

**TOXICOLOGICAL INVESTIGATION IN LEGAL CONTEXT FOR VETERINARIANS**

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ABSTRACT

Due to rapid industrial expansion and change in socio-economic dynamics of human population, the death and also cruelty to animals due to toxicant, poisons or industrial or agricultural chemicals is very common social phenomenon now a days. The prevention of such incidences depends on investigation of incidence, finalizing causes of death and cruelty to animals and formulating policy based on findings and experiences. Under such toxicological investigation, Veterinarian plays key role by offering his services to law enforcing agency for performing the post-mortem, collection of samples, submitting the samples to forensic laboratory and interpretation of results hereby obtained. All these functions become very crucial when there is involvement of legal disputes or conflicts. Under such condition, Veterinarian is authorized sources of expert evidence and opinions as per Expert Witness Act of India. Veterinarian has to present his findings and related opinions in the presence of court jury. Admissibility of expert evidences and opinions depends on accuracy of Veterinarian's professional functions performed during investigation of cases. It is a challenging job for Veterinarian to investigate veterolegal cases in field condition in order to protect the interest of animal or owner or society or government. So the intersection of knowledge of toxicological and legal matters is of vital for Veterinarians to understand and to conduct such investigation in most correct ways. The present review is focused on blending of technicality of toxicological investigation and its legality for the use of court and jury.

Keywords: Toxicological investigation, Veterinarians and Veterolegal.

INTRODUCTION

Veterinary professionals clearly have ethical and legal responsibility that go along with privilege of the profession. By careful attention to details of his professional activities, he can contribute to avoid most ground for malpractice or other unpleasant legal action in modern litigious environment of society. The changes in socio-economical dynamics of human population have substantially affected the ways in which animals are insulted, injured, tortured or murdered by criminals of society. The incidences of crime related to animals are on rising due to such changes. Death of animals due to toxicant/poisons is not uncommon in Indian environment. This happens due to intercommunity/interpersonal, social and

economical conflict at rural level among farming community. Rapid expansion of industrial area over grazing and farming lands has increased incidences of death due to pollutants/industrial waste and other chemicals. Under such incidences or epidemics, the investigation of causes of death and indentifying the real culprits is of paramount importance for society which always seeks to locate, prevent and correct cause in our world. Case/cause must be investigated with a toxicological relevance and context. When toxicological investigation involves legal disputes, the case becomes veterolegal importance. Death of animal is suspected due to exposure to poisons or toxicants and this may be malicious poisoning or accidental due to managerial or administrative errors/omissions/negligence/ ignorance. Veterinarians

play key role in entire process to meet the challenge of investigation of veterolegal cases in field condition in order to protect the interest of animal or owner or society or government. Attention to such causes and incidences offers possible tool for early identification of victims and perpetrators of crimes and cruelty. ^[1]

Additionally, Veterinarians are entitled to give evidences at inquest or in any court of law as an expert under section 45 of Indian Evidence Act 1872 on any matter related to Veterinary medicine. ^[2] The Veterinarians functions as a medical/Veterinary examiner, veterolegal investigator and crime scene investigator in animal cruelty and death cases. So much of action depends on Veterinarian's action and findings. The natural and learned skill of investigation, logical and deductive reasoning ability is very essence of duty of Veterinary profession. The Veterinarian job is to assemble all the piece of information from the body and put them together to determine the most probable scenario. All piece of information gathered must be accounted for and explained. ^[3] So the intersection of knowledge of toxicological and legal matters is of vital for Veterinarians to understand and to conduct such investigation in most correct ways. The comprehensive reviews of all the laws and legal steps are difficult task. At the same time, it is not possible to discuss in detail individual case of many kind of veterolegal cases. The general aspect which is more required in all types of veterolegal case would be helpful to generate good sense of understanding among Veterinarians for such issues.

The toxicological investigation is collaborative task involving Veterinarians, toxicologist, pathologist and court. Toxicology is a science of poisons or it deals with study of adverse effects of any chemicals on living organism including domestic animal and birds. The toxicologists are trained for analysis of samples sent to laboratory from Veterinarians and based on findings they can generate interpretations and likelihood of occurrence of toxicosis. ^[4] The interpretations are made based on knowledge of exposure, dose response and variability. Their interpretation can be valuable assets in chain of evidence to prove the legality of veterolegal cases.

