Feasibility of Using Evidence-Based Vrtopsy to Answer the Possible Clinical and Post-Mortem Questions, in Veterinary Practice

Mohammad Molazem1, 2*, Arezoo Ramezani1, Sarang Soroori1, Zahra Jafari Giv1, Sara Shokrpoor3, Urs Geissbuehler

1 Department of surgery and radiology, Faculty of veterinary medicine, university of Tehran, Tehran, Iran
2 Department of Clinical Veterinary Medicine, Division of Clinical Radiology, Vetsuisse Faculty of Bern, University of Bern, Bern, Switzerland
3 Department of pathology, Faculty of veterinary medicine, university of Tehran, Tehran, Iran

Abstract

Postmortem examination is an important part in evidence-based medicine to understand deterioration of clinical signs or causes of death in euthanized or deceased individual animals or even populations.

The object of postmortem analysis is to improve clinical treatment and therapy, to confirm suspected diagnosis, to manage breeding strategies or to clarify in forensic cases the conditions, which had led to death (neglect, animal abuse). In analogy to virtopsy in human medicine, Diagnostic imaging modalities have been applied in post mortem veterinary medicine, which we call Vtvtopsy.

We hypothesize, that Vtvtopsy can be used as a method for certain clinical/post mortem questions to improve the reliability of the diagnosis. In some questions, Vtvtopsy actually can replace conventional necropsy. The aim of this overview study is to compare Vtvtopsy to conventional necropsy in variable causes of death in animals and to define its possibilities and limitations.
Deceased or euthanized pets and wild animals were collected. The methods used are imaging techniques such as postmortem digital radiography, postmortem ultrasound, postmortem computed tomography and postmortem magnetic resonance tomography in combination with image-guided tissue sampling to address the open questions about clinical symptoms or causes of their death.

The case series in this project shown that using diagnostic imaging technique is feasible in answering different clinical ante-mortem and post-mortem clinical and forensic questions; however, there is an interdisciplinary collaboration between the diagnostic imaging and sampling under imaging guidance.
Introduction

Postmortem examination is an important technique in veterinary evidence-based medicine to identify causes of death and improve medical treatment or breeding strategies. In some cases, (animal abuse, neglecting or suffering), the postmortem examination has also a forensic aspect (Watson et al., 2017; Benetato et al., 2011). In addition, it is useful for confirmation of the suspected disease in poor prognostic cases with several differentials diagnosis, in cases of limited economic owner possibilities, unknown history, and forensic cases (animal protection).

Autopsy is the current standard in veterinary medicine (Hostettler et al., 2015); however, the numbers of conventional necropsies at the Institute of Veterinary Pathology of the Vet Suisse Faculty Zurich decreased markedly in the past years. In the years from 2010 until 2013, the yearly average of conventional necropsies was 350 dogs and cats. In 2014, 300 and in 2015 only 151 dogs and cats (until 26.11.15) underwent conventional necropsy (Pewsner et al., 2017).

In the past years, respect for and ethical values of pets are continuously increasing in a humanistic view of the world. In today’s society, the position of the animal changes more and more to a social partner and family member. In addition, religious reasons could also explain the decrease in conventional necropsies (Thali et al., 2009).

In human forensic medicine, cross sectional imaging techniques, such as postmortem computed tomography, postmortem magnetic resonance imaging, photogrammetry-based 3 dimensional optical surface scanning, CT-guided biopsies and minimally invasive CT- or MR- angiography are used on a regular base to complement or replace autopsy (Thali et al., 2007).

Literature on comparative studies between 2D and/or 3D postmortem imaging procedure and conventional necropsies in veterinary medicine are very rare (Ibrahim et al., 2012).

The term “Virtopsy” is compound from the words “virtual” and “autopsy”. Virtual means in this context artificial, produced by a computer. Autopsy is a combination of the Greek words “autos” for self and “opsomei” for I will see. Autopsy means to see with one’s own eyes (Ibrahim et al., 2012).

The process of an autopsy is an unrepeatable, operator depending act (Thali et al., 2010). Therefore, important, in the external examination nonobvious or unexpected findings can be lost. Such findings include for example small fractures, pathologic gas or fluid accumulations, relevant thrombi and decent soft tissue trauma.

The use of virtopsy in veterinary medicine was very limited until 2004 and was often used for diagnosis confirmation (Buck et al., 2009; Delannoy et al., 2012). In recent years, however, studies have shown that virtopsy, especially in small animals, is an efficient method to prevent necropsy and save time, resources and energy (Ibrahim et al., 2012).
The aim of the study was to prove the essential role of virtopsy in the question based determination of death animals.

For this purpose, a case series of virtopsy was performed in routine veterinary practice to demonstrate its potential use in different clinical or forensic questions.

MATERIALS AND METHODS

The study population consists of deceased or euthanized pets and wild animals referred to the small animal hospital of the university of Bern and small animal hospital of University of Tehran between 2017 and 2020. While the authorities requested for wild animal post mortem examination, the request for pet post mortem animals arised from medical interest. In all pets a post-mortem examination consent was obtained from the owners. History, time of death or euthanasia and open questions about clinical symptoms or causes of death were recorded in a survey sheet for each case prior to the imaging examination. Based on this information, the cases were categorized in 3 groups in order to see, if it is possible to detect the cause leading to prognostically hopeless clinical signs in pets. This question is frequently asked in research autopsy with the primary goal of collecting tissue to support basic or translational research. This approach has increasingly been used to investigate the pathophysiological mechanisms of cancer evolution, metastasis formation and distribution and treatment resistance (Watson et al., 2017). In the second group we were interested, if it is possible to detect the cause of death in found dead animals. Owners, clinicians and authorities may oppose an autopsy for a variety of reasons, including fear of animal abuse or suffering, or for caring of the animals in danger of extinction.

