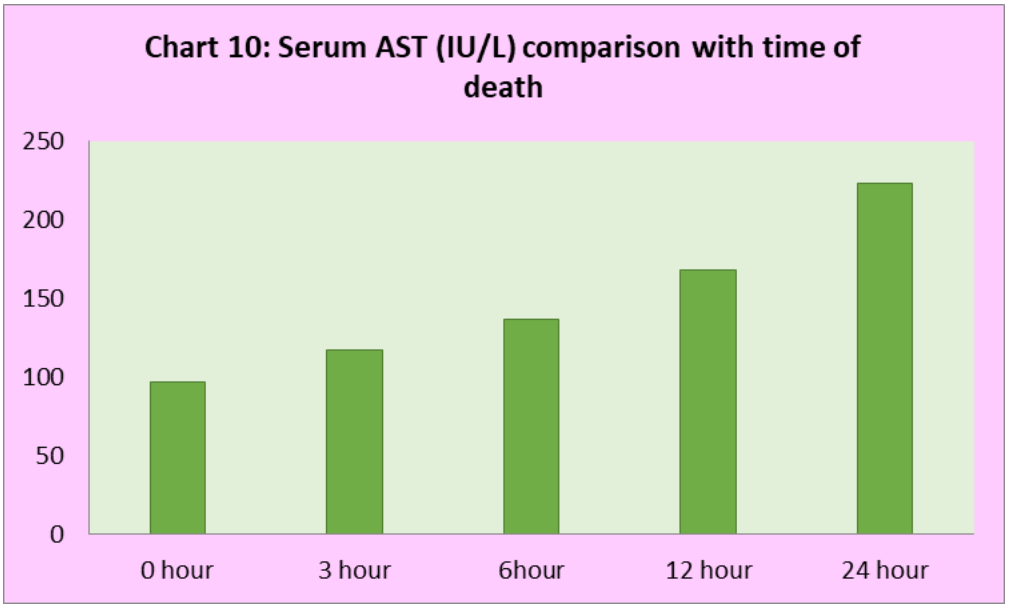
Table 6. Mean±SE value of different Biochemical enzymes of serum in post-mortem blood (P≤0.05)

Biochemical Parameter	0 hour	3 hours	6hours	12 hours	24 hours
AST (IU/L)	97.33^{d}± 6.33	117.17^{cd}±9.29	137.00^{c}±8.09	168.50^{b}±8.25	222.83^{a}±12.79
ALT(IU/L)	23.5^{e} ±1.74	35.50^{d} ±2.40	49.83^{b}±5.19	83.83^{b} ±4.35	133.50^{a}±4.70
ALP (IU/L)	98.00^{d}±12.12	116.17^{d}±14.65	166.67^{c}±9.15	207.67^{b}±12.38	261.17^{a}±6.44
LDH (IU/L)	92.00^{d}± 9.51	111.83^{d}±6.19	162.6^{c}7±4.35	192^{b}±4.43	256.67^{a}±6.28



Aspartate aminotransferase (AST)

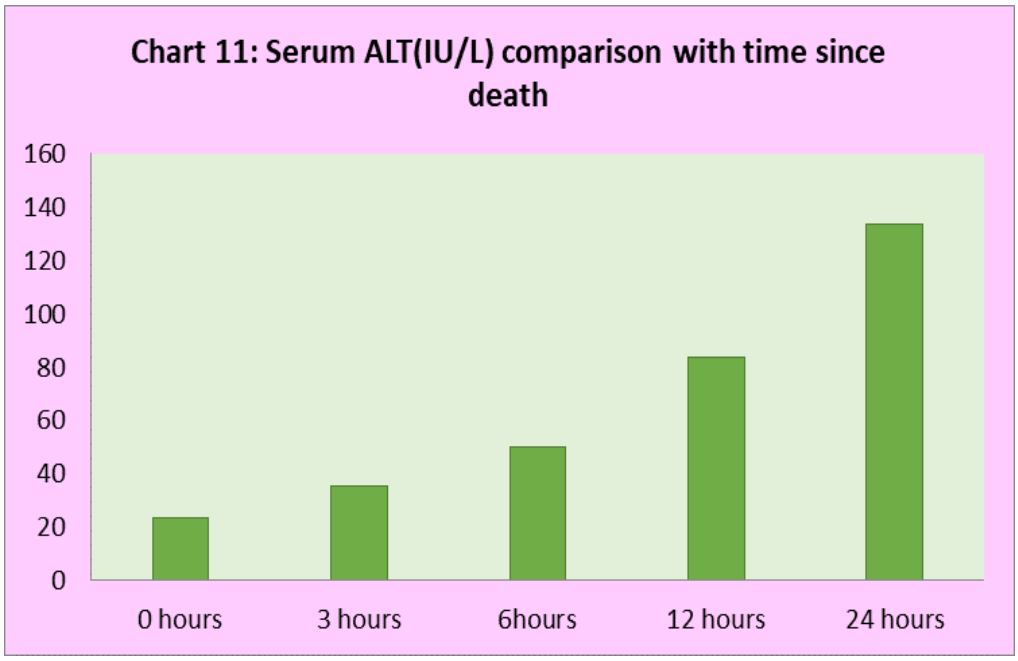
Serum AST (IU/L) values at 0 hrs, 6 hrs, 12 hrs, 18 hrs & 24 hrs post-mortem were 97.33 ± 6.33, 117.17± 9.29, 137.00± 8.09, 168.50 ± 8.25 and 222.83 ± 12.79 (Table.6, Chart 10). There was significant increase in serum AST values at 3hrs, 6hrs, 12hrs and 24hrs ($P \leq 0.05$) than 0hr with a significant difference between the groups.



Alanine aminotransferase (ALT)

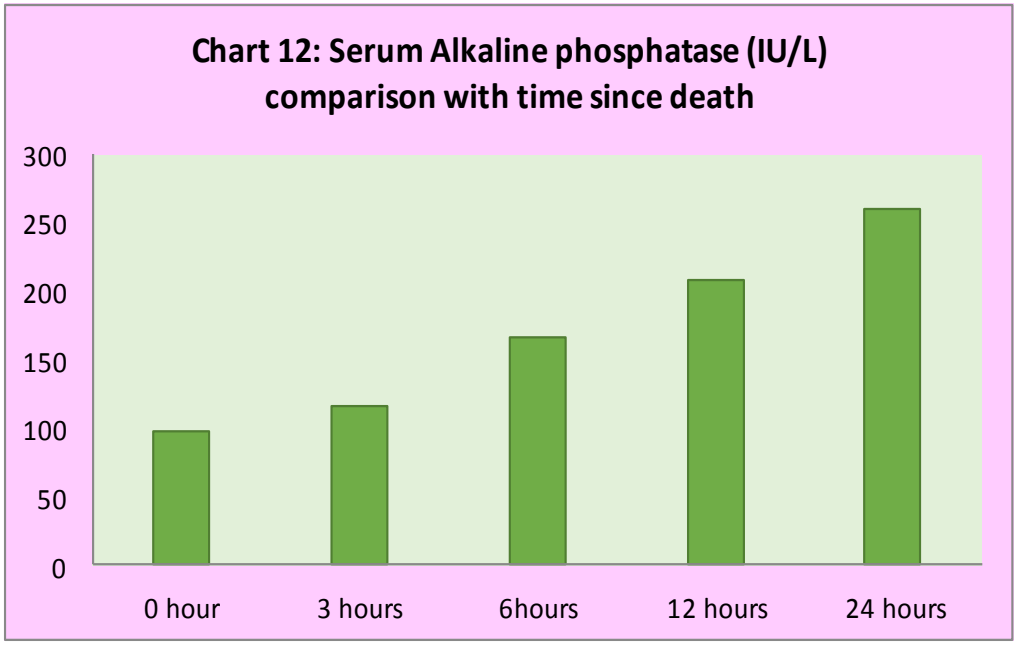
Serum ALT (IU/L) values in 0 hrs, 6 hrs, 12 hrs, 18 hrs & 24 hrs post-mortem were23.5±1.74, 35.50±2.40, 49.83±5.19, 83.83±4.35 and 133.50±4.70 (Table. 6,

Chart 11). These values of different time interval showed significant difference and also there was significant increase at 3hrs, 6hrs, 12hrs and 24hrsthan 0hr (P≤0.05).



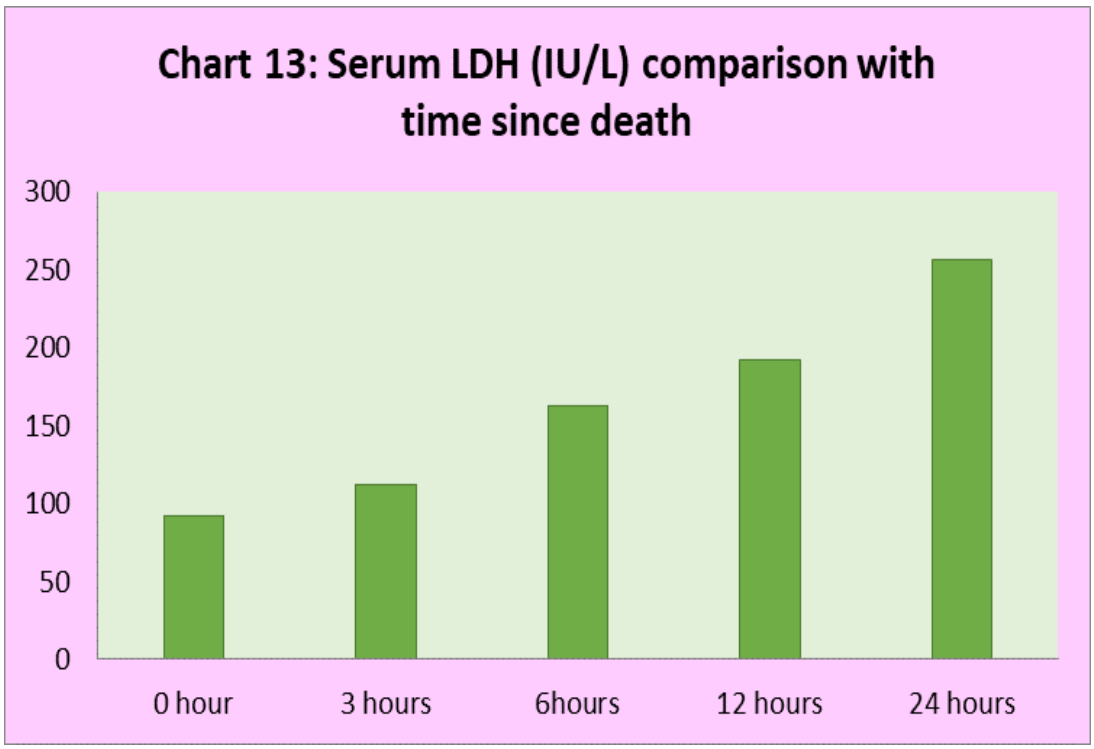
Alkaline phosphatase

Serum alkaline phosphatase (IU/L) values in 0 hrs, 6 hrs, 12 hrs, 18 hrs & 24 hrs post-mortem were 98.00 ± 12.12, 116.17 ±14.65, 166.67 ±9.15, 207.67 ±12.38 and 261.17 ±6.44 (Table 6, Chart 12). There was significant increase in serum Alkaline phosphatase values at3hrs, 6hrs, 12hrs and 24hrs than 0hr (P≤0.05) with a significant difference between hours.



Lactate dehydrogenase (LDH)

Serum LDH (IU/L) values in 0 hrs, 6 hrs, 12 hrs, 18 hrs and 24 hrs of post-mortem were 92.00 ± 9.51, 111.83 ±6.19, 162.67±4.35, 192 ±4.43 and 256.67±6.28 (Table. 6, Chart 13). There was significant increase in serum LDH values at 3 hrs, 6hrs, 12 hrs and 24hrs than 0hr with a significant difference between the groups of 6 hrs, 12 hrs & 24 hrs ($P \leq 0.05$). However there was no significant difference of LDH value between 0 hour and 3 hours interval.