PURPOSE OF TOXICOLOGICAL ANALYSIS

Toxicological investigations are conducted for varieties of purposes in order to peruse the welfare of human and animal or even environment. The methods of analysis and investigation differ for different purposes. Nature of biological samples

collected also varies greatly. The basic purposes of toxicological investigations are as under:

- (1) Legal purpose/Veterolegal.
- (2) Enforcement (Adulteration/fake drug/spurious drug/drug residues).
- (3) Diagnosis (Clinical diseases arising from over exposure to poisonous substance).
- (4) Research (Pre-clinical and clinical toxicity studies of drug).

Circumstance under which investigation is conducted varies. Collection of samples to be carried out when reasons for testing the samples is known and differs from when reasons for testing samples are not known. In first set of condition, the spectrum of toxicological test is limited and biological material needed to be collected is also specific and limited. So Veterinarians can collect the samples as prescribed by analytical laboratory. In second condition, when reasons for sample collection are not know or it changes after sample collection, the validity of such investigation may or may be acceptable. Under such condition, the tracing of sample collection/other documentation and testimonials may not be available because of lack of knowledge of purpose of samples. ^[5]

STANDARD OF PROOF

Legal disputes are subjected to judicial trial. The Trier of court learns facts regarding disputes largely through evidences. All evidences recorded or submitted are not acceptable. The credential of evidences and qualification of persons who recorded them is determined by judges. Based on the recommendations of judges, the evidences becomes either admissible or non admissible in the court of trial. The admissibility of evidences is determined based on relevancy of evidences. If evidences are relevant, they are admissible otherwise else, they are not relevant. ^[5]

SOURCES OF EVIDENCES

The main sources of evidence are as under:

Documents: The authenticated documents related to cases can be used as evidences if they are considered as admissible by Trier. In clinical set up, the office/laboratory register (Post-mortem register, case paper, prescription, post-mortem reports, specimen registrar, laboratory finding report, other formats dully signed by competent authority/officer are major sources of evidences in veterolegal cases. The drug vials, labels, pack inserts and cash memo of drug purchase from pharmacist store may provide accessory evidences in future course of investigation

and may be useful as link evidence. The government issues guideline and standard operating procedures regarding maintenance of required register/forms/file/formats for execution of Veterinary dispensary. All the correspondences/papers/newspaper clips/related to case are also good sources of evidences as documents.^[6]

Post-mortem: A toxicology case frequently depends on the post-mortem examination for a diagnosis. Although practitioners are trained to perform a necropsy to identify disease and traumatic conditions, if the case is accepted as a veterolegal case, a complete necropsy should be done, not a partial, "keyhole" necropsy. Such an incomplete job may cause a practitioner to miss an essential sample or observation, perhaps leading to professional and legal embarrassment. If an animal is insured, the insurance company should be contacted before the examination to find out what samples, testing, observations, or procedures must be conducted. Photographs of the animal and findings may be helpful in assessing the case.^[4] Animals submitted for necropsy should be positively identified (record or save ear tags, identify brands and other markings) to help track results. The necropsy itself is conducted systematically. These cases may involve a great deal of emotion and money. Thus, it is imperative for the clinician to keep an open mind and not to focus on only one diagnosis or on poisoning possibilities alone.^[7] Post-mortem examination involving internal and external examination of carcasses is to be conducted by registered Veterinarian on receiving the legal order/request from Police Officer or the Executive Magistrate.^[6] Each system should be examined thoroughly. The clinician should look for evidence of struggle (e.g., head, injuries, damage to coralls), and search the subcutis and muscles for bruising and trauma.^[7]

All the details observed by Veterinarians should be included in post-mortem report with time, date and place. The reports including all post-mortem, environmental and third party artefacts help to avoid misinterpretations and confusions at the time of judgement. Burning, corrosion and maceration are very commonly caused artefacts which are introduced after occurrence of death. Deliberate mutilation of dead body as efforts to mask or change the identifying characteristics of dead animals includes cutting of piece of ears, tail, damaging scars or tissue marks shaped due to treatment and surgery tried before death of animals.^[6]

Specimens: The most important and crucial sources of evidences are specimens collected by