In the third group of cases we wanted to know, if it is possible to identify the animal and the probable cause of death bog bodies?

A non-destructive method enables gentle examination of fragile bog bodies. Based on the open questions, the cadavers underwent an examination of one or several regions of interest or whole body imaging by choosing one or several of the following diagnostic imaging modalities. Radiographs were performed with a direct (Kodak casestream directview classic) or computed radiography or (Fuji smart CR-XG-1) system.

Computed tomography was either performed with a 16 slice (Philips Brilliance 16) or a 2 slice (Siemens Somatom) helical machine. Selection of slice thickness depended on the body weight and measured 1.5 mm in body weights from 1 to 10, 2.5 mm in cadavers from 10 to 30 and 3 mm from 30 to 60 kg.

Magnetic resonance imaging was performed with a 1.0 Tesla device (Philips panorama, 1.0 T, HFO). STIR sequences were performed in the region of interest.

Possible examination areas in CT and MRT were “head, neck and front limbs”, “thorax” and “abdomen and hind limbs”. The decision for ultrasound or CT-guided tissue biopsies was based on the open questions and imaging findings and assessed by the Institute of Veterinary Pathology of the University of Bern or by the Department of Veterinary Pathology of the University of Tehran.
Analysis of the imaging findings were based on the ability to answer (yes) or inability (no) the clinical question.

**Results**

During a period of 6 years, from 2017 to 2020, virtopsy was performed on a total of 20 carcasses of 12 species of animals with different age and sex (table 1).

The protocols included 11 whole-body and partial CT scans, 4 partial MRI examination, 1 ultrasonography, 4 ultrasound-guided sampling and 18 radiography (table 1).

Neoplasia (3 cases), infectious (3 cases), Trauma (8 cases), hallow organ rupture (2 cases), were the fetal pathologies noted in this study (table 1).

Six cases were assigned to the first group; in all 6 cases, virtopsy could figure out the cause of hopeless clinical signs by the following necropsy confirmation.

Case no 1:

A 7-year-old Flat-coated retriever female dog was evaluated for her inability to chew and pain when chewing. Additionally recurrent ear infections occurred shortly after starting treatment. The exact location of the pain was not recognizable. The animal was euthanized due to the poor quality of life and prognosis and the owner gave consent to postmortem examination. An hour after death in post mortem laterolateral radiography of the skull a decent, round, granular soft to mineral opaque lesion was seen caudal to the bullae, partly superimposed to the nasopharynx and the retropharyngeal soft tissues (figure 1).

In the computed tomography, a monostotic aggressive bone lesion of the left condylar process mainly affecting the mandibular fossa with soft tissue mineralization and otitis media was detected. Differentials included neoplasia or osteomyelitis (figure 2). Subsequently, CT-guided bone biopsy (2x) using a bone marrow biopsy set 11G x 100 mm from the left mandibular condyle was performed for histopathologic examination. In histopathological specimen study, osteosarcoma was confirmed.
Figure 1 post mortem LL radiography of the skull of a 7-year-old Flat-coated retriever female dog. An ill-defined decent soft tissue to mineral opaque lesion was seen caudal to the tympanic bullae (Black arrow).
Figure 2 Transverse CT image of the temporomandibular joints of case 1: aggressive bone lesion of the left condylar process with granular-like mineralization ventral to it. The soft tissues of this area are increased and the left lateral aspect of the nasopharynx is flattened.

Case No 2:

An 11-year-old male Persian cat with a history of chronic cough and lameness of the right forelimb for one week was presented. Regarding age and financial limits the animal was euthanized at the request of the owner. No palpatory lesion could be found in the gross examination.

Postmortem MRI and CT imaging was performed to assess if there exists a lesion at the right forelimb and if there are signs for metastases.

On whole body CT a large mass in the right supraspinatus muscle with predominantly proliferative bone changes in the scapula and enlargement of the right ipsilaterally axillary lymph node were detected (figure 3).

On whole-body MRI (STIR) examination soft tissue lesions were identified in the right fore- and left and right hindlimbs and in the left pectoral, right epiaxial and left gluteal muscles. Consolidation of the entire left lung lobes was also visible (figure 4).

An ultrasound-guided biopsy was performed from the lung lesion, which turned out to be a mast cell carcinoma (solid carcinoma) but the affected tissue could not be assessed by biopsy; it might be intercostal or diaphragmatic muscles.
Figure 3 Transverse and parasagittal post mortem CT-images of a 11-year-old male Persian cat at the level of the right scapula, bone window; predominantly proliferative bone lesion of the right scapula.

Figure 4: Dorsal postmortem CT reconstruction image (left side) and dorsal MRI SPIR image of the right sacular region of the same case in Fig 3 showing proliferative scapular changes and marked heterogenous hyperintenisty in the supra- and infraspinam muscle.

Case No 3:

A sixteen-years-old female cat with a history of anorexia, epileptic seizures, ataxia, and vomiting presented on clinical examination a heart murmur and abdominal tenderness. Due to poor prognosis she was euthanized. Postmortem left lateral and VD radiographs of the chest were performed immediately after euthanasia for further examination of the thorax. A large thoracic volume with gas accumulation in the esophagus and the stomach, and diffuse bronchointerstitial lung pattern with soft tissue opacity of the right middle lung lobe were detected. The lung changes were most likely consistent with allergic bronchitis such as feline asthma (figure 5).

A post sagittal mortem MRI STIR sequence of the cervical, thoracic and lumbar spine and a transverse
SPIR sequence of the lumbosacral junction were performed. Signal intensity was increased in the left humerus (Figure 6), paravertebral muscles, left and right lateral soft tissues of L7, and ventral aspect of the vertebral canal of the L7

Post mortem abdominal ultrasonography showed generalized small intestinal muscular layer thickening up to 1.5 mm (figure 7). Ultrasound guided fine needle aspirates of the small intestinal muscularis confirmed high grade lymphoma. Therefore, the pre-mortem clinical signs are were most likely due to multicentric distribution of the neoplasia.