4.2 DISPOSAL OF CARCASS

4.2.1 Disposal of carcass by burial method

After 4 months of burial the soft tissues were in process of decomposition or in a stage of active decay. The bones and hairs remained as such. During this stage the carcass had a characteristic wet appearance. A strong odour of putrefaction was associated with the carcass. Then at 8 months there was almost complete decomposition of internal organs and other soft tissues with remnants of bones and cartilage. The carcass lost most of the mass during this stage with lesser odour. At 12 months there was complete decomposition of soft tissues and carcass that has mixed with the soil leaving few remnants of long brittle bones. There was little to no odour associated with remains in the soil.

The changes in soil nutrient (0 month, 4 months, 8 months, 12 months) of the soil sample taken from the burial site are presented in Table.7

Table 7. Changes in soil parameters at different interval in burial method

Soil Parameter	0 month	4 months	8 months	12 months
pH	6.42	7.66	8.2	7.81
Electrical Conductivity (Ds/m)	0.012	0.059	0.22	0.14
Organic Carbon (g/kg)	5.07	7.62	1.82	1.7
N(kg/ ha)	294	486	433.44	555.63
P (kg/ ha)	25.4	72.3	79.73	24.14
K (kg/ ha)	282	203	199	188
S (kg/ ha)	16.7	104	0.0163	0.0021
Ca (kg/ ha)	2.15	9.7	9.1	8.9
Mg (kg/ ha)	0.29	1.084	1.4	1.1

The pH and Electrical Conductivity (EC) of the soil increases gradually up to 8months and then there was slight decrease at 12months. As EC increased up to 8 months indicating increase in nutrients of soil which is decreased afterwards. All other nutrients like N, P, K, S, Ca, Mg increased highly at 4 months. The N increased gradually till 12 month. But there was decrease in P and Organic Carbon (OC), S, K 8 months as well as in 12 month.

3.2.2 Disposal of carcass by composting

Composting was complete within 45 days. Around one tractor load of compost could be produced during the study period. The prepared compost was dark brown in colour (Fig. 13) and having no soft tissue or bones fragments larger than 15 cm or 6 inch in any dimension. Smaller bone fragments were brittle. The resulted compost was devoid of any offensive odour. The nutrient analysis of the compost was done to evaluate the fertility as biofertilizer.

Result of analysis of the compost sample is expressed in the Table 8.

Table 8. Values of different parameters of compost

Parameters of the compost	Value
pH	7.9
EC	1.32
Moisture(%)	19
Total organic carbon (C)(%)	25.2
Total Nitrogen (N)(%)	2
Total phosphorous (P_2O_5)(%)	4.58
Total Potash (as K_2O)(%)	3.28
C:N Ratio	12
S (%)	0.73
Ca(%)	3.2
Mg(%)	1.9
Cd (mg/kg)	1.01
Pb (mg/kg)	7
Zn(mg/kg)	40
Cu(mg/kg)	Not detected
Cr(mg/kg)	1.1
As(mg/kg)	Not detected
Hg(mg/kg)	Not detected
Ni(mg/kg)	Not detected

The C:N ratio of compost was 12:1 which was in between the reference value (20:1 or less).All other nutrients like total organic carbon(C) (%), total nitrogen (N) (%), total phosphorous (P_2O_5)(%), total Potash (as K_2O) (%), S (%), Ca(%), Mg(%) were 25.2%, 2%, 4.58%, 3.28%, 0.73%, 3.2% and 1.9% respectively which were in between the standard values of the compost (organic fertiliser)issued by govt. of India.(Schedule IV). The toxic parameters like As, Hg, Ni were not detected. But Cd, Cr, Pb and Zn were 1.01 mg/ kg 1.1 mg/ kg, 7mg/kg and 40 mg/kg respectively which were much less than the maximum permissible values.

Fig. 1: Liver kept in sterile beaker

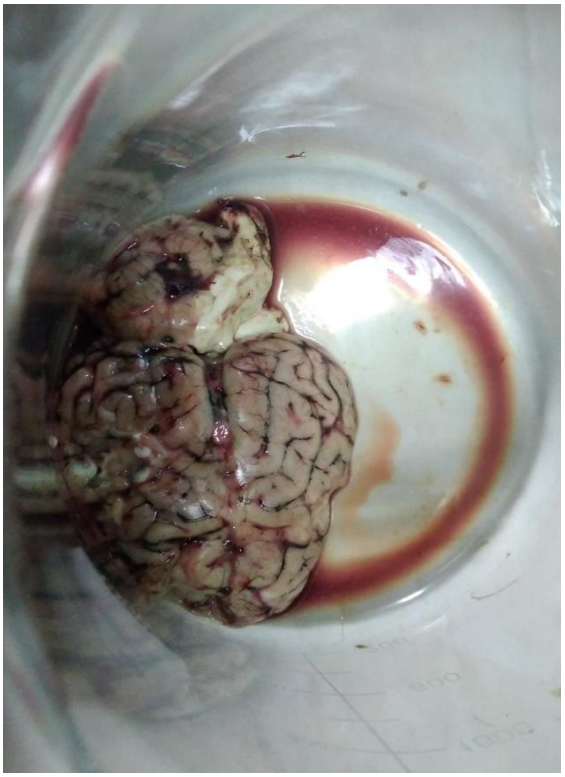


Fig. 2: Brain kept in sterile beaker

Fig. 3: Liver and brain samples kept in incubator

Fig. 4: Burial pit for carcass disposal

Fig. 5: Carcass disposed in burial pit

Fig. 6: Sampling of soil from burial pit

Fig. 7: Soak pit on which composting bin was prepared

Fig. 8: Prepared compost bin

Fig.9: Carcasses on co-composting materials

Fig.10: Carcasses covered with co-composting materials

Fig. 11: Measuring temperature of compost

Fig. 12: Turning of compost

Fig. 13: Finished compost

Fig. 14: Weighing of soil sample

Fig. 15: Analysis of soil sample

Fig. 16: Analysis of soil sample

Fig. 17: Analysis of soil sample

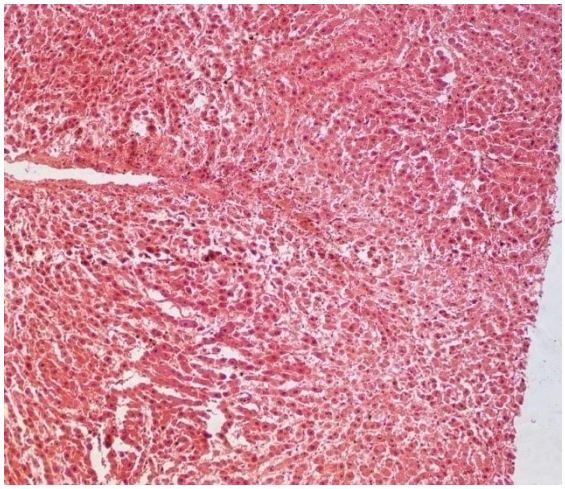


Fig. 18: Photomicrograph of liver showing condensation of nucleus with increase granularity (H & E × 100)

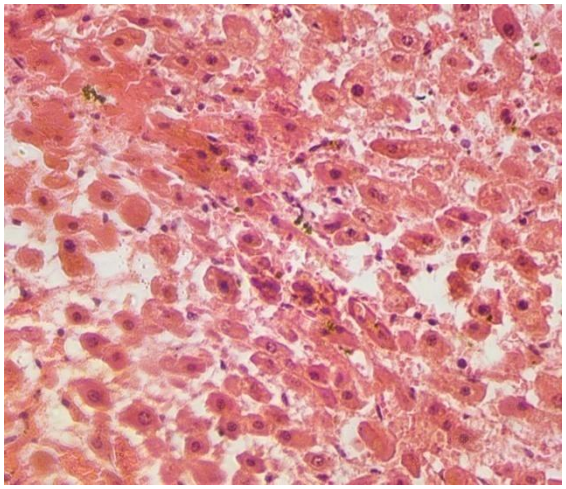


Fig. 19: Photomicrograph of liver showing condensation of nuclei in hepatocytes (H & E × 400)

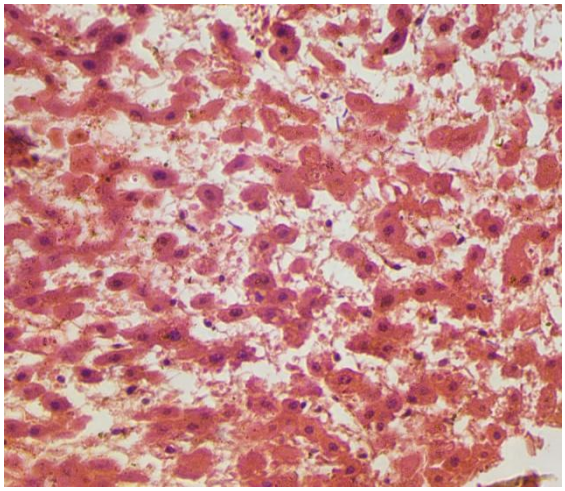


Fig. 20: Photomicrograph of liver showing disintegration of nucleus in some hepatocytes with disruption of chords and increase sinusoidal space (H & E × 400)

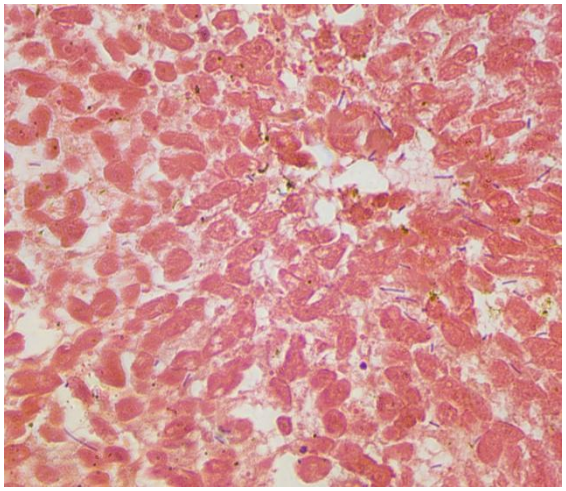


Fig. 21: Photomicrograph of liver showing complete disintegration of nucleus with many saprophytes (H & E × 400)

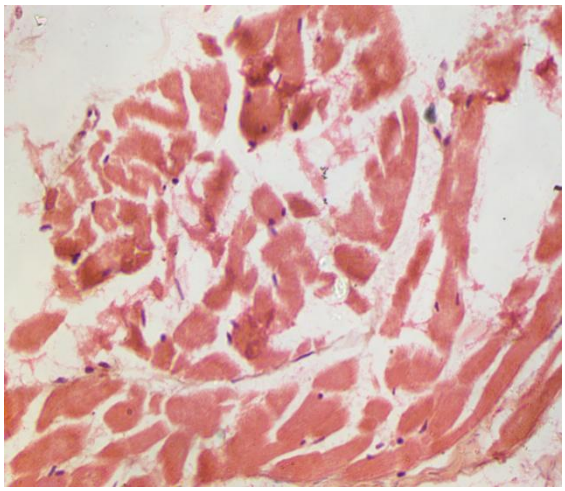


Fig. 22: Photomicrograph of heart showing pyknotic nuclei, loss of granularity of cytoplasm in myofibrils (H & E × 400)