Veterinarians from affected or dead animals. The specimens must be collected as per the guideline issued by analytical laboratory. The Veterinarian has to follow this guideline. The shipping of samples with prescribed preservatives also influences accuracy of analysis and there by credibility of evidences generated from this analysis. Generally brain, liver, kidneys, heart, lung, skins, hairs, stomach contents, intestinal contents, blood and urine fats are collected using appropriate preservatives. Generally saturated salt solution, alcohol mercury chlorides are used for preserving the tissues samples. For collection of blood, sodium fluoride (20 mg/ml), sodium citrate (5 mg) and mercury chloride mixture (0.1 mg) per ml of blood is preferred for toxicological investigation. For specific analysis, the instruction of laboratory analysts must be followed. The preservatives used in collection should be sent as a control testing. Alcohol should not be used in poisoning cases involving volatile poisons like phenol. Many times solid salts are also used to preserve the pieces of tissues which are embedded with dry salt powder.^[6]

Samples of tissue should be fixed in formalin for histology testing and frozen fresh in separate containers for toxicology testing. Formalin-fixed tissue is usually useless for toxicology testing. However, fixed tissue should be collected for histology examination, which is also critical to a toxicology diagnosis. Because toxicology laboratories differ in some tests and test methods, a Veterinary toxicologist and the state or regional laboratory should be consulted about proper sampling before an investigation is initiated.^[7, 6]

All samples should be saved despite any initial findings. For the legal case, duplicate samples should be frozen to compensate for shipping losses. Each sample is labelled as to origin, tissue type, date, animal, and clinician.^[7] Unless the viscera and other articles are forwarded to chemical analysis laboratory they are to be preserved for a period of six month and are then to be destroyed after obtaining the permission from Magistrate.^[8]

Testimony: Testimony may be from lay witness or expert witness. They are generated during discovery portion of legal investigation. It may oral under oath before Trier or one may submit them in written form dully sworn statement (affidavit).^[5]

a) Lay witness

Generally farmers/clients give lay evidences in form of information on nature, time and cause of death and observed symptoms. The lay testimony need to be verified by expert

testimony thoroughly. The Veterinarian often is called upon to testify in legal proceedings. As a general rule of evidence, a witness may testify only to facts known to him or her. The witness may offer opinions solely on the basis of what he or she has observed. Such a witness is called a “lay witness”.

b) **Expert witness**

When court has to form an opinion upon a point of foreign law, science, or art, or as to identity of hand writing, the opinions upon that point of person especially skilled in such foreign law, science or art are relevant facts. Such person is called expert. ^[11]

However, an expert need not be a paid professional expert who makes earning by giving such evidences, but he must have devoted sufficient time and study to the subject so that he can make his evidence trustworthy. A person having a special knowledge of the value of land through by experience but not by profession can be treated as expert. ^[12] The Veterinarian/toxicologist is referred to as an “expert witness.” A court recognizes a witness as an expert if that witness possesses knowledge or experience in a subject that is beyond the range of ordinary or common knowledge or observation. An expert witness may provide two types of testimony: objective testimony and “opinion.” Objective testimony by a Veterinarian/toxicologist usually involves a description of his or her analytic methods and findings of Post-mortem examination, and correlation between findings and facts. When a toxicologist testifies as to the interpretation of his or her analytic results or those of others, that toxicologist is offering an “opinion.” Lay witnesses cannot offer such opinion testimony, as it exceeds their ordinary experience. ^[4]

Before a court permits opinion testimony, the witness must be “qualified” as an expert in his or her particular field. In qualifying someone as an expert witness, the court considers the witness’s education, on-the-job training, work experience, teaching or academic appointments, and professional memberships and publications as well as the acceptance of the witness as an expert by other courts. Qualification of a witness takes place in front of the jury members. ^[4] The Veterinarian as an expert must be well

prepared for court room appearance. The logical and systemic structure of opinion qualitatively needs professional competence, objectivity and impartiality, intellectual integrity and intelligibility of speech and duty to strictly personal delivery of opinion and strict adherence to theme. ^[13] The Veterinarians may also be challenged as to his competency and his credit may be impeached on the ground that he was not in fir start to form an opinion that he was interested, corrupted or expressed a different opinion at other times. The few set of golden rules for expert are: ^[14]

- a) If you have an opinion, be firm about it.
- b) If there is a room for argument, admit it freely.
- c) Be as concise as possible.
- d) Use non technical terms without loss of accuracy of facts and findings.
- e) If you have to give ground, do so with honeys and with good grass.