Figure 5 Immediate post mortem radiographs of a sixteen-years-old female cat show a large thoracic volume with a considerable amount of gas in the stomach and a diffuse bronchointerstitial lung pattern. The right middle lung lobe is soft tissue opaque (consolidation)
Figure 6 Focal STIR hyperintensity of the same case as Fig 5 in the left proximal humerus metaphysis in a dorsal MRI image (arrow).

Figure 7 Transabdominal post mortem ultrasound with a linear probe demonstrates concentric muscularis thickening in two jejunal loops.

Case No 4:
A recently deceased 6 years old African grey parrot of unknown sex was referred with a history of chronic apathy and anorexia. The bird died during clinical examination. In clinical post mortem exam a mass was observed between the mandibles. The owner requested, virtopsy to get further informations about the cause of death.
Lateral and VD radiographic projections of the whole body were taken. A space occupying soft tissue lesion was observed caudal to the beak. Furthermore a large volume of gas was seen in the intestines, which could be a post mortem change.

CT scans of the skull performed almost 4 hours postmortem showing a well-defined homogeneous soft tissue attenuating (HU=12) mass which was visible at the ventroproximal aspect of the trachea causing narrowing of its lumen. At the same level an additional irregular soft tissue attenuating mass was visible in the dorsal wall of the trachea. Both lesions caused partial obstruction of the upper airway tract (Figure 8). Ultrasound guided fine needle aspiration of the ventral tracheal lesion was performed. Cytological examination revealed an abscess. A focused necropsy at the area of the mass confirmed the findings.

Figure 8 Reconstructed sagittal CT image (bone window) and photograph of a 6 years old African grey parrot of unknown sex in the region of skull showing a homogenous soft tissue attenuating mass ventral to the ventral and in the dorsal tracheal wall (arrows).

Case No 5:
A 12-year-old euthanized male mixed breed dog with a history of chronic bilateral forelimb lameness has been referred for virology to verify the suspicion of a leishmaniasis.
Post mortem lateral and craniocaudal radiographs of both stifles were performed.
On the images of the right stifle, there was a smooth periosteal reaction on the distal metaphysis of the femur and inhomogeneous moth-eaten lysis and patchy sclerosis of the cancellous bone of the distal metaphysis of the femur and the proximal metaphysis of the tibia. The bone cortex was interrupted. A
bone defect was also visible at the distal end of the patella. Multiple small subcutaneous gas opacities were delineated in the lateral aspect of the distal part of the femur. The lytic changes in the left stifle were significantly less pronounced and the periosteal changes and the gas opacities were not visible (figure 9).

The differential diagnosis of the aggressive bone lesion in the distal femur and proximal tibia were more likely related to fungal osteomyelitis and neoplasia was unlikely. Bone biopsy was performed under ultrasound guidance. Histopathological studies detected multifocal osteolysis by osteoclasts with fibrosis of the bone tissue and multifocal infiltrates with macrophages, lymphocytes and isolated polygonal cells. These findings were compatible with an inflammatory process with pronounced bone resorption. Biopsy of the bones confirmed Leishmania infection.
Figure 9 bilateral aggressive bone lesions in the distal femurs and proximal tibiae of a 12-year-old euthanized male mixed breed dog.
A 7-month-old female pig was referred with tetraplegia. On neurological examinations myelopathy of C6 to T2 and pain in the neck were observed. The animal was euthanized with the consent of her owner. The carcass of the animal was subjected to post-mortem imaging studies to determine the cause of the neurological complication. According to the history, lateral radiographs of the cervical vertebrae were taken. The body of C7 was shortened with narrowing of the intervertebral disc space of C6/C7 and focal dorsoventral narrowing of the trachea at the thoracic inlet associated with moderate ill-defined increased ventral soft tissue swelling minimally compresses the dorsal trachea lumen (figure 10). The findings were consistent with a collapse of C7, IVDD C6/7 and space-occupying soft tissues ventral to this region. The radiographic findings might be compatible with osteomyelitis/discospondylitis, compression fracture and soft tissue inflammation or hematoma. Due to age and species a bone tumor appeared less likely. Post mortem MRI of the cervicothoracic spine confirmed a shortened C7 vertebral body, a large amount of extradural material at the level of C7 severely compressing the spinal cord from ventral and right, C6/7 discospondylopathy, and left-sided supra- and infraspinatus and longus coli muscles myopathy (figure 11). These findings were compatible with pathologic fracture of the C7 and disc and probably bone material extrusion due to C6/7 discospondylitis.

Afterwards, a CT examination of the cervicothoracic part of the vertebral column was performed for CT guided tissue probing. Aggressive bone changes and compression fracture with collapse of C7 were confirmed and were compatible with bacterial discospondylitis or abscess formation and a consequent pathologic fracture. CT-guided fine needle aspiration (2x) from the C7 vertebral body confirmed acute, purulent discospondylitis and chondritis (figure 12).

Figure 10 Lateral projection of a 7-month-old female pig in the region of cervical vertebra, C6/C7 intervertebral disc space and C7 vertebral body is narrowed.
Figure 11 The same case as Fig 10: pathologic fracture of C7 and disc and probably bone material extrusion due to suspected C6/7 discospondylitis. The C6/7 intervertebral nucleus pulposus is smaller than the previous and following ones.

Figure 12 transverse image of the CT guided aspiration of C6 by a spinal needle.

Second group: in found death animals, is it possible to detect the cause of death?

Clinical Question: What is the cause of death?

A total of 12 cases (12/20) were evaluated; in 11 cases virtopsy figured out the pathology leading to animal death compatible with the following necropsy studies.