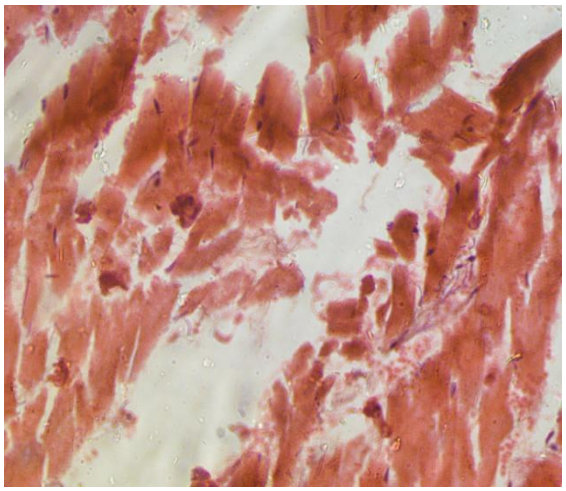


Fig. 23: Photomicrograph of heart showing loss of granularity polarity, detachment of myofibrils and pyknosis,disintegration of nucleus (H & E × 400)

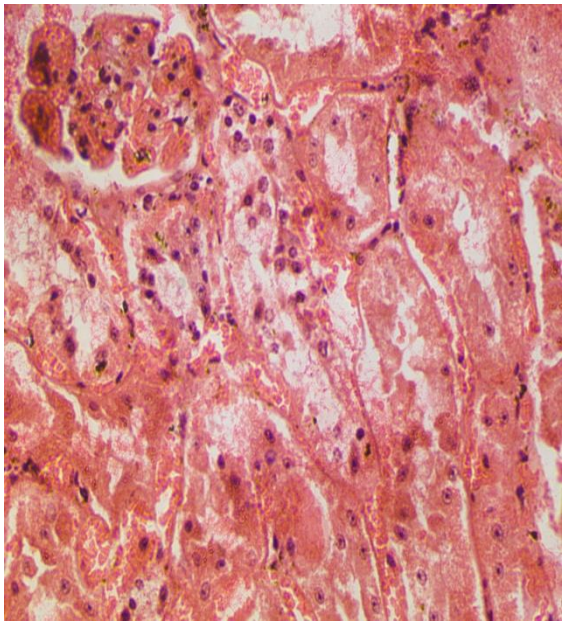


Fig. 24: Photomicrograph of kidney showing cellular swelling with increase granularity of cytoplasm of tubular epithelium and disruption of tubular epithelium at many places (H & E × 400)

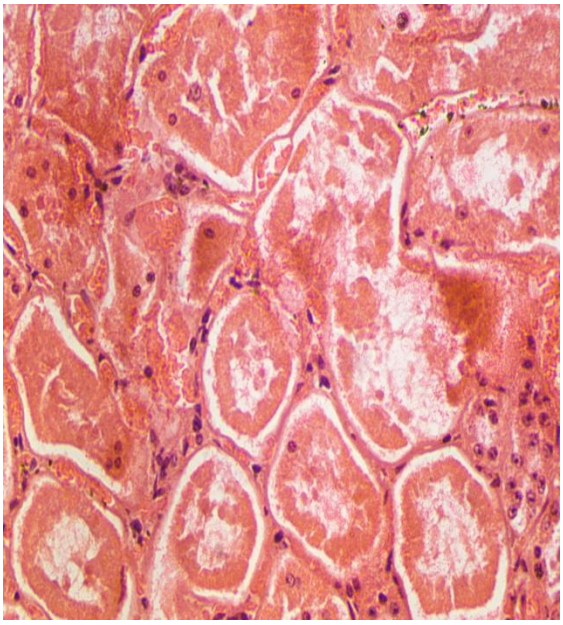


Fig. 25: Photomicrograph of kidney showing disintegration of tubular epithelium (H & E × 400)

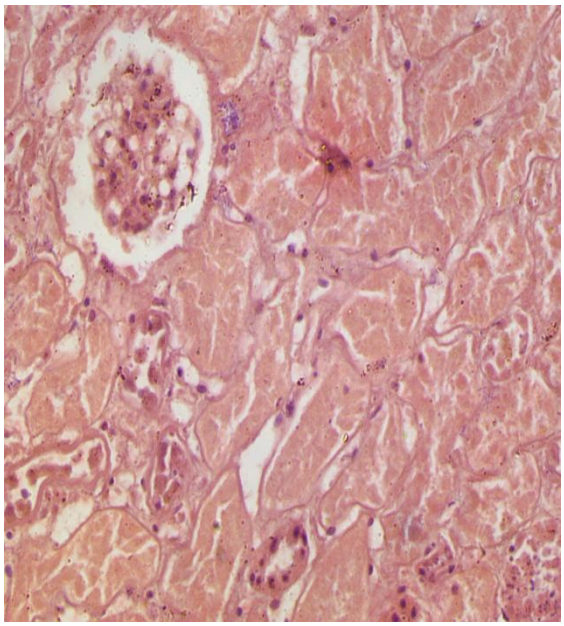


Fig. 26: Photomicrograph of kidney showing disintegration and dissolution of nuclei of some tubular epithelial cells with collapse of glomeruli (H & E × 400)

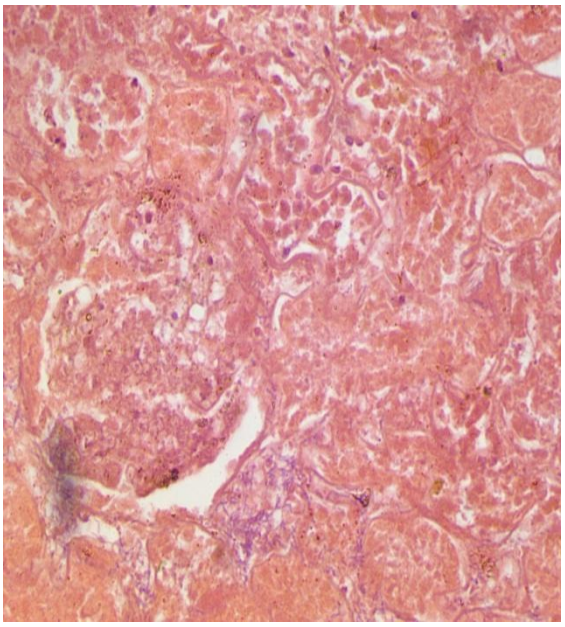


Fig. 27: Photomicrograph of kidney showing complete disintegration and dissolution of nuclei of tubular epithelial cells with collapse of glomeruli (H & E × 400)

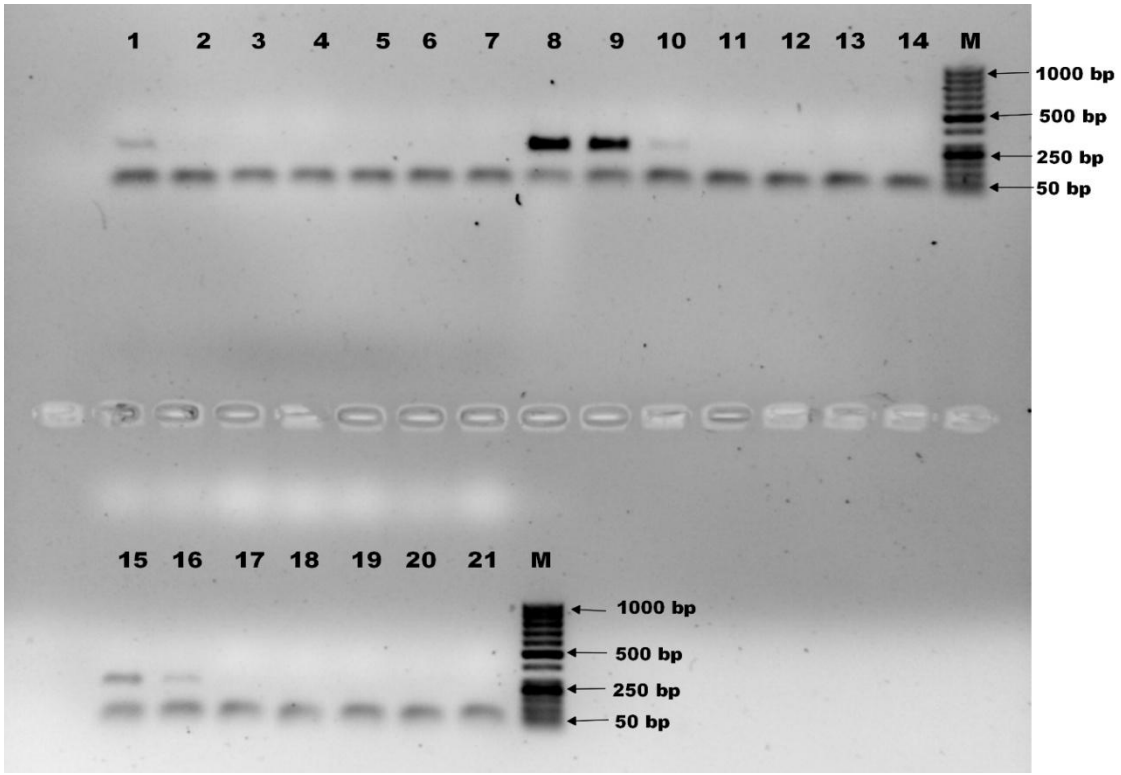


Fig. 28: Agarose gel electrophoresis (1.2%) showing PCR amplification of β-actin gene of liver

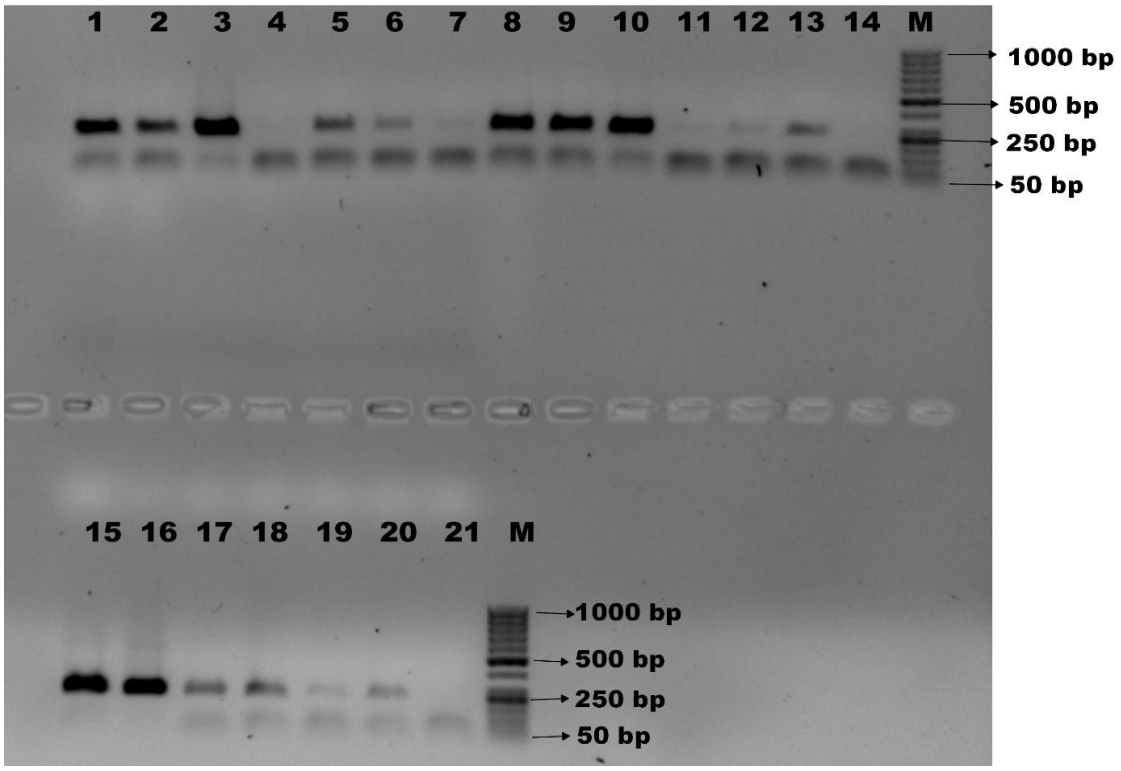


Fig. 29: Agarose gel electrophoresis (1.2) showing PCR amplification of β-actin gene of brain