The opinion of medical man includes the cause of disease, age of animal, time of death, the nature of injury, type of weapon used to injure animals and resultant patterns of wounds, use of weapon to cause death, use of poisons, medicines or other chemicals ¹². The opinion needs to throw enough light on; independence of evidences, objective and unbiased opinion, presentation of material facts, disclosure of issue beyond the witness’s expertise, any lack of detector qualification of truth, any change of opinion, exchange of report with opposing expert, and sharing of material evidences on which opinion is based. ^[14]

General Guidelines for Collection of Samples for toxicological investigation: Consistent sampling procedure is important for quality specimen collection. Sampling procedure is very specific job and requires specific protocol for specific purpose and types of investigation. But some generalized guidelines can be made and adopted in many instances to make sample collection very consistent and effective for toxicological investigation. Such generalized guidelines are described as under: ^[9, 15]

- (1) As the distribution of toxicants suspected in body tissues is unknown, it is compulsory to take more than one tissue sample in large quantity.
- (2) Extraction protocol for every toxicant differs widely, so one extraction protocol may not

work simultaneously for other toxicants. That is why it is necessary to collect large amount of tissue samples for toxicological analysis.

- (3) Do not wash the sample with water before packing and dispatching.
- (4) The selection of sample of ingesta and/or chemical analysis depends on the results of necropsy. When in doubt, it is always better to send too much rather than too little.
- (5) Most samples should be frozen unless specific analyzes require other treatment. Blood samples should not be frozen but transported under refrigeration. Freezing prevents loss of volatile agents (e. g. ammonia or cyanide) and generally prevents enzyme or bacterial activity that could inactivate toxicants. Conversely, freezing can inactivate some sensitive tests involving enzyme activity. The toxicologist or analytic laboratory should be consulted if doubt exists about the recommended preservation.
- (6) Always give information regarding circumstantial evidences to the investigator.
- (7) Each sample must be sealed in clean, individual container.
- (8) Glass containers are preferred over plastic container for pesticide or low level organic contaminants.
- (9) Preservatives must be added to samples unless laboratory instruction specifically.
- (10) Serum should be separated from the clot because interpretation of serum results may be affected by leaching of material from the erythrocytes.
- (11) For some trace elements, special element free tubes may be necessary.
- (12) Feed and forage samples that are high in moisture (>15%) should be dried or refrigerated to retard mold growth.
- (13) Adequate coolant must be used. At least a 4:1 ratio of coolant to specimens is recommended.
- (14) Biologic specimens should be double bagged and sealed to prevent leakage of blood and tissue fluids.
- (15) Sample integrity and chain of custody may be important for cases involving insurance claims or litigation.

THE BASIC APPROACH TO LEGAL CASES

The Veterinarians being an expert evidence has to deal with the all expert evidences in the court for setting up legal dispute. The three main fundamental approaches to deal with legality of veterolegal cases are as under: ^[5]

(1) General Causation and specific causation of death

The expert witness or evidences as admissible by court must able to prove cause of death in general condition. The standard reference materials or research generals or treatises on subjects or monograph issued by official bodies can be used as supportive materials to prove the thing right in court. The specific causation considers the cause of death in given specific sets of circumstances. This can only be proven by correlating different evidences and logical technical interpretations.

(2) Rule out and Rule in

One should able to prove that the death is not due to all this cause i.e. ruling out all possible likely causes of death based on evidences. At the same time, it is to be proven that the death under investigation is most likely due to this cause among all possible cause. The strong rule out and in requires technical knowledge, logical skill of interpretation and subject wisdom.

(3) Differential diagnosis

The ability of expert witness and evidences to establish clear cut diagnosis/case of death requires differential diagnosis. The disease/cause of death is differentially determined based on some diseases or causation specific symptoms or post-mortem findings. The differential diagnosis profile is very important for Veterinarian to ascertain the cause of death from causes "likely to be" causes related to investigation.