Case No 7:

A skeletally immature female Eurasian lynx (Lynx lynx) was found dead. Lateral and dorsoventral radiographs of the skull and lateral views of thorax and abdomen were taken. Several sharpnels associated with multifragmented right mandible, increased opacity of the right tympanic bulla, and a
small mineral opacity rostral to the odontoid process were detected (figure 13). Some post mortem changes were already visible including lung atelectasis, intestinal functional ileus, and pneumomilia.

Given the distribution of the sharpnels and the soft tissue swelling, the bullet trajectory could be from the coudodorsomedial aspect of the occiput to the right rostroventrolateral aspect to the right mandible.

Figure 13 orthogonal views of the skull of a female Eurasian lynx (Lynx lynx) showing the trajectory (arrows) of the gun shot sharpnels.

Case No 8:

A carcass of a bear was found and undertaken radiographic study of the skull. Radiography revealed the presence of a metal bullets in the ventral aspect of the calvarium at the level of tympanic left bullae and behind the left mandible (figure 14). Computed tomography performed later and identified multiple fractures in the midline of the frontal bone indicating the gunshot trajectory through the skull, nasal cavity, ethmoidal bone, and left perpendicular plate of the palatine bone. Bullets were visible in the soft tissue of the ventral aspect of the calvarium, left lateral aspect of the trachea slightly behind the left mandible and Left TMJ, and slightly rostrally to the left tympanic bullae (figure 15). The shrapnles could not be seen in the radiographs due to small sizes and superimposition on the surrounding bones.
Figure 14 orthogonal radiographs of the skull of the bear with a metal bullets in the ventral aspect of the calvarium at the level of tympanic left bulla and behind the left mandible.

Figure 15 The same case as Fig 14: multiple fractures in the midline of the frontal bone indicating the gunshot trajectory through the skull, nasal cavity, ethmoidal bone, and left perpendicular plate of the palatine bone (arrows).

Case No 9 and 10:
The cadaver of two crows (Corvus) were found and presented to detect the reason of death. In case no 9, a metal density was visible at the right shoulder joint. In case 10, four bullets in the right humerus, left radius, right femur, and the coelomic cavity was detected. Also, transverse fracture at tibiotarsal bone was visible which could be due to falling on the ground after being shot (figure 16).
Case No 11:

Figure 16 Two crews with multiple gunshots.
A female roe deer (Capreolus capreolus) was found dead. Post mortem study consists of one lateral view of the thorax which includes the caudal cervical region, almost the entire thorax, the ventrocranial abdomen, the entire right forelimb and part of the left forelimb (until the mid-3rd metacarpus).

Luxation of the metacarpophalangeal joint of digit III and IV, right-sided and subacute to chronic ossifying periostitis along the dorsal aspect of the right-sided 3rd metacarpus were found on the post mortem radiographic study. Metallic structures confirming gunshot was not detected (figure 17). Post mortem lung lobe atelectasis produced alveolar lung pattern with airbronchograms is also noted.

Figure 17 Luxation of the metacarpophalangeal joint of digit III and IV, right-sided and ill-defined smooth and solid periostal reaction along the dorsal aspect of the right-sided 3rd metacarpus of a roe deer consistent with periostitis. Note the alveolar lung pattern with airbronchograms.
The carcass of a flamingo was found and was referred to investigate the complication created in the legs and its connection with the death. Radiographic images of both legs were taken in the craniocaudal view. Complete spiral fracture was observed at the end of the left tibiotarsal bone. No metal density was found indicating the bullet (figure 18).

![Image of radiographic images](image)

**Figure 18** Complete spiral fracture was observed at the end of the left tibiotarsal bone of a death flamingo.

**Case No 13:**

A cadaver of a 7-month-old female outdoor domestic short hair cat which had been found death by the owners in the yard referring for forensic investigations on the same day.

In the whole body CT, the right pleural cavity was filled with space occupying hyperattenuating fluid which had displaced the mediastinum to the contralateral side. In addition, there was marked soft tissue swelling around the left forelimb associated with diffuse subcutaneous hyperattenuating lesions and multiple small gas inclusions. The skeletal structures were within normal limits.

The changes were compatible with acute right sided pleural hemorrhage and diffuse soft tissue swelling and hemorrhage around the left forelimb which could be related to multicentric trauma (possible cat biting). (Figure 19 and 20).

In the necropsy hemothorax due to a small perforating trauma has been concluded.
Figure 19 Almost immediate post mortem image of a 7-month-old female outdoor domestic short hair cat with right sided pleural hemorrhage.

Figure 20 Almost immediate post mortem image of the same case as Fig 19 with soft tissue swelling and hemorrhage around the left forelimb.

Case No 14:
A cadaver of a 6-year-old Jack Russell Terrier male dog presented for forensic studies. Initially, postmortem CT scan study of the whole body revealed multiple fractures on the right zygomatic arch, lateral wall of the occipital condyle, the atlas, axis, and C4 with multiple gas inclusions in the soft tissues on the right lateral side of the skull and extensive soft tissue lacerations on the right lateral side of the neck. The right globe and the lens were deformed.

The urinary bladder wall was noticeably thickened and only a little inhomogeneous content with small gas pockets in the urinary bladder was visible. A soft tissue density (intervertebral disc, dorsal and ventral intervertebral ligament) protruded on lumbosacral junction.

Post mortem MRI of the skull and cervical spine was performed to evaluate probable brain and spinal cord damages. Large skin lacerations at the level of C2-C6 with lacerations and interruption of the spinal
cord at C1 and C2/3, multiple fractures of the right parietal bone and the skull base with marked compression and dislocation and moderate destruction of the right brain hemisphere and adjacent soft tissue associated with several hemorrhagic lesions and gas inclusion was detected. Based on the signal characteristic of the visible hemorrhages, acute bleeding was suspicion (T1w iso, T2w hyperintens with T2* susceptibility artifact).

It indicates a possible trauma to head and neck and no evidence of metal fragments that suggest bullets was detected (Watson et al., 2017).