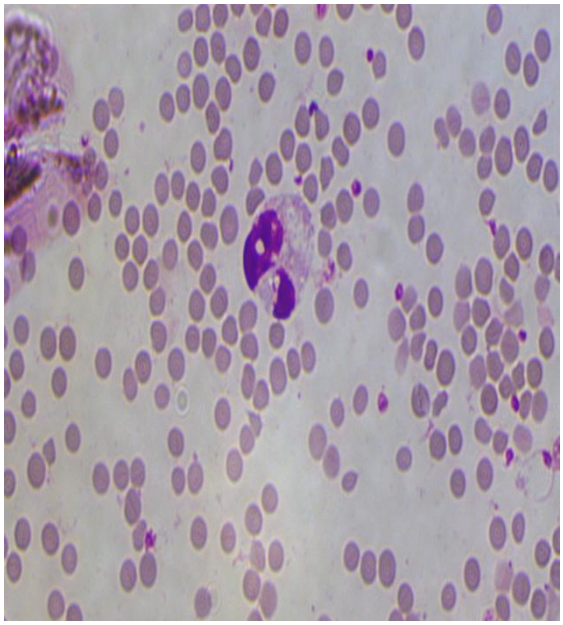


Fig. 30: Photomicrograph of blood smear showing cytoplasmic and nuclear vacuolation of neutrophil (1000X)

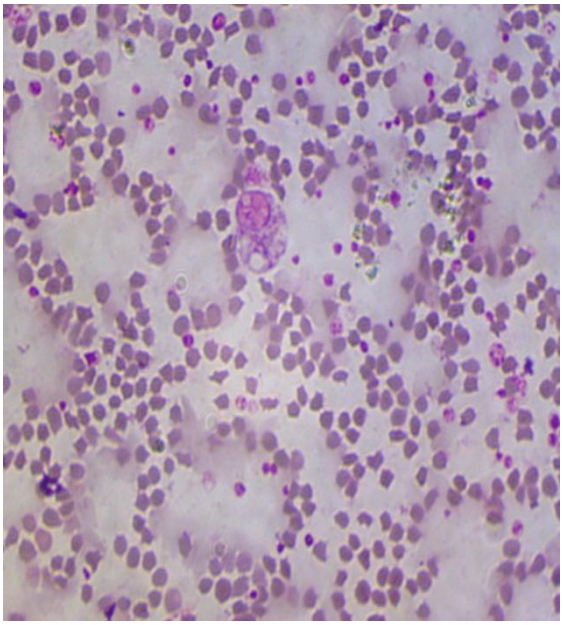


Fig. 31: Photomicrograph of blood smear showing cytoplasmic and nuclear vacuolation of monocyte (1000X)

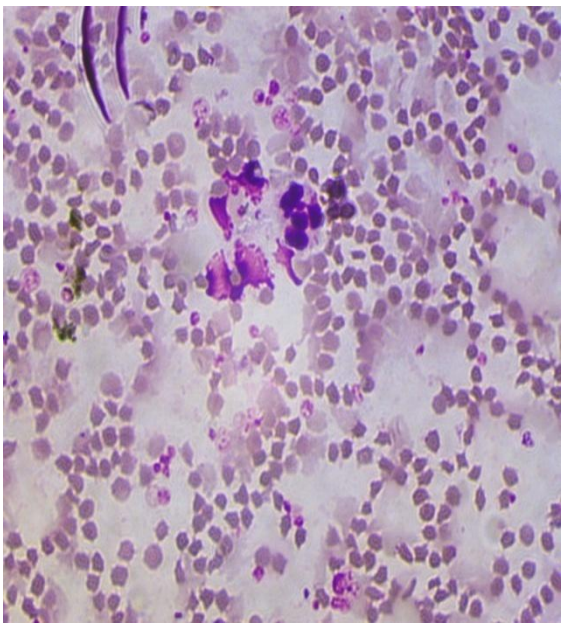


Fig. 32: Photomicrograph of blood smear showing nuclear fragmentation of neutrophil (1000X)

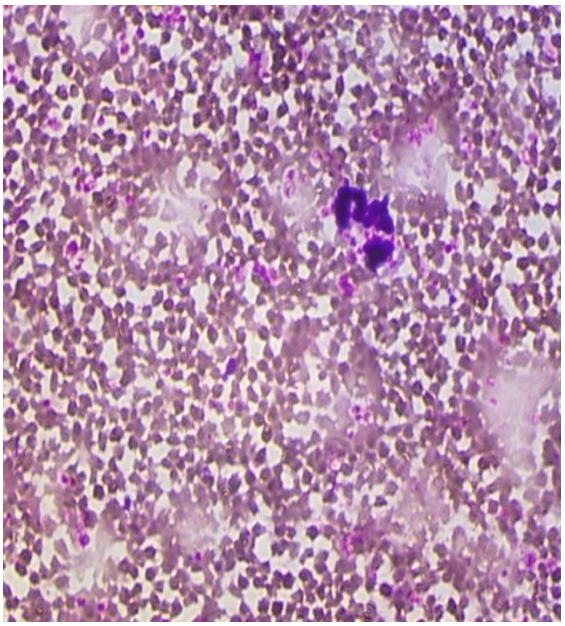


Fig. 33: Photomicrograph of blood smear showing nuclear fragmentation of eosinophil (1000X)

CHAPTER V

DISCUSSION

As many external and internal factors influences post-mortem changes hence prediction of exact death time is critical task to be evaluated from a single method. So intensive study on each aspect is very much needed to correlate different changes to avoid misdiagnosis. Again, by correlating these changes with time passed since death we can predict approximate time of death or post-mortem interval (PMI). Different parameters of post-mortem changes were studied for accurate determination of post-mortem interval or time of death. Studies were also conducted on disposal of carcasses by burial and composting methods for a suitable method of disposal of carcass.

For accurate determination of postmortem interval, tissue enzymatic parameters like alanine aminotransferase (ALT) and alanine aminoaspartate (AST) in the heart and liver samples collected at 0 hr, 6 hrs and 12 hrs of slaughter were evaluated from twenty goats. The mean ±SE of ALT in liver at 0 hrs, 6 hrs and 12 hrs of slaughter were 269.10±3.61, 301.72 ±4.59 and 336.25±4.49. This showed there was significant increase from 0 hr to 12 hr. The value of AST (as mean±SE) were 67. 65 ± 2.77, 103.35± 3.49, 131.20±4.70 at 0 hrs, 6 hrs and 12 hrs respectively indicating significant increase at 6 hrs and 12 hrs. The ALT concentration in the liver increased significantly at 6-hours as well as in 12 hours of slaughter. The result of this enzymatic study showed that there was significant increase in tissue enzymes (ALT & AST) in liver as well as in heart upto 12 hrs of post death. In a similar study done by Gandhi and Patnaik (1997) found that the ALT and AST in liver & heart tissue raised and then declined followed by either same or reverse pattern in between the 4-38 hrs post death. Normally theses enzymes present in cytosol, mitochondria etc., but after death there is cell disintegration, autolysis or putrefaction of the tissue so these enzymes are released hence on estimation their concentration rises. So this may be the reason of sharp increase of these tissue enzymes at 6 hrs and 12 hrs of death.

Histopathological examination of liver sample collected after 12 hrs of showed that there was increase in granularity of hepatocytes with condensation of nucleus and after 24 hrs it was observed that there was disintegration of nucleus of some

hepatocytes with increase in sinusoidal spaces. In a similar histopathological study on goat by Chowdhury *et al.* (1970) showed that after 6 hrs of post death. There was focal areas of autolysis with increase granularity of hepatocytes Then after 12 hrs disintegration and rarefaction of nuclei in hepatocytes started and the hepatocyte cytoplasm became eosinophilic with replacing the basophilic character of liver cells. At 24 hrs nuclear pyknosis begin with merging of cell outline of hepatocytes. They tried to correlate these histopathological changes with time passed since death. According to Kushwaha *et al.,* at 30^0C mild autolytic changes were seen beginning form 24 hrs & b/w 36 - 48, hrs, the changes were moderately advanced, then after 72 hrs there were severe autolytic changes with loss of cellular detail as well as cellular architecture. In case of human beings in a study of post death Yamamoto *et al.* (1997) found that there was autolytic changes, shrinkage of hepatocytes and disruption of hepatic chords with wavy transformation of hepatocytes.

On histopathological examination of heart sample collected after 12 of slaughter showed autolytic changes with pyknotic nucleus and loss of granularity of cytoplasm of myofibrils. After 24 hrs of heart showedloss of granularity polarity, detachment of myofibrils and pyknosis, disintegration of nucleus. There were partial liquefaction of myofibrils in some places. Fakhruddin (2002) observed autolytic changes of heart samples collected at 12 hrs of post death. He also reported that there was pyknotic nucleus with granularity of myofibril cytoplasm with detachment of myofibrils. At 24-hours, advanced autolytic changes with loss of polarity and disintegration nucleus was observed in heart samples. There was also partial liquefactions of myofibrils in some places.

At 12 hours kidney showed, cellular swelling with increase granularity of cytoplasm of tubular epithelium and disruption of tubular epithelium at many places. There were swollen glomeruli in some places but architecture was maintained. At 24 hours disintegration and dissolution of nuclei of some glomerular epithelial cells with collapse of glomeruli. The architecture was disturbed and disruption of epithelium was prominent at some places. Tandon (1985) studied the histological changes of kidney tissue after death at 30^0C. According to his study at 12 hrs there was cloudy swelling of PCT (proximal convoluted tubules) and DCT (Distal convoluted tubules). Then at 24 hrs diffuse cloudy swelling of renal tubules, glomeruli and also in blood

vessels was observed. At 30 hrs the these changes became more intense and diffuse. Then severe autolysis was observed after 48 hrs of post death. Beyond 72 hrs there was complete liquefaction of kidney with bacterial invasion

Molecular studies of tissue samples like brain and liver was carried out through semi quantitative PCR & qRT-PCR. Amplification of β-actin was carried out with brain and liver tissues collected at different time intervals after sacrificing the goats using semi quantitative PCR. In goat, β- actin gene was selected for study of RNA degradation as it is a house keeping gene and present in every tissue so that we can correlate degradation pattern of same gene in different tissues. Also this gene being housekeeping gene present in every animal so result can be applied in other animals. Actb, Gapdh, Hprt, Pipa and Cyp2E1 are five commonly used genes in different study as these are widely accepted as stable expressed genes (Huggett *et al*., 2005; Dheda *et al*., 2004 and Vennemann & Koppelkamm, 2010). The expression of β-actin was slowly down regulated till 6 h of post-sacrification as compared to 0 h, and it could not be in detectable limit from 12 h till 96 h in liver tissue. In brain tissue, the expression of β-actin was detected at all time periods. The highest level of expression was detected in 0 h which was gradually decreased in subsequent time periods having lowest expression at 96 h of post-sacrification. The result was further validated using real-time PCR which is more sensitive as compared to semi-quantitative PCR. In liver tissue a significant decrease in expression was noticed from 0h to12 hours rand attends constant expression till 96 hrs. In brain tissue the expression was constant in 0 hrs, 3 hrs and 6 hrs of post-sacrification. As the RNA of dead animal started degrading after 6 hrs, the expression decreased at later time points with the lowest expression was detected at 96 hrs. Now it is suggested that correlation of degradation of nucleic acid after death with time passed since death act as elegant an elegant alternative to other classical methods of estimation of time of death. Indeed, besides the endogenous and exogenous ribonucleases, RNA degrades more rapidly after death because of chemical and thermal conditions. In goat, general PCR results depicts that the RNA of liver tissue degraded more rapidly as compared to brain tissue after death. The amount of RNA in liver can be detectable upto 6 hrs after death whereas in brain tissue the RNA was intact and degrades slowly after 3 hrs of post death. In real-time PCR the time of death can be predicted upto 12 hrs whereas as in brain there is no gradual degradation observed. So, liver tissue found to be more

effective to co-relate the time of death with RNA degradation till 12 hrs. Bauer M (2007) stated that as after death loss of RNA transcripts or degradation of RNA seems to be rapid and time depended the estimation of time since death by studying RNA degradation may be within the reach.