(4) Interpretations

Test results are not always indisputable as their mystique might infer. Diagnosis of death due to poisoning must always be synthesis of history, post-mortem lesions, test results, response to therapy and professional judgement based on knowledge and experience with toxicology problems. Similarly, testing May sometimes indicates a seemingly significant concentration of poison but animal may not be poisoned by it. This happens in cases, when toxicants may be natural substances in body (nitrates, urea, ammonia, DDT) or animal may have developed tolerance to it. So test results may be contradictory to tentative/presumptive diagnosis. The critical review of cases and circumstantial evidences helps to arrive at errorless judgement and diagnosis of cause of death. ^[16] Toxicants cause a variety of lesions ranging from no lesions to specific changes in tissue structure. No lesions in per-acute poisoning/neurotoxicosis/residue cases are commonly encountered and produce great confusion in

interpretations and judgement. At another end presence of non specific lesions for example, hemorrhagic gastroenteritis (for example in arsenic or even salt poisoning) is also divert accuracy of diagnosis towards false positive of negative. Presence of specific lesions associated with well defined etiological agents should be correlated with laboratory diagnosis. In addition to the pattern and type of lesions, the chronicity of those lesions can also yield clues about the nature of the toxin and exposure pattern.^[7] Once the examination is complete, it may help to refer to a checklist prepared

before the examination to be sure all organ systems were properly examined and sampled. A preliminary diagnosis may be given after all laboratory testing is complete. For example, gross findings and an initial check on the history may be misleading and could be embarrassing later if a diagnosis is made too soon. A written history and description of signs and lesions should be included with the samples to help the laboratory diagnostician provide the best advice regarding selection of tests and interpretation of findings.^[7]

Table: Specimens and Samples for Diagnostic Toxicology^[9, 10]

Sample or Specimen	Amount	Comments
ANTE-MORTEM		
Blood	5-10 ml	Useful for detecting exposure to most metals, trace metals cholinesterase, pesticides, and ethylene glycol, and for evaluating erythrocyte and leukocyte morphology.
Serum	5-10 ml	Useful for evaluation of erythrocytes, urea nitrogen, ammonia nitrogen, and organ function; exposure to metals, drugs (e.g., antibiotics) and vitamins.
Urine	50 ml	Useful for detecting exposure to alkaloids, metals, electrolytes, antibiotics, drugs, sulphonamides, and oxalates.
Feces	250 g	Useful for detecting recent oral exposures or drugs or toxicants excreted primarily in bile.
Vomit	250 g	Useful for detecting ingested poisons of all types, especially those that cannot be measured in tissue (e.g., organophosphates, ionophores). Indicator of recent oral exposure.
Hair	5-10 g	Useful for detecting dermal exposure to pesticides; chronic accumulation of some metals (e.g., arsenic, selenium). Must be interpreted based on time of years, area of body where sampled, and external contamination.
POST-MORTEM		
Liver	100 g	Major organ of biotransformation. Accumulates, pesticides, alkaloids, phenols, and some mycotoxins; bile may be useful for detecting toxicants concentrated by bile (e.g., lead).
Kidney	100 g	Major organ of excretion for antibiotics and other drugs, metabolized toxicants, alkaloids, herbicides, some metals, phenolic compounds, oxalates.
Stomach contents	500 g	Confirmation of recent oral toxicant exposure.
Rumen contents	500 g	Confirmation of oral toxicant exposure. Rumen may degrade some toxicants (e.g., nitrates, mycotoxins). Quantitative analysis is difficult as a result of variability of concentrations and lack of correlation with toxic levels in tissues. Samples should be collected from several locations in the rumen and kept frozen until analysis.
Fat	250 g	Useful for detection of cumulative fat-soluble toxicants (e.g., chlorinated pesticides, dioxins).
Ocular fluids	Entire eye	Useful for evaluating electrolytes (e.g., sodium, calcium, potassium, magnesium), ammonia, nitrogen, nitrates, and urea nitrogen; ocular potassium and urea have been used to estimate time since death. Both aqueous and vitreous humors are useful, but should be collected separately.

Brain	Entire brain	Useful for detecting some neurotoxicants (e.g., chlorinated pesticides, pyrethrins, sodium, mercury). Brain should be separated by midline sagittal section, and the caudate nucleus collected for cholinesterase determination. Half of brain should be frozen, half fixed in 10% buffered formalin.
ENVIRONMENTAL		
Feeds	2 kg	Multiple representative samples should be taken and then either combined and mixed for a composite sample or retained as individual samples (to detect variability in the source), or both.
Forages (e.g., pasture, hay, silage)	5 kg	Samples should be taken from multiple locations in a pasture or storage facility, using a forage (core) sampler for baled hay or stacks. Silage should be frozen to prevent mold and deterioration.
Baits	All	Entire suspect bait and label should be submitted if available.
Water	0.5-1 L	Useful for detection of nitrates, sulphates, total solids, metals, algae, and pesticides.

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