The shape of the wounds associated with the depression fractures were speaking for a trauma happened by a blunt instrument (e.g. hammer) (Watson et al., 2017; Benetato et al., 2011). (Figure 21 and 22)

Figure 21 Three dimentional rendering image of a 6-year-old Jack Russell Terrier with skull compression fracture.
Figure 22 Transverse T1w, T2w and T2* and a sagittal T2w showing several hemorrhagic lesions (arrow) and gas inclusion (arrow head) in the left temporal aspect of the skull and the cerebrum.

Case No 15:

A skeletally mature Persian male cheetah body was referred for forensic assessment, two days after an acute longitudinal L5 fracture open reduction and stabilization by internal fixation.

Post mortem radiography and CT performed to found out the reason of death; a severely displaced L5 and minimally displaced T8 fractures associated with implant failure and additional small fragments of L5 compared to the pre-operative radiographs were detected. In addition, abdominal effusion, solitary cavitary lung lesion, and Mega esophagus including some food material were visible.

The abdominal effusion aspirated under ultrasound guidance and acute abdominal hemorrhage was found. The cavitary lung lesions were consistent with traumatic lung bulla and the esophageal changes were most likely due to be a post mortem change. Implant failure creating hemorrhage have considered to be the main reasons of death. (Figure 23 and 24).
Figure 233 Lateral radiograph of lumbar spine of a Persian Cheetah with implant failure and severely displaced L5 fracture.

Figure 244 Three dimensional rendering reconstructed images of the lumbar spine of a Persian Cheetah with implant failure and severely displaced L5 fracture.

Case No 16:

An 11 years old female herd guard dog was found death and the carcase presented to detect the cause of death. Severe post mortem autolysis developed prior to the post mortem study. Whole body post mortem CT scan revealed, bilateral moderate to severe osteoarthritis in the stifle joints with signs of joint effusion with the Hansfield Unit = 45 (figure 25) compatible with erosive arthritis (rheumatoid, immune-mediated, leishmaniosis), lesion of the cranial cruciate ligament, or primary osteoarthritis. There were bilateral partially obstruction of the external ear canal by soft tissue attenuating material (figure 26) which might be due to chronic otitis externa or neoplastic process (ceruminoma).
The findings could not explain the main reason for death in this case. Necropsy also failed to found the main fetal pathology.

Figure 25 Orthogonal CT scan reconstructed images of the right stifle joint of a death female herd guard dog with moderate degenerative or inflammatory osteoarthritis.
Case No 17:

The body of a skeletally mature European shorthair female cat was presented for necropsy. Post mortem radiography and CT scan of the whole body revealed patchy hyper densities in both nasal cavities (L>R), hyper dense material in the lateral compartment of the left bulla, subcutaneous and connective gas inclusions in the region of the pharynx, neck, and the thoracic wall and along the trachea and esophagus, pneumomediastinum and connecting severe bilateral pneumothorax and Subluxation of the left stifle joint with rupture of the cranial cruciate ligament.

There was a defect in the dorsal aspect of the trachea in its most cranial part (figure 27 and 28) Since no signs of skin lacerations, tracheal rupture should have done by a blunt trauma or passage of a traumatic material through the esophagus.
Figure 27 Lateral view of the skull of a European shorthair female cat with retropharyngeal and subcutaneous emphysema and pneumomediastinum.

Figure 288 Three planar reconstructed image of the skull of the cat as Fig 27 with a defect visible in the dorsal wall of the proximal trachea (arrow) associated with large amounts of surrounded emphysema.

Case No 18:

12-year-old Californian sea lion (Zalophus californianus) has been found death without prior significant clinical signs.

Primary full-body radiography revealed a metal density superimposed on the intestinal lumen.
Post mortem CT confirmed the presence of one irregular linear metallic foreign body in the stomach associated with small amounts of pneumoperitoneum with peritoneal effusion. These findings were compatible with perforating foreign body causing infectious peritonitis which has been confirmed later by necropsy (figure 29 and 30).

Figure 29 Transverse CT images of the abdomen of a dead Californian sea lion showing 2 metallic foreign body segments in the gastric body, the ventromedial one in the pyloric antrum is perforated. There is moderate amount of gas in the peritoneal and retroperitoneal regions.
Figure 30 Dorsoventral radiograph of the same case as Fig 29 with an irregular linear metallic foreign body superimposed on the intestinal loops.

The most common indication for the referral cases with death of unknown cause was being shot by gun (table 1).