In haematological study total 60 post-mortem blood samples were taken in different time interval after death from 12 postmortem cases. Different haematological parameters like Hb, TEC, TLC, PCV as well as pH of blood samples were studied. The average value of all haematological parameters at different time interval in post-mortem blood of goat were compared with 0 hour of death which is equivalent to antemortem blood. Haemoglobin (Hb%) values in were 10.62± 0.37, 10.62±0.37, 10.43± 0.37, 10.23± 0.39 and 9.96±0.36 at 0 hrs, 6 hrs, 12 hrs, 18 hrs and 24 hrs respectively indicating no much difference of Hb at different time interval as compared to 0hr which is equivalent to antemortem blood of death. According to Kundu (2017) the values of Haemoglobin and Haematocrit in post-mortem blood were not correlated with time passed since death.

TEC (10^5 μl) values in 0 hour, 6 hour, 12 hour, 18 hour and 24 hour post-mortem were 11.44±0.57, 11.09±0.50, 8.32±0.41, 5.00±0.27 and 2.83±0.25 indicating decrease in TEC with increase in time after death. In this TEC different time interval were compared with 0 hour of death which is equivalent to antemortem blood. Here there is no significant difference between 0 hour and 6 hour of post-mortem but there is significant difference afterwardsi.e18 hour & 24 hour. TLC (10^4 μl) values in 0 hour, 6 hour, 12 hour, 18 hour and 24 hour post-mortem were 9.64±0.59, 9.44±0.57, 8.6±0.57, 7.02±0.51 and 5.17±0.44 indicating there is significant decrease in TEC with increase in time after death. In this TLC different time interval were compared with 0 hour of death which is equivalent to antemortem blood. Here there is no significant difference between 0 hour 6 hour and 12 hour of post-mortem but there is significant difference afterward i.e. 18 hour & 24 hour. As with time passed since death there is lysis of RBC as well as WBC occur due to degenerative changes so their total number gradually decreases. Kundu SD (2017) said that hemogram in blood after death act as a tool to estimate Time Passed since Death. She found that total number of RBC and WBC strongly correlated with Time passed since Death, though they are inversely correlated.

pH values were7.42±0.04, 7.27±0.05, 6.99±0.07, 6.55±0.12 and 6.12±0.07 in 0 hour, 6 hour, 12 hour, 18 hour and 24 hour post-mortem indicating significant decrease in pH with increase in time upto 24 hrs of post death. The pH of blood decreases as after death there is anaerobic glycolysis occur so lactic acid releases which decrease the pH of blood. Due to autolysis of body after death the metabolites and ions such as hydrogen ions, dihydrogen phosphate ions, bicarbonate and carbon dioxide were produced and accumulated in blood with lowering the Ph. In a study Donalson and Lamont (2013) observed the changes in blood Ph upto 96 hrs of post death in blood collected from pig, rat corpses and also in blood from human and rat stored in-vitro. They showed that blood Ph decreases from 7.4 to 5.1 in the blood collected from corpse and rate and extend of changes varied with different species. The concentration of lactate increased and then remained at an elevated level, so pH decreases as time passes after death and in human and pig the changes in concentration were different as compared to rat.

There were marked morphological changes occur in leukocytes in the post-mortem blood collected at different time since death (0 hr, 6 hrs, 12 hrs, 18 hrs and 24 hrs). Identifiable degenerations of neutrophils, monocytes and eosinophils were first noticed at 6 hrs of death. At 6 hrs there was pyknosis of nucleus of neutrophils, eosinophils and also in monocytes. At 12 hrs there was cytoplasmic and nuclear vacuolation in neutrophils as well as in eosinophils and monocytes. At 18 hrs there was nuclear fragmentation of neutrophils, eosinophils and monocytes. Beyond 24 hrs there was disintegration and fragmentation of nucleus in these cells. But lymphocytes were quite stable than other leukocytes with first identifiable degeneration noticed at 24 hrs post death. Dokgoz *et al.* (2001) reported that identifiable degenerative changes were first observed in eosinophils at 6 h after death then became unidentifiable after 72h of death. In case of neutrophils identifiable degenerative changes were observed at 6 h after death then became unidentifiable beyond 96h of death. For lymphocytes identifiable degenerative changes were observed after 24 hrs of post death. But they were identifiable even after 120 hrs of post death. Henssge *et al*. (1995) stated that after death, Due to post-mortem putrefaction and autolysis like other cells of the body blood cells also lose their normal morphology and became unidentifiable. Hence for estimating PMI these sequence of changes of blood cells (normal morphology to unidentification period) can be used as an useful aid.

Serum samples of total 30 were analyzed for different serum enzymes i.e., ALT, AST, LDH, Alkaline phosphatase from 6 post-mortem cases. Serum samples were taken in different time interval (0 hour, 3 hours, 6 hours, 12 hours and 24 hours) after death. There was gradual significant increase in serum AST values at 3hrs, 6hrs, 12 hrs and 24 hrs than 0 hr with a significant difference between the groups. Serum ALT values of different time interval showed significant difference and also there was gradual significant increase at 3 hrs, 6 hrs, 12 hrs and 24 hrs than 0 hr. Ramakrishnan *et al*. (1980) stated normally in serum ALT and AST enzymes are present in less concentration but in case of extensive damage the enzymes (AST & ALT) were released to serum with raising of the concentration. They also stated that these two enzymes are having clinical importance. So according to him AST and ALT in serum rises after death due to post-mortem damage of tissues. According to Mukherjee (1994) & Nandy (2010) serum ALT value raised upto second to third day of postmortem but for postmortem clocking these observations are very asymmetrical and erratic. Serum Alkaline phosphatase values at3hrs, 6hrs, 12hrs and 24hrs revealed gradual rise. Enticknap (1960) observed that after 30 hrs of death alkaline phosphatases value increased to 40 kA from 8 kA and upto 40hrs the concentration raised steeply (even upto peak 70 Ka) then after that the concentration fell downwards. There was gradual significant increase in serum LDH values at 3hrs, 6hrs, 12hrs and 24hrs.According to Enticknap (1960) after death serum lactate dehydrogenase concentration raised almost linearly upto 60 hrs with peak value at about 60 hrs. This linear change in concentration of LDH helps in calculation of PMI. According to Mukherjee (1994) & Nandy (2010) all these enzymatic values increase with time passed from death. As after death there is autolysis, putrefaction of different organs so the enzymes releases to blood and their values rises than normal.

Conventional method of disposal of carcass by burial method was carried out in dead calf. After 4 months of burial, the soft tissues were in process of decomposition or in a stage of active decay. The bones and hairs remained as such. During this stage the carcass had a characteristic wet appearance due to the liquefaction of tissues and purging of decompositional fluids. A strong odour of putrefaction was associated with the carcass. Then at 8 months there was almost complete decomposition of internal organs and other soft tissues with remnants of

bones and cartilage. The carcass lost most of the mass during this stage with lesser odour. At 12 months there was complete decomposition of soft tissues and carcass that has mixed with the soil leaving few remnants of long brittle bones. There was little to no odour associated with remains in the soil. The pH and Electrical Conductivity (EC) of the soil increased gradually up to 8months and then there was slight decrease at 12months. As EC increased upto 8 months indicating increase in nutrients of soil which is decreased afterwards. All other nutrients like N, P, K, S, Ca, Mg increased highly at 4 months. The N increased gradually till 12 months. But there was decrease in P and Organic Carbon (OC), S, Kat 8 months as well as in 12 months. According to the soil analysis report the parameters in the soil at burial site before burial of carcass changed a lot after decomposition. Mainly there was much increase in N that is because animal carcass is source of high N. The C:N ratio altered such that the soil could not be suitable for agriculture.

Disposal of carcass by composting method was carried out with poultry birds in a newly developed compost unit utilizing used paddy husk poultry litter. Composting was complete within 45 days. According to Wiedemann (2008) the compost process must go for a minimum of six weeks and the materials must be composted within 6 weeks. Murphy and Handwerker (1988) said that carcass composting dead chickens were fully biodegraded in only 30 days. The prepared compost was dark brown in colour. There was no soft animal tissue as well as no bones or bone fragments larger than 15 cm (6 inch) in any dimension. Smaller bone fragments were brittle. The resulted compost was devoid of any offensive odour. The C:N ratio of compost was 12:1 which was in between the reference value (20:1 or less).All other nutrients like Total organic carbon (C) (%),Total Nitrogen (N) (%), Total Phosphorous (P_2O_5)(%), Total Potash (as K_2O)(%), S(%), Ca(%), Mg(%) were 25.2%, 2%, 4.58%, 3.28%, 0.73%, 3.2% and 1.9% respectively which were in between the standard values of the compost (organic fertiliser) issued by govt. of India (Schedule IV). The toxic parameters like As, Hg, Ni were not detected. But Cd, Cr, Pb and Zn were 1.01, mg/ kg 1.1 mg/ kg, 7mg/ kg and 40 mg/kg respectively which were much less than the maximum permissible values.

Carcasses in a compost are having low C:N (excess N) , high moisture, low C:N and having no porosity whereas paddy husk (used poultry litter) as co-

composting material have high C:N(low N), relatively low moisture and having sufficient porosity. Carbon rich paddy husk & Nitrogen rich carcass contribute for balancing required C:N ratio for compost. Porosity present in co-composting materials required for aerobic decomposition. Another important point in composting is that it was devoid of any bad odour. No offensive odour as the odorous gases and liquids diffuse into drier and aerobic co-composting materials (poultry Litter). There the micro-organisms ingested these gases and liquid resulting into simple organic compounds and ultimately to water and CO_2 which are harmless. According to Keener *et al.* (2000) after absorption of odorous gases and liquids by drier co-composting materials micro-organisms ingested and degraded into CO_2 and water which are simple organic compounds and not harmful. As during composting the temperature rises up to 55-58^{0}C and this temperature killed the pathogenic bacteria and even the spores. According to Henri and Bitney (2010) an internal temperature of 130^{0}F is needed to kill disease organism. Bonhotal *et al.* (2012) stated the, the destruction of pathogenic bacteria fungi, parasites and even seeds of plants depends upon the internal temperature of the compost pile. They also stated that the rate of decomposition depends on the temperature of the compost. According to them temperature in between 40^{0}C-60^{0}C is the most sufficient for compost preparation. Ward (2009) reported that the core of the compost pile commonly reaches the temperature of 55^{0}C–65^{0}C or higher. This temperature range is important for destruction of pathogenic micro-organisms in the organic matters. Lu *et al.* (2003) stated that Avian Influenza virus can be killed when kept at temperature of 56^{0}C for 90 mins or 60^{0}C for 10 mins. At this high temperature insects, maggots even rove beetles are also discouraged. The finished compost can act as a good biofertilizer and biologically safe which can be used as soil amendment. Glanville (1999) said that compost has valuable source of different crop nutrients like other animal manure. The resulted compost should be analyzed for it's nutrient content and should be used accordingly. According to Collins (2009) in compost method of carcass disposal there is no ground or surface water contamination and there is minimum chance of disease transmission as well as insect problems. The compost results into a useful organic biofertilizer by stabilizing the ingredients which will not attract dogs flies or rodents.