<table>
<thead>
<tr>
<th>number</th>
<th>Species (genus)</th>
<th>Imaging modality by time order (part)</th>
<th>Sampling</th>
<th>Age</th>
<th>Imaging modality by time order (part)</th>
<th>Sampling</th>
<th>Final Diagnosis</th>
<th>Type of pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dog/F</td>
<td>Rad (WB)</td>
<td>CT-guided biopsy</td>
<td>7 ys</td>
<td>Rad (WB) CT (WB)</td>
<td>CT-guided biopsy</td>
<td>Neoplastic</td>
<td>osteosarcoma</td>
</tr>
<tr>
<td>#</td>
<td>Species</td>
<td>Exam</td>
<td>Procedure</td>
<td>Age</td>
<td>Exam</td>
<td>Procedure</td>
<td>Age</td>
<td>Exam</td>
</tr>
<tr>
<td>----</td>
<td>---------</td>
<td>------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>2</td>
<td>Cat/M</td>
<td>MRI (WB)</td>
<td>US-guided biopsy</td>
<td>11 ys</td>
<td>MRI (WB)</td>
<td>US-guided biopsy</td>
<td>Neoplastic</td>
<td>Mast carcinoma</td>
</tr>
<tr>
<td>3</td>
<td>Cat/F</td>
<td>Rad (thorax)</td>
<td>US-guided FNA</td>
<td>16 ys</td>
<td>Rad (thorax)</td>
<td>US-guided FNA</td>
<td>Neoplastic</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>5</td>
<td>Dog/M</td>
<td>Rad (stifle)</td>
<td>US-guided biopsy</td>
<td>12 ys</td>
<td>Rad (stifle)</td>
<td>US-guided biopsy</td>
<td>Infectious</td>
<td>Lishmanios</td>
</tr>
<tr>
<td>6</td>
<td>Pig/F</td>
<td>Rad (spine), CT (spine), MRI (spine)</td>
<td>US-guided biopsy</td>
<td>7 ms</td>
<td>Rad (spine), CT (spine), MRI (spine)</td>
<td>US-guided biopsy</td>
<td>Infectious</td>
<td>Purolent discospond</td>
</tr>
<tr>
<td>7</td>
<td>Eurasian lynx/M</td>
<td>Rad (WB)</td>
<td>Skeletally immature</td>
<td>-</td>
<td>Rad (WB)</td>
<td>-</td>
<td>Trauma</td>
<td>Gunshot</td>
</tr>
<tr>
<td>8</td>
<td>Bear/F</td>
<td>Rad (skull)</td>
<td>Skeletally mature</td>
<td>-</td>
<td>Rad (skull)</td>
<td>-</td>
<td>Trauma</td>
<td>Gunshot</td>
</tr>
<tr>
<td>9</td>
<td>Crow/U</td>
<td>Rad (WB)</td>
<td>-</td>
<td>Rad (WB)</td>
<td>-</td>
<td>Trauma</td>
<td>Gunshot</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Crow/U</td>
<td>Rad (WB)</td>
<td>-</td>
<td>Rad (WB)</td>
<td>-</td>
<td>Trauma</td>
<td>Gunshot</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Roe deer/F</td>
<td>Rad (forelimbs)</td>
<td>Skeletally mature</td>
<td>Rad (forelimbs)</td>
<td>-</td>
<td>Trauma</td>
<td>Chronic lux</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Flamingo/U</td>
<td>Rad (legs)</td>
<td>-</td>
<td>Rad (legs)</td>
<td>-</td>
<td>Trauma</td>
<td>Acute frac</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Cat/F</td>
<td>CT (WB)</td>
<td>US-guided aspiration</td>
<td>7ms</td>
<td>CT (WB)</td>
<td>US-guided aspiration</td>
<td>Trauma</td>
<td>Bite</td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>Exam 1</td>
<td>Exam 2</td>
<td>Age</td>
<td>Exam 3</td>
<td>Exam 4</td>
<td>Disease</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>---------------</td>
<td>----------</td>
<td>------------</td>
<td>-----</td>
<td>----------</td>
<td>------------</td>
<td>----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Dog/M</td>
<td>CT (skull) MRI (skull)</td>
<td>-</td>
<td>6ys</td>
<td>CT (skull) MRI (skull)</td>
<td>-</td>
<td>Trauma Blunt hard</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Persian Cheetah/F</td>
<td>Rad (WB) CT (WB)</td>
<td>necropsy</td>
<td>Skeletally mature</td>
<td>Rad (WB) CT (WB)</td>
<td>necropsy</td>
<td>Iatrogenic Implant fail</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Dog/F</td>
<td>CT (WB)</td>
<td>necropsy</td>
<td>11 ys</td>
<td>CT (WB)</td>
<td>necropsy</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Cat/M</td>
<td>Rad (WB)</td>
<td>necropsy</td>
<td>Skeletally mature</td>
<td>Rad (WB) CT (WB)</td>
<td>necropsy</td>
<td>Hallow organ rupture Tracheal perforation</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>California Sealion/U</td>
<td>Rad (WB) CT (WB)</td>
<td>necropsy</td>
<td>12 ys</td>
<td>Rad (WB) CT (WB)</td>
<td>necropsy</td>
<td>Hallow organ rupture Perforating foreign bod</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Mammified fetus (Calf)</td>
<td>Rad</td>
<td>-</td>
<td>-</td>
<td>Rad</td>
<td>-</td>
<td>Congenital Bullgod syndrome</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Covered box</td>
<td>Rad</td>
<td>-</td>
<td>-</td>
<td>Rad</td>
<td>-</td>
<td>Canine bon</td>
<td></td>
</tr>
</tbody>
</table>

- Third group: in case of bog bodies, is it possible to identify the animal and the probable cause of death?

**Question:** Which kind of animal species?

**Case No 19:**

A mummified animal carcass was found and sent as a possible wolf or lynx. Post mortem orthogonal view radiographs of whole body were taken with severe abnormal position due to rigidity of the dead body.

The body and the limbs were shortened and compressed due to reduced length of the spine and the long bones while the head was markedly enlarged compared to the rest of the body.

The appendicular skeleton was grossly deformed. The distal parts of the limbs bilaterally and symmetrically deviated and there were two digits present in the distal limbs.

The face showed severe dysplasia. The calvarium was prominent and dome-shaped and there was an almost 90° angular ventral deviation of the facial bones to the axis of the brain. The frontal and occipital bones were thick and the fontanel was wide.

The mandible and maxilla were the most developed structures with severe pragmatism. Mandibular incisors, premolars and molars were present. Maxillary incisors were absent and the premolar and molars formed but were severely crowded due to the skull deformity. The canine teeth were absent. The dorsal border of the nasal bone had a "bullterrier-like" convex shape. Nasal and ethmoid turbinate bones were present with heterogeneous parenchyma.

The spine and long bones of the limbs showed severe dysplastic features. Their diaphysis was small and misshapen with diffusely increased opacity and the tibiae and the tarsal bones were significantly
thickened. The metacarpal and metatarsal bones were severely shortened and the proximal. The vertebrae were compressed. There was widespread failure of fusion of the right and left parts of the dorsal spinous processes of the thoracic and lumbar spine. Some ribs were present with cranial orientation. The thorax and abdomen volume was reduced and the enclosed organs were not delineated.