In a comparative study it was found that composting method was better in all the ways. In burial method the carcass decomposed in 4 month but in composting there was complete decomposition of carcass within 35-45 days. There was the offensive odour remain in the soil of burial site but in composting it was completely devoid of bad odours. After decomposition the soil at burial site was having very high C:N ratio (1: 67). But the reference value of C:N is 20:1 or less. All other nutrients also rise sharply which indicate altered soil at burial site became unsuitable for agriculture. But in case of composting method of disposal a finished bio-compost was prepared which has C:N 12:1 again all other nutrients of compost are within the reference range of a fertiliser. So it can be used as a good biofertilizer. In burial method due to seepage of liquids released from carcass during decomposition to the surrounding soil it became contaminated. But in composting during degradation of carcass there was rise of temperature (55^0C) which killed the pathogenic bacteria even parasites and spores.

CHAPTER VI

SUMMARY AND CONCLUSION

Different parameters of post-mortem changes were studied for accurate determination of post-mortem interval or time of death. Studies were also conducted on disposal of carcasses by burial and composting methods for a suitable method of disposal of carcass.

For accurate determination of postmortem interval, tissue enzymatic parameters like alanine aminotransferase (ALT) and alanine aminotransferase (AST) concentration in the heart and liver samples which were collected from 20 goats at 0 hrs, 6 hrs and 12 hrs of slaughter were collected. The mean ± SE of AST at 0 hrs, 6 hrs and 12 hrs of slaughter were 67. 65 ± 2.77, 103.35± 3.49, 131.20 ± 4.70 indicating significant increase at 6 hrs and 12 hrs. The mean ± SE in liver at 0-hour, 6-hours and 12 hours of slaughter were 269.10 ± 3.61, 301.72 ± 4.59 and 336.25 ± 4.49 showing significant increase from 0 hr to 12 hr. The ALT concentration in the liver tissue significantly increased at 6 hrs as well as in 12 hrs of slaughter. The mean ±SE values of AST concentration in the heart tissue were 151.28 ± 3.44, 174.99 ±3.58 and 209.45 ±5.47 IU/L at 0-hour, 6 hour and 12 hours of slaughter. The AST concentration in the heart increased significantly at 6-hours and 12 hours of slaughter. The mean values of ALT level in the heart were 231.72± 2.00, 268.05±3.39 and 308.79 ±4.45IU/L at 0 hour, 6 hours and 12 hours of slaughter showing significant increase at 6hrs and 12 hours.

Histopathological examination of liver tissues collected at 12 hours after slaughter showed increase granularity in cytoplasm of hepatocytes with condensation of nucleus and there was increased sinusoidal space. After 24 hours of slaughter the there was perinuclear halo in the hepatocytes with advanced autolytic changes and also there was disruption of hepatic chords with increased sinusoidal space. There was complete disruption of nucleus in hepatocytes with invasion of saprophytes in some places. In heart tissue which was collected at 12 hrs showed that increased granularity of the cytoplasm in myofibrils, pyknotic nuclei and also autolytic changes. After 24

hrs in the heart tissue there was loss of polarity of myofibrils along with their detachment and advanced autolytic changes with disintegration of nuclei. There was partial liquefaction in myofibrils at some places. At 12 hours kidney showed, cellular swelling with increase granularity of cytoplasm of tubular epithelium and disruption of tubular epithelium at many places. There were swollen glomeruli in some places but architecture was maintained. At 24 hours disintegration and dissolution of nuclei of some glomerular epithelial cells with collapse of glomeruli. The architecture was disturbed and disruption of epithelium was prominent at some places.

Molecular studies of tissue samples like brain and liver was carried out through semi quantitative PCR & qRTPCR. Amplification of β-actin was carried out with brain and liver tissues collected at different time intervals after sacrificing the goats using semi quantitative PCR. The expression of β-actin was slowly down regulated till 6 h of post-sacrification as compared to 0 h, and it could not be in detectable limit from 12 h till 96 h in liver tissue. In brain tissue, the expression of β-actin was detected at all time periods. The highest level of expression was detected in 0 h which was gradually decreased in subsequent time periods having lowest expression at 96 h of post-sacrification. The result was further validated using real-time PCR which is more sensitive as compared to semi-quantitative PCR. In liver tissue a significant decrease in expression was noticed from 0h to12 hours rand attends constant expression till 96 hrs. In brain tissue the expression was constant in 0 hrs, 3 hrs and 6 hrs of post-scarification. As the RNA of dead animal started degrading after 6 hrs, the expression decreased at later time points with the lowest expression was detected at 96 hrs. Post mortem degradation of nucleic acids has been suggested as an elegant alternative to classical methods for PMI estimation. Indeed, besides the endogenous and exogenous ribonucleases, RNA degrades more rapidly after death because of chemical and thermal conditions. In goat, general PCR results depicts that the RNA of liver tissue degraded more rapidly as compared to brain tissue after death. The amount of RNA in liver can be detectable upto 6 hrs after death whereas in brain tissue the RNA was intact and degrades slowly after 3 hrs of post death. In real-time PCR the time of death can be predicted upto 12 hrs whereas as in brain there is no gradual degradation observed. So, liver tissue found to be more effective to co-relate the time of death with RNA degradation till 12 hrs.

For haematological study total 60 post-mortem blood samples were taken in different time interval after death from 12 postmortem cases. Different haematological parameters like Hb, TEC, TLC, PCV as well as pH of blood samples were studied. The average value of all haematological parameters at different time interval in post-mortem blood of goat were compared with 0 hour of death which is equivalent to antemortem blood. Haemoglobin (Hb%) values in 0 hours, 6 hours, 12 hours, 18 hours and 24 hours post-mortem were 10.62± 0.37, 10.62±0.37, 10.43± 0.37, 10.23± 0.39 and 9.96±0.36 indicating no much difference of Hb at different time interval as compared to 0 hr which is equivalent to antemortem blood of death. TEC (10^5 μl) values in 0 hour, 6 hour, 12 hour, 18 hour and 24 hour post-mortem were 11.44±0.57, 11.0 ±0.50, 8.32±0.41, 5.00±0.27 and 2.83±0.25 indicating decrease in TEC with increase in time after death. In this TEC different time interval were compared with 0 hour of death which is equivalent to antemortem blood. Here there is no significant difference between 0 hour and 6 hour of post-mortem but there is significant difference afterwards i.e. 18 hour & 24 hour. TLC (10^4 μl) values in 0 hour, 6 hours, 12 hours, 18 hours, 24 hours post-mortem were 9.64±0.59, 9.44±0.57, 8.6 ±0.57, 7.02 ± 0.51 and 5.17±0.44 indicating there is significant decrease in TEC with increase in time after death. In this TLC different time interval were compared with 0 hour of death which is equivalent to antemortem blood. Here there is no significant difference between 0 hour 6 hour and 12 hour of post-mortem but there is significant difference afterward i.e18 hour & 24 hour.

pH values in 0 hours, 6 hours, 12 hours, 18 hours and 24 hours post-mortem were7.42^{a}±0.04, 7.27^{b}±0.05, 6.99^{c}±0.07, 6.55^{d}±0.12 and 6.12^{e}±0.07 indicating significant decrease in pH with increase in time i.e. 6 hours, 12 hours, 18 hours and 24 hours after death.

There were marked morphological changes occur in leukocytes in the post-mortem blood collected at different time since death (0 hr, 6 hrs, 12hrs, 18 hrs and 24 hrs). Identifiable degenerations of neutrophils, eosinophils and monocytes were first noticed at 6 hrs of death. At 6 hrs there was pyknosis of nucleus of neutrophils, eosinophils and also in monocytes. At 12 hrs there was cytoplasmic and nuclear vacuolation in neutrophils as well as in eosinophils and monocytes. At 18 hrs there

was nuclear fragmentation of neutrophils, eosinophils and monocytes. Beyond 24 hrs there was disintegration and fragmentation of nucleus in these cells. But lymphocytes were quite stable than other leukocytes with first identifiable degeneration noticed at 24 hrs post death.

Serum samples of total 30 were analyzed for different serum enzymes i.e., ALT, AST, LDH, Alkaline phosphatase from 6 post-mortem cases. Serum samples were taken in different time interval (0 hour, 3 hours, 6 hours, 12 hours and 24 hours) after death. There was gradual significant increase in serum AST values at 3hrs, 6hrs, 12hrs and 24hrs than 0hr with a significant difference between the groups. Serum ALT values of different time interval showed significant difference and also there was gradual significant increase at 3 hrs, 6 hrs, 12 hrs and 24 hrs than 0 hr. Serum Alkaline phosphatase values at 3 hrs, 6 hrs, 12 hrs and 24 hrs revealed gradual rise. There was gradual significant increase in serum LDH values at 3hrs, 6hrs, 12hrs and 24hrs.

Conventional method of disposal of carcass by burial method was carried out in dead calf. After 4 months of burial, the soft tissues were in process of decomposition or in a stage of active decay. The bones and hairs remained as such. During this stage the carcass had a characteristic wet appearance due to the liquefaction of tissues and purging of decompositional fluids. A strong odour of putrefaction was associated with the carcass. Then at 8 months there was almost complete decomposition of internal organs and other soft tissues with remnants of bones and cartilage. The carcass lost most of the mass during this stage with lesser odour. At 12 months there was complete decomposition of soft tissues and carcass that has mixed with the soil leaving few remnants of long brittle bones. There was little to no odour associated with remains in the soil. The pH and Electrical Conductivity (EC) of the soil increased gradually up to 8months and then there was slight decrease at 12months. As EC increased up to 8 months indicating increase in nutrients of soil which is decreased afterwards. All other nutrients like N, P, K, S, Ca, Mg increased highly at 4 months. The N increased gradually till 12 month. But there was decrease in P and Organic Carbon (OC), S, K at 8 months as well as in 12 months.

Disposal of carcass by composting method was carried out with poultry birds in a newly developed compost unit utilizing used paddy husk poultry litter. Composting was complete within 45 days. The prepared compost was dark brown in colour. There was no soft animal tissue as well as no bones or bone fragments larger than 15 cm (6 inch) in any dimension. Smaller bone fragments were brittle. The resulted compost was devoid of any offensive odour. The C:N ratio of compost was 12:1 which was in between the reference value (20:1 or less). All other nutrients like Total organic carbon (C) (%), Total Nitrogen (N) (%), Total Phosphorous (P_2O_5)(%), Total Potash (as K_2O) (%), S(%), Ca(%), Mg(%) were 25.2%, 2%, 4.58%, 3.28%, 0.73%,3.2% and 1.9% respectively which were in between the standard values of the compost (organic fertiliser) issued by govt. of India.(Schedule IV). The toxic parameters like As, Hg, Ni were not detected. But Cd, Cr, Pb and Zn were 1.01, mg/ kg 1.1 mg/ kg, 7 mg/ kg and 40 mg/kg respectively which were much less than the maximum permissible values. In a comparative study it was found that composting method was better in all the ways.

It may be concluded from the study that:

- Different parameters of post-mortem changes were studied for accurate determination of post-mortem interval or time of death. AST & ALT of liver and heart tissue gradually increased at 6 & 12 hrs. Serum enzymes like AST, ALT, LDH and alkaline phosphatase increased with increase in time after death upto 24 hrs of post death.
- Haematological parameters like TEC & TLC decreased with increase in time since death within 24 hrs. pH of blood, decreased with increase in time since death upto 24 hrs from 7.42 ±0.04 to 6.12 ±0.07.
- Degenerative and necrotic changes observed in liver, heart and kidney after death.
- Morphological changes of leukocytes like pyknosis at 6 hrs, nuclear & cytoplasmic vacuolation at 12 hrs, fragmentation & disintegration of nucleus at 24 hrs except lymphocytes.
- In molecular study it was observed in semi-quantitative PCR expression of β-actin was slowly down regulated till 6 hrs of post-sacrification and it could not

be in detectable limit from 12 hrs till 96 hrs in liver tissue but in brain tissue the RNA was intact and degrades slowly after 3 hrs of post death.

- As real time q- PCR is more sensitive so the result was further validated with real time q- PCR. There was gradual decrease in expression of b-actin gene in liver. So by real-time PCR the time of death can be predicted upto 12 hrs in liver whereas as in brain there is no gradual degradation observed. So, liver tissue found to be more effective to co-relate the time of death with RNA degradation till 12 hrs.
- This molecular study is first time applied for estimation of time of death which is novelty of the work, besides other parameters are also unique for application in other forensic animals as well as in wild life death cases.
- Simple, innovative, economical & biologically safe compost unit for poultry carcass disposal was designed.
- Result of finished compost in our study showed C:N ratio of 12:1 which can be well utilised as a soil biofertilizer.

REFERENCES

Anderson GS and Van Laerhoven SL. 1996. Initial studies on insect succession on carrion in South Western British Columbi,. *J. Forensic Sci Int*., **41**: 617-625.

Anderson GS and Cervenka VJ.2001. Insects associated with the body :their use and analysis. In: Haglund W and Sorg M, editors. *Advances in forensic taphonomy*. New York: CRC Press p. 174-200.

Anderson GS and VanLaerhoven SL. 1996. Initial studies on insect succession on carrion in southwestern British Columbia. *Journal of Forensic Sciences*. **41** (4): 617–625.

Anderson GS. 2005. Effects of arson on forensic entomology evidence, *Canadian Society of Forensic Science Journal*, **38**(2):49–67.

Anderson GS. Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). J. Forensic Sci. 2000, 45: 824-832.

Anderson WAP. Pathology. 5th ed. Mosby Company, 1946: 74.

Babapulle CJ, Jayasundera NPK. 1993. Cellular changes and time since death death. *Medicine Science Law*,**33**.:213-222.

Bauer M .2007. RNA in forensic science. *Forensic Sci Int Genet* ,**1**: 69–74.

Bauer M, Gramlich I, Polzin S, Patzelt D .2003. Quantification of mRNA degradation as possible indicator of postmortem interval–a pilot study. *Leg Med (Tokyo)* **5**: 220–227.

Berenbaum MC.1956. Bone marrow after death. *J. Clinical Path* ,**9**: 381.

Bishop CP. 2015. Estimating Postmortem Interval: A Molecular Approach.. National Institute of Justice. *National Criminal justice* Reference Service (NCJRS)

Blocks GH, Wensing T and Van Logtestin, JG. 1988. Potassium ion concentration in the vitreous body as an indicator of the post-mortem interval in cattle. *Tijdschrift Voor Diergeneeskunde.* **113**(7)-. 359-363.

Bonhotal J, Schwarz M, Williams C, Swinker A, 2012. Horse Mortality: Carcass Disposal Alternatives Cornell Waste Management Institute. Department of Crop and Soil Sciences http://cwmi.css.cornell.edu cwmi@cornell.edu

Bourel B, Callet B, Hedouin V and Gosset D. 2003. Flies eggs: a new method for the estimation of short term postmortem interval? *Forensic Sci Int*. **135**: 27-34.

Brooks JW. 2016. Postmortem changes in animal crasses and estimation of the postertem interval, *Veterinary Pathology*, **58**(5): 929-940

Byrd JH. 2010. Forensic entomology: Insects in legal investigations. Available at: http://www. forensicentomology.com/definition.htm (accessed April 19, 2010).

Campobasso CP, Linville JG, Wells JD and Introna F.2005. Forensic genetic analysis of insect gut contents. *J Forensic Med Pathology* , **26**:161-165.

Carvalho LML, Thyseen PJ, Goff ML and Linhares AX. 2004.Observations on the succession patterns ofnecrophagous insects on pig carcass in an urban area of southern Brazil, *J Forensic Med Toxicol.* **5**:33-39.

Catts, E. P. 1990. Analyzing entomological data, in Entomology and Death: A Procedural Guide, Catts,E. P. and N. H. Haskell, Eds., Joyce's Print Shop, Clemson.

Chatterjee PC and Goyal VK. 1977. Histological study of the spleen tissue with regard to determining time of death. *JAF Sci* . **35** (2): 20.

Chowdhari S, Chatterjee PC and Banerjee PK.1974. Histological study of brain tissue with regard to determining time of death, *Jap J Legal Med*, **28** (2): 137.

Chowdhury S, Chatterjee PC and Banerjee PK. 1970. Histological study of liver tissue with regard to determining time of death. *J. Indian Acad, of Forensic Sci*., **9**(1): 19-23.

Clark MA, Worrell MB and Pless JE .1997. Post-mortem changes in soft tissue. In: Froede RC (ed) *Handbook of forensic pathology*, 2nd edn. CAP, Illinois.

Davies K and Harvey ML.2013. Internal morphology analysis for age estimation of blow fly pupae(Diptera : Calliphoridae) in post mortem interval estimation. *J. Forensic Sci.,* **58**(1):79-84.

Day DM and Wallman JF. 2006. Width as alternative measurement for length for postmortem interval estimation using Calliphora augur (Diptera: Calliphoridae) larvae. *Forensic Sci Int.,* **159**:158-67.

Dettmeyer RB. 2011. Forensic Histopathology: Fundamentals and Perspectives. New York: *Springer*;

Dheda K, Huggett JF, Bustin SA, Johnson MA and Rook G. 2004. Validation of housekeeping genes for normalizing RNA expression in real-time PCR. *Biotechniques*, **37**: 112–114, 116, 118–119.

DiMaio VJ, DiMaio D. Forensic Pathology. New York: CRC Press; 2001.

Dokgoz H, Arican N, Elmas I and Fincanci SK. 2001. Comparison of morphological changes in white blood cells after death and invitro storage blood for the estimation of postmortem interval. *Forensic Science International*, **124**: 25-31.

Dokgoz H,Arican N, Elmas I and Fincanci SK. 2001. Comparison of morphological changes in white blood cells after death and in vitro storage of blood for the estimation of postmortem interval, *Forensic Science International*, **124.** 25-31.

Dokgoza H, Aricanb N, Elmasb I, and Fincancib SK. 2001. Comparison of Morphological Changes In White Blood Cells After Death And In Vitro Storage Of Blood For The Estimation of Postmortem Interval. *Forensic Science International*, **124**(1): 25-31.

Donaldson AE and Lamont IL 2013. Biochemistry Changes That Occur after Death: Potential Markers for Determining Post-Mortem Interval. *POLS.*

Donaldson AE, Lamont IL .2013. Biochemistry Changes That Occur after Death: Potential Markers for Determining Post-Mortem Interval. *PLoS ONE* **8**(11)

Enticknap JB. 1960. Biochemical changes in cadaver sera *J. Forensic Med*, **7**:135-146.

Enticknap JB. 1960. Biochemical changes in cadaver sera, *J. Forensic Med*, **7:**135-146.

Erlandsson M and Munro R. 2007. Estimation of the post-mortem interval in beagle dogs. *Sci Justice*, **47**(4):150–154.

Fatteh A. Handbook of Forensic Pathology. Handbook publisher. J B Lippincott Company: Phialdelphia, 1973: 20-29.

Ferris MM, Habbersett RC, Wolinsky M, Jett JH, Yoshida TM and Keller RA. 2004. Statistics of single-molecule measurements: applications in flowcytometry sizing of DNA fragments. Cytometry A. **60**:41-52.

Florey H. *General Pathology*. 3rd ed. Lloyd Lahe Medical Books Ltd: London, 1962: 389.

François G. Gallois-Montbrun, Denis R. Barrès Michel Durigon. 1988.Postmortem interval estimation by biochemical determination in birds muscle. . *ELSEVIER .Forensic Science International,* **37**(3):189-192.

Gallois-Montbrun FG, Barrès DR and Durigon M.1988. Postmortem interval estimation by biochemical determination in birds muscle. Forensic Sci Int., **37**(3): 189

Gandhi R and Patnaik KA. 1997. A study of organ enzyme concentration in relation to time of death. *J. Forensic Med. Toxicol.,* **24**(1): 5-8.

Garg SDP, Arora A , Dubey BPJ. 2005. A study of serum enzymal changes after death and its correlation with time since death, *iafm,*: **27** (1).

Geberth VJ. 1996. Practical Homicide Investigation, CRC Press LLC, Boca Raton, FL.

Glanville, T. D. and D. W. Trampel. 1997. Composting alternative of animal carcass disposal. Journal of the American Veterinary Medical Association. 210:1116 – 1120.

Goff ML. 1993. Estimation of postmortem interval using arthropod and successional patterns. *Forensic Science Review,* **5**(2):82–94.

Goff ML.2009. Early post-mortem changes and stages of decomposition in exposed cadavers, *Exp Appl Acarol* , **49**:21–36.

Gomes L, Godoy W and Von Zuben CJ. 2006. A review of post feeding larval dispersal in blowflies: Implications for forensic entomology. *Naturwissenschaften,* **93**:207–215.

Green RL, Roinestad IC, Boland C and Hennessy LK. 2005. Developmental validation of the quantifiler real-time PCR kits for the quantification of human nuclear DNA samples. *J. For. Sci*., **50**:809-25.

Hall WEB.1958. The medicolegal application of the serum transaminase test, *J. Forensic Sci.,* **3:**117-122.

Harper AF, DeRouchey JM, Glanville TD, Meeker DL, Straw BE. 2008. Swine Carcass Disposal Options for Routine and Catastrophic Mortality. Council for Agricultural Science and Technology (CAST). Issue Paper 39. CAST, Ames, Iowa. Thiemann

Harper AF, Estienne MJ and GoUine, ER. 2002. Gomposting as an environmentally safe means of dead pig disposal on Virginia swine farms. Virginia Tech Tidewater Agricultural Research & Extension Center Suffolk. Virginia. Retrieved June 16, 2003 from: http:/ /vmirl. vmi.edu / ev / Paper%20Sessions / Early%20Bird / Absracts / Harper_Abstract.htm

Harper HA. 1969. Review of pliysiological cliemistry. Large Medical Publication, 12'^ed. pp.128-137.

Heinrich M, Matt K, Lutz-Bonengel S and Schmidt U. 2007. Successful RNA extraction from various human postmortem tissues, *Int J Legal Med* , **121**: 136– 142.

Henri CG and Bitney L.L. 2010 Disposal methods of livestock and poultry mortality.http://extension.unl.edu./publication.

Henry JB and Smith FA. 1980. Estimation of the post-mortem interval by chemical means. *Am. J. Forensic Med. Pathol.,* **1**: 341-347.

Henssge C, Knight B, Krompecher T, Madea B and Nokes L. 1995. The Estimation of the death in the Early Postmortem Period. Edward Arnold, London. p 224.

Higley LG and Haskell NH. 2001.Insect development and forensic entomolgy. The utility of athropods in legal investigation. Boca Raton:CRC Press: p.287-300.

Imaede, N. 1999. Characterization of serum and enzyme activities and electrolyte levels in broiler chickens after death from sudden death syndrome. *Poultry Sci.,* **78**(1): 66-69

Introna F, Altamura BM. Dell'Ebra A and Dattoli V. 19.89 Time since death by experimental reproduction of Lucillia seriata cycles in growth cabinet, *J. Forensic Sci.,* **34**:478-80.

Itani M, Yamamoto Y, Doi Y, and Miyaishi 2011. Quantitative Analysis of DNA Degradation in the Dead Body. *S. Acta Med. Okayama,* **65** (5): 299-306. Huggett J, Dheda K, Bustin S, Zumla A .2005. Real-time RT-PCR normalisation; strategies and considerations, *Genes Immun,* **6**: 279–284.

Jaffe FA. 1962. Chemical post-mortem changes in the intra-ocular fluid. *J. Forensic Sci.,* **7**: 231-237.

Kashmanian, R. M. and R. F. Rynk. 1996. Agricultural composting in the United States: Trend and driving forces. *Journal of Soil and Water Conservation*. **51**(3):194-201

Kashyap VK and Pilai VV.1989. Efficiency of entomological method in estimation of postmortem interval: a comparative analysis, *Forensic Sci Int*., **40**:245-50.