The radiographic appearance of the generalized congenital chondrodysplasia associated with absence of the maxillary incisors and canine teeth and presence of 2 distal phalanxes in the limbs were in favor of a ruminant. The deformities of the skull and limb conformation resemble changes seen in "bulldog calf syndrome" (Watson et al., 2017) (figure 31)

Figure 31 A lateral radiograph of a weird mummified animal carcass with shortened and compressed limbs, small misshapen vertebral bodies and markedly disproportional enlarged skull compared to the rest of the body.

Case No 20:

A Pandora’s Box of bones, submitted to search for projectiles, fractures, or other trauma. To prevent potential loss of a projectile or other important forensic material, the bag could not be opened.

Multiple mature bones with associated soft-tissues and multiple gas inclusions were randomly distributed and partially superimposed with each other in a squared like-recipient. A normal body conformation was not visible. No obvious fractures were identified; instead, multiple joints were completely luxated. Both mandibular rami were separated from the rest of the skull and maxilla. Only a short vertebral segment with three vertebral segments being completely misshapen and decreased in size and an additional one being subluxated was visible. A few coccygeal vertebrae were identified. No visible scapulae. One independent humerus and another humerus with an associated radius/ulna and carpus/manus were visible. An additional radius/ulna was also visible close by, without associated carpus/manus. The hip was separated from the sacrum and femora. Subluxation of one stifle with
subluxation of the ipsilateral tarsus/foot and complete separation of the other stifle a tarsus/phalanges were notable. Two elongated metal opaque structures are partially superimposed on the animal. No metallic bullets or fractured bones were found. Based on the shape of the shape of the skeletal structures and closed growth plates in addition to presence of several luxations, a skeletally matured canine species was the final answer (figure 32).

![Radiology of a bag of bones without identification.](image)

**Figure 32 Radiology of a bag of bones without identification.**

Discussion

Radiography has been used frequently in veterinary virtopsy (Cooper et al., 2008; Wolosker et al., 2021; Thali et al., 2007; Ibrahim et al., 2012). Serial postmortem imaging of the thorax and abdomen in dogs and postmortem radiographic findings in the abdomen of cats are described (Cavard et al., 2011; Cooper et al., 2008; Wolosker et al., 2021; Hamano et al., 2014; Ibrahim et al., 2012; Heng et al., 2009). Postmortem imaging of projectile trauma in wildlife is also performed routinely (Thali et al., 2007; Heng et al., 2008). Postmortem CT imaging confirmed sudden death due to skull trauma in caged layer chickens by a moving feeder hopper (Morrow et al., 2012). A few reports of postmortem evidence based cross-sectional imaging to find the cause of death found in the veterinary literature (Hostettler et al, 2015; Thali et al., 2007; Hamano et al., 2014). Postmortem CT-guided biopsy was described in Bernese mountain dogs with suspected histiocytic (Hostettler et al, 2015). Histiocytic sarcoma was confirmed in 10 of 11 dogs by this technique. Two additional reports involve postmortem imaging of exotic animals leading to final diagnoses have also been published (Hamano et al., 2014; Gascho et al., 2020). In our series the post mortem protocols included 11 whole-body and partial CT scans, 4 partial MRI examination which is unique in case of the number. The use of CT and MRI are also described to be valuable imaging modalities for noninvasive necropsy in animals (Watson et al., 2017). CT and MRI have been performed to examine a gravid Boa to confirm fetal death (Gascho et al., 2020). Virtopsy also allowed chronological age estimation by the degree of epiphyseal ossification and closure as well as
bone mineral density evaluation by quantitative CT, both of which may contribute to the assessment of biological health and profiles c

In our study, virtopsy was successful in detecting three neoplastic and three infection ante-mortem pathologies out of the 6 referred cadavers which can open a horizon in post mortem veterinary clinical investigations. Based on the modality selection, in 5 cases, plain radiology was the first modality of choice followed by MRI, CT scan, and/or ultrasonography based on the radiographic findings.

Our results also show that, in a series of unselected deaths referred, that the discrepancy exists between autopsy and imaging causes of death in 91.7 % of cases. Overall, radiology was the most used modality. The most common reason for death of unknown cause were being shot by gun following by trauma which were unique in our casemix. This is almost agreed with the other studies, which discussed the importance of virtopsy in the evaluation of gunshot injuries (Watson et al., 2017; Benetato et al., 2011). It is also mentioned as the most accurate means of forensic veterinary analysis of gunshot wounds in animals (Watson et al., 2017).

The identification of wired and bizarre cadavers are occasionally involved in forensic investigations. If there is a suspicion of human body remnants, clinic-legal examinations are required. However, these containers are occasionally locked (Watson et al., 2017). Computed tomography was able to reveal the content of a highly radiopaque forensic Pandora’s Box successfully (Gascho et al., 2018). In the present study, radiology was able to reveal the contents of such a strange corpses.

In general, we recommend to follow the below guideline in daily veterinary practice:
- Get the clear history and the main forensic, clinical or legal question(s).
- Take plain radiographs from the whole body and try to find:
  - The general aspect of the cadaver (dry bones, mummified body, fragmented, putrefied, intact, carbonized).
  - The body conformation and the proportion of the organs compared to the axial skeleton.
  - Investigate the skeletal structure to find age, spices and if possible sex.
- If the radiographs could not address the questions, chose cross sectional imaging following by ultrasound-guided sampling of the suspicious lesions.

Postmortem imaging in veterinary practice has one clear advantage and one major problem as compared to clinical imaging: absence of motion artifacts, whereas lack of standard projection and lack of circulation; however, couple of publications describing using polyethylene glycol iodinated contrast medium have been recently released (Wolosker et al., 2021; Gascho et al., 2018).