Keener HM and Elwell DL. 2000. Mortality composting principles and operation. In: Ohio's Livestock and Poultry Mortality Composting Manual. Produced by The Ohio State University Extension, Ohio. Extension, 7/2000-200.

Keener HM, Elwell DL and Monnin MJ. 2000. Procedures and equations for sizing of structures and windrows for composting animal mortalities. *Appl Engin Agric* **16**(6):681–692.

Kouadjo KE, Nishida Y, Cadrin-Girard JF, Yoshioka M and St-Amand J .2007. Housekeeping and tissue-specific genes in mouse tissues, *BMC Genomics*, **8**: 127.

Kumar B, Mahto T and Kumari V. 2015. Determination of Time Elapsed since Death from Changes in Morphology of Red blood cells in Ranchi, Jharkhand. J Indian Acad Forensic Med. **37**(2).

Kumar B, Mahto T and Kumari V.2015. Determination of Time Elapsed since Death from Changes in Morphology of Red blood cells in Ranchi, Jharkhand . *J Indian Acad Forensic Med*.. **37**(2) 971- 973.

Kundu SD. 2017. Changes in Haemogram in Subjects After Death As A Tool To Estimate Time Passed Since Death. *IOSR Journal of Dental and Medical Sciences (IOSRJDMS)*, **16**(10): 19–27

Kushwaha V, Yadav M, Srivastava AK and Agarwal A.Time passed since death from degenerative changes in liver .*J Indian Acad Forensic Med*, **31**(4) :320-325.

Langhlin MPS and Mc Langhlin, BG. 1987 . Chemical analysis of bovine and porcine vitreous humors. Correlation of normal values with serum chemical values and changes with time and temperature. *Am. J. Vet. Res*., **48**(3): 467-473.

Langston, J, Garman,D, VanDevender, K and Boles JG. 2002. Disposal of swine carcasses in Arkansas. MP3975M-9-97N, Cooperative Extension Service, Division of Agriculture, University of Arkansas. Little Rock, AR 72204. Retrieved April 6, 2003 from: http://www.uaex.edu/ Other_Areas / publications / HTML / MP397 / composting_swine_carcasses.asp#Recipe.

Langston, J, Garman,D, VanDevender, K. and Boles JG. 2003. Disposal of swine carcasses in Arkansas. MP3975M-9-97N, Cooperative Extension Service, Division of Agriculture, University of Arkansas. Little Rock, AR 72204, http://www.uaex.edu/ Other_Areas/publications/HTML/MP397/composting_swine_carcasses.asp#Recipe.

Lew EO and Matshes EW. 2005.Postmortem changes. In: Dolinak D, Matshes EW, Lew EO, eds. *Forensic Pathology: Principles and Practice.* New York: Elsevier/ Academic Press: 527–554.

Lew EO and Matshes EW. Postmortem changes. In: Dolinak D, Matshes EW and Lew EO, eds.2005. Forensic Pathology: Principles and Practice. New York: Elsevier/ Academic Press : 527–554

Liab C, Wanga Q ,Zhanga Y, Lina H, Zhanga, Huangb P and Wanga Z. 2016. Research progress in the estimation of the postmortem interval by Chinese forensic scholars. *Forensic Sciences Research*, **1:** 3–13.

Liu L, Shu X, Ren L, Zhou H and Li Y. 2007. Determination of the early time of death by computerized image analysis of DNA degradation: which is the best quantitative indicator of DNA degradation? J Huazhong Univ Sci Technolog Med Sci **27**: 362–366.

Looper M. 2003. Whole animal composting of dairy cattle. Dairy Business Communications. Guide D- 108: l-4, http://www.dairybusiness.com / western / NovOl / NovWDBcompost.htm.

Martins PA, Ferreira F, Natal Jorge R, Parente M and Santos .A. 2015 Necromechanics: Death-induced changes in the mechanical properties of human tissues. *Proc Inst Mech Eng H*, **229**(5): 343–349.

Mason JK, Harkness RA, Elton RA and Bartholomew S. 1980. Cot deaths in Edinburgh: Infant feeding and socio-economic factors. *J. Epid. Comm. Health*., **34**: 35-41.

Merrit RW. 2000. Entomology. In :Higgins MJ, Siegel JA, Saukko PJ, Knupfer GC, Editors. *Encyclopedia of Forensic Science*, **2**: 200.

Millner P. 2003. Composting: Improving on a time-tested technique. Available: http://www.ars.usda.gov/is/AR/archive/aug03/ time0803.htm. Accessed May 25, 2004.

Morse DL. 2009. Composting Animal Mortalities. Agricultural Development and Financial Assistance Division Minnesota Department of Agriculture. www.mda.state.mn.us.

Munro R and Munro HM. . 2013.Some challenges in forensic veterinary pathology: a review. *J Comp Pathol*, **149**(1):57–73.

Munro R, Munro HMC. 2008. Animal Abuse and Unlawful Killing: Forensic Veterinary Pathology. Philadelphia: Saunders, *Elsevier*.

Nandy A. 2010. Principles of Forensic Medicine including Toxicology 3rd edition: 280. 12.

Nashelksy M and McFelley P .2003. Time of death. In: Froede RC (ed) *Handbook of forensic taphonomy*, 2nd edn. CAP, Illinois

Naumann H.N. Postmortem. 1956. Liver Function Tests, *Am. J. Clin. Pathol*. **26**: 495-505.

Nemilow AW. 1928.Time of death from tissue changes. *Med Welt,* **3**: 1337.

Nemilow AW.1928. Time of death from tissue changes. *Med. Welt*., **2** : 1337 (Cited by Chowdhury etal., 1970).

Nunno DR, Costantinides F, Bernasconi P, Bottin C and Melato M. 1998. Is flow cytometric evaluation of DNA degradation a reliable method to investigate the early postmortem period? *Am. J. For. Med. Pathol*., **19**:50-53

Nuorteva P. 1977. Sarcosaprophogous insects as forensic indicators. In :Tedeschi CG, Eckert WG. Editors. Forensic Medicine. A study in trauma and environmental hazard, physical trauma, Philadelphia, *Saunders:* **2:** 1072-95.

Oliveira M, JA and Santos-Martin, CC. 1995. Enzyme histochemistry of the liver in autopsy material at different post-mortem times, *Med. Sci. Law*., **35** (3): 201-206.

Ortmann J, Deberentz E and Mdea B. 2017. Immunohistochemical as an aid n estimating time since death, *Forensic Science International*, *ELSEVIER,* **273:** 71–79.

Partemi S, Berne PM, Batlle M, Berruezo A and Mont L. 2010. Analysis of mRNA from human heart tissue and putative applications in forensic molecular pathology. *Forensic Sci Int.,* **203**: 99–105.

Pentilla A And Lahio K, Autolytic Changes In Blood Cells Of Human Cadavers II. Morphological Studies., Forensic Science International.1981 Mar-Apr; **17**(2):121-132.

Penttila A and Laiho K. 1981. Autolytic changes in blood cells of human cadavers. II. Morphological studies. *Forensic Sci Int.,* **17**(2):121-32.

Platt MS. 1989. Postmortem cerebrospinal fluid pleocytosis. *Am J Forensic Med Pathol*, **10**: 209-12?

Preece P and Cairns NJ. 2003. Quantifying mRNA in postmortem human brain: influence of gender, age at death, postmortem interval, brain pH, agonal state and inter-lobe mRNA variance. *Brain Res Mol Brain Res,* **118**: 60–71.

Rakesh T. 1985.Time and Temperature controlled histopathological changes in tissue & organs of Rabbit. *I.J.F. Sci*., 20-25.

Ramakrishnan S, Prasannam, KC and Ranjan R. 1980. Text Book of Medical Biochemistry. Orient Longman Ltd., Delhi, Calcutta, pp. 128-155.

Reiter C and Hajek P. 1984. Age dependent changes in the intestinal contents of blow fly maggots. A study method in the framework of forensic determination of time of death, .*Rechismed,* **92**: 39-45.

Ritz C and Worley J. 2005. Poultry mortality composting management guide. Publication B-1266. University of Georgia Cooperative Extension Service.

Rodriguez WC and Bass WM. 1983. Insect activity and its relationship to decay-rates of human cadavers in east Tennessee. *J Forensic Sci*.; **28**(2):423–432.

Šaňková M and Račanská M. 2016. Molecular genetics and determination of time since death - short communication. *Pub Med,* **61**(3):28-9.

Saukko PJ and Knight B. 2004.The pathophysiology of death. In: Saukko PJ, Knight B, eds. *Knight's Forensic Pathology*. 3rd ed. New York: Oxford University Press: 52–97.

Schleyer F .1963. Determination of the time of death in the early postmortem interval. *Methods - Forensic Sci*, **2**: 253-293.

Schoning P and Strafuss AG. 1980. Post-mortem biochemical changes in canine vitreous humor. *J. Forensic Sci*., **25**:53-59.

Senne, DA, Panigrahy B and Morgan RL. 1994. Effect of composting poultry carcasses on survival of exotic avian viruses: highly pathogenic avian influenza (HPAI) virus and adenovirus of egg drop syndrome-76. *Avian Dis*. **38**:733–737

Sharma R, Garg RK and Gaur JR. 2015. Various methods for the estimation of the postmortem interval from Calliphoridae: A review, *Egyptian Journal of Forensic Sciences, ELSEVIER*, **5**:1-12.

Shearer JK. 2006. Euthanasia and Carcass Disposal .Proceedings 3rd Florida & Georgia Dairy Road Show. PP 79-83

Singh D and Bala M . Studies on larval dispersal in two species of blow flies (Diptera: Calliphoridae). *J. Forensic Res*, **010**: 1:1-3.

Sinha M, Lalwani S, Mir R , Sharma S, Dogra YD and Singh TP. 2012. A Preliminary Molecular Study on Protein Profile of Vital Organs: A New Direction for Post Mortem Interval Determination. *J Indian Acad Forensic Med*., **34**(4).

Vegad JL. 2012.Postmortem changes. *Veterinary General Pathology*. 2nd edition. pp. 89-91.

Vennemann M and Koppelkamm A. 2010. Postmortem mRNA profiling II: Practical considerations, *Forensic Sci Int*, **203**: 76–82.

Wehner F, Eingrenzungder and Leichenliegezeitim S.2009. Postmortalen Intervall – Neue AnsätzeMittels immunhistochemischen Verfahren, *Med.Welt* **60** :402–406. *J. Ortmann et al., Forensic Science.*

Wehner F, Wehner, HD, Schieffer MCand Subke J. 2001. Delimitation of the time since death by immunohistochemical detection of calcitonin, *Forensic Sci. Int*.**122** : 89–94.

Wehner F, Wehner, HD, Schieffer MCand Subke J. 2001. Delimitation of the time since death by immunohistochemical, Detection of glucagon in pancreatic b - cells, *Forensic Sci. Int.***124**: 192–199.

Wehner F,Wehner, HD, Schieffer MCand Subke J. 1999. Delimitation of the time since death by immunohistochemical detection of insulin in pancreatic ß-cells, *Forensic Sci. Int.* **105** :161-169

Wehner F,Wehner, HD, Schieffer MCand Subke J. 2000.,Delimitation of the time since death by immunohistochemical detection of thyreoglobulin, *Forensic Sci. Int*. **110**: 199–206.

Wolff M, Uribe A, Ortiz A and Duque PA.2001. preliminary study of forensic entomology in Medellin Colombia, *Forensic Sci Int* , **120** :53-9.

Wyler D, Marty W and Bar W.1994. Correlation between the post-mortem cell content of cerebrospinal fluid and time of death. *Int J Legal Med.*, **106**(4):194-9.

Yan X, Habbersett RC, Cordek JM, Nolan JP, Yoshida TM, Jett JH and Marrone BL. 2000. Development of a mechanism-based, DNA staining protocol using SYTOX orange nucleic acid stain and DNA fragment sizing flow cytometry, *Anal. Biochem*., **286**:138-48.