**Conclusion:**

The case series in our “virtopsy” project shown that using diagnostic imaging technique is feasible in answering different clinical ante-mortem and post-mortem clinical and forensic questions; however, there is an interdisciplinary collaboration between the diagnostic imaging and sampling under imaging guidance. In addition, this study was the first to provide SOP guideline in veterinary virtopsy to reduce the time, manage the quality and facilitate the entire workflow in a more effective and efficient manner.

Acknowledgement:

The authors appreciate the efforts of Dr. Iman Memariyan (Chief Veterinarian of Tehran zoo and Pardisan Rehabilitation Centre).
امکان سنجی استفاده از ویرتوپسی مبتنی بر شواهد برای پاسخ به سوالات احتمالی بالینی و پس از مرگ، در دامپزشکی

چکیده:

معاینه پس از مرگ بخش مهمی در پزشکی مبتنی بر شواهد برای درک بدترب شدن علائم بالینی یا علل مرگ در حیوانات یا حتی جمعیت‌های کشتندگی به مرده است.

هدف از تجزیه و تحلیل پس از مرگ، به‌ویژه درمان و درمان بالینی، تأیید تشخیص‌های مورد شک، مدیریت استراتژی‌های اصلاح نزدیک یا روشن شرایطی است که منجر به مرگ در موارد پزشکی قانونی شده است. در قیاس با ویروسپسی در پزشکی انسانی، روشهای تصویربرداری تشخیصی در زمان‌های پس از مرگ که ما آن را Vetvirtopsy می‌نامیم، استفاده شده‌است. ما افرادی که علی‌رغم چگونگی گذشت پس از مرگ، البته ممکن است در سوالات بالینی/پس از مرگ خاص برای افزایش اطمینان Vetvirtopsy در واقع می‌تواند به عنوان روشن‌کننده برای سوالات مورد استفاده مطرح شود. هدف از این مطالعه مرور مقایسه ای Vetvirtopsy با کالبد‌شناسی معمولی در علی‌متغیر مرگ و معیار در حیوانات و تعیین امکانات و محصولاتی که از آن استفاده می‌شود.

بدین منظور حیواناتی که محروم مرده یا معدوم شده و حیوانات بالینی جمع آوری شده، روش‌های مورد استفاده تکنیک‌های تصویربرداری مانند رادیوگرافی دیجیتال پس از مرگ، سونوگرافی پس از مرگ، توموگرافی کامپیوتری پس از مرگ و توموگرافی تشخیصی پس از مرگ در ترکیب با نمونه‌برداری بافت (هدایت‌شده با تصویر) برای پاسخ‌گویی به سوالات پاسخ می‌دهند.

مجموعه موارد این مطالعه نشان داد که استفاده از تکنیک تصویربرداری تشخیصی در پاسخ به سوالات مختلف بالینی پیش و پس از مرگ و پزشکی قانونی امکان‌پذیر است.
References:


امکان سنجی استفاده از ویرتوبسی مبتینی بر شواهد برای پاسخ به سوالات احتمالی بالینی و پس از مرگ، در دامپزشکی

محمدملازم،1 ارزو رمضانی،2 سارناگ سروری،1 زهرا جعفری گیو،3 سارا شکوری،2 اورس گیسپوهلر2

گروه جراحی و رادیولوژی دانشگاه دامپزشکی، دانشگاه تهران، تهران، ایران

گروه دامپزشکی بالینی، بخش رادیولوژی بالینی، دانشگاه Vetsuisse برن، دانشگاه برن، برن، سوئیس

گروه بالینی، دانشگاه دامپزشکی، دانشگاه تهران، تهران، ایران

معاینه پس از مرگ بخش مهمی در پزشکی مبتینی بر شواهد برای درک بدتر شدن علل علائم بالینی یا علل مرگ در حیوانات یا حتی جمعیت‌های کشتی‌نشین یا مرده است. هدف از تجزیه و تحلیل پس از مرگ، بهبود درمان و درمان بالینی، تأیید تشخیص‌های مورد شک، مدیریت استراتژی‌های اصلاح نژاد یا روش‌کردن شرایطی است که منجر به مرار در موارد پزشکی قانونی شده است. در قیاس با ویرتوبسی در پزشکی انسانی، روش‌های تصویربرداری تشخیصی در دامپزشکی پس از مرگ که در حالی است که می‌تواند به عنوان روشی برای سوالات بالینی/پس از مرگ، خاص برای افراد مشاهده تمایل از شخص اطمنان تشخیص استفاده شود. در برخی سوالات، Vetvirtopsy می‌تواند به گونه‌ای که Vetvirtopsy ما فرص می‌کنیم که می‌تواند جایگزین کالبدگشایی معمولی شود. در برخی سوالات، Vetvirtopsy با کالبدگشایی معمولی در علی‌مغز مرگ و میر در حیوانات و تعیین امکانات و محدودیت‌های آن است.

پدید نظر جریان‌های خانگی مرده به مقدم شده و حیوانات وحشی جمع‌آوری شده. روی‌های مورد استفاده تکنیک‌های تصویربرداری مانند رادیوگرافی دیجیتال پس از مرگ، سونوگرافی پس از مرگ، توموگرافی کامپیوتری پس از مرگ و توموگرافی تشدید مغناطیسی پس از مرگ در ترکیب با نمونه‌برداری بلافاصله پس از مرگ و پاسخ‌گویی به سوالات پس از مورد علائم بالینی یا علل مرگ جامع موارد این مطالعه نشان داد که استفاده از تکنیک تصویربرداری تشخیصی در پاسخ به سوالات مختلف بالینی پیش و پس از مرگ و پزشکی قانونی امکان‌پذیر است.

ویرتوبسی، رادیوگرافی، سونوگرافی، توموگرافی کامپیوتری، توموگرافی رزونانس مغناطیسی