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Role of PET-CT imaging system in diagnosis of spontaneous lesion in laboratory New Zealand White rabbit: A case report

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Abstract

Recently procured New Zealand White rabbits were brought to the department and kept for quarantine in the animal house facility. Out of 5 rabbits, one rabbit was anorexic, corner seating setting, and could not respond to the physical stimuli. On physical examination, we found the raised subcutaneous nodule covered under fur at lateral side of the face. PET-CT imaging was done which indicated the central area of dead tissue with peripheral uptake of 18F-FDG. Fine needle aspiration was performed and collected sample was subjected for bacteriology and cell cytological examination. Cell cytology showed many degenerated heterophils, few RBC's, and other cellular debris. Predominantly Klebsiella and few *Escherichia coli* bacterial colonies were grown on agar. A broad spectrum Enrofloxacin antibiotic was given to the other animals as a preventive measures to control the infection

Key words: Bacteria, cell cytology, PET-CT imaging, rabbit.

Introduction:

Commonly, all animal facility investigators expect disease-free animals in their facility. A new infection can arise in the animal house facility where the quarantine procedure is compromised. In rabbits, diseases are occurred mostly with the involvement of respiratory and Gastrointestinal system infected by *Pasteurella multocida, Escherichia coli*, *Klebsiella pneumoniae*, *Bordetella bronchiectasis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Marlier *et al.,*2000). Enterobacteriaceae family bacteria is most prevalent in the natural environment and infecting to humans and animals, where in *Klebsiella pneumoniae* and *Escherichia coli* are the most commonly occurring opportunistic pathogens infecting dogs, cats, foal, cow, birds and laboratory animals like rabbit. *Klebsiella pneumoniae* bacteria was most commonly isolated from pneumonic lung, suppurative infections, cervicitis, metritis, mastitis, wound infections, urinary tract infections and septicaemia (Wu *et al*., 2019). As it covers wide-range of hosts and exhibits the drug resistance to many antibiotics (Partridge, 2011) its prevalence has seen an increasing trend over the period. Hence, its early diagnosis is most important to prevent the case fatality and high mortality in the animals (Reiss *et al*., 2000). The rationale of this study was to explore the utility of a non-invasive PET/CT imaging technique for accurate diagnosis of a spontaneously observed skin lesion in laboratory New Zealand White rabbits and its correlation with cell cytology, tissue histology for confirmation and to establish the further line of treatment.

Case History:

An 8-weeks old, male rabbit with an average body weight of 1 kg and having a traveling history was housed for the quarantine in the institutional animal house facility. The rabbit was maintained under controlled environment of 22 ± 1^0 C., humidity of 55 ± 5% in a 14 h light/10 h dark cycle. The animal was provided with fresh green Lucern grass, carrots and germinated pulses as feed and filtered drinking water ad libitum. On clinical examination rabbit was anorexic, weak, emaciated and responding to the external stimuli. There was no abnormal discharge from natural orifice and normal body temperature. On physical palpation, round subcutaneous, soft nodule with 0.8 cm diameter was observed near the left side of the nostril. After physical examination, a fine-needle aspiration of the subcutaneous nodule was conducted for cytological and bacterial examination. Further, Positron Emission Tomography/Computed Tomography (PET/CT) imaging following administration of [^{18}F]-FDG was performed for analysis of the nodule. The rabbit was anesthetized with Ketamine (22 mg/kg) and Xylazine (5 mg/kg) body weight combination intramuscularly. Around 1.0 mCi of [^{18}F]-FDG was injected intravenously by using central ear vein. Imaging was carried out at various time points with constant supervision of animal till the recovery. Animal expired on the 3rd day of quarantine period, hence tissue samples were collected for histopathology. Macro and microscopic evaluation was done with the aspirated sample. Methanol fixed thin cytological smear was prepared and stained with Geimsa Stain and examined under light microscope for evaluation of microscopic tissue reaction in the nodule.

Results

In this investigation, a spontaneously observed nodule in laboratory animal was analysed by the routine diagnostic techniques. The nodule was round in shape with soft consistency. Fine needle aspiration cytology showed a thick white greenish coloured semisolid fluid (Fig.1. B, C). It was further confirmed by microscopic observation of cell cytology which showed high incidence of degenerated heterophils, few lymphocytes and RBCs (Fig.1. A). Haematology showed relatively increased total neutrophil counts with marginally increased white blood cell count, other parameters were normal except pack cell volume was increased. The histopathological findings demonstrated the area of necrosis, infiltration of polymorphonuclear cells with proliferation of connective tissue (Fig 1. D, E).

In image analysis CT revealed non-invasive round nodular tissue mass with well circumscribed capsule at periphery. It showed no invasion, no bone or peripheral muscle involvement with central area necrosis (Fig. 2). PET image analysis showed peripheral uptake with no uptake in the central area. Normal accumulation of [^{18}F]-FDG in brain, bone marrow, submandibular jaws and guts was observed. Bacterial culture and staining examination revealed lactose fermenting, gram negative, rod to coccobacillus shaped, capsulated and non-motile bacterium suggestive of *K. pneumoniae* and few *E. coli* colonies on agar.

Discussions

A newly procured young age laboratory rabbit was evaluated to find the cause of weakness and emaciation to provide the supportive treatment. During the physical examination a spontaneous nodule was seen and assessed for its type and nature. A keen observation and palpation is a very important step in the quarantine period to provide better care to the animals. Usually, laboratory animals having subclinical infection do not show any clinical signs and symptoms, hence physical examination during the quarantine period can add important insights in the diagnosis for laboratory animals (Hampshire, 2015). Gross palpation of the nodules gives an idea about the soft tissue consistency suggestive of subcutaneous abscess, hematoma and cyst (Vegad, 2007). The greenish white colour of aspired fluid indicates the accumulation of pus at site. This was further confirmed by microscopic cytological observation with degenerated neutrophils and other inflammatory cells suggestive of pyogenic inflammation of subcutaneous tissue with development of abscess (Fig. 1A). Haematological observation goes hand in hand with cell cytology.

In order to get a deeper insight, the nodule was subjected to PET-CT imaging analysis. CT analysis helped to understand the location, extent of the lesion and non-invasive behaviour of the nodule (Huber-Wagner *et al.,*2009). [^{18}F]-FDG uptake in peripheral border of capsule indicate the pyrogenic membrane of the abscess with active cell metabolism of connection tissue and inflammatory cells. However, no uptake in the central part of tissue suggested liquefactive necrosis and accumulation of dead tissue. The overview of these findings is indicative of the inflammatory nature of the lesion (Fig. 1 & Fig. 2). [^{18}F]-FDG accumulation is observed in infection and inflammatory cells such as neutrophils and macrophage where cell metabolism is augmented (Saha, 2010). This finding corroborates well with the FNAC and haematology. The causative organism was bacteria of enterobacteriaceae such as *Klebsiella pneumoniae* and *E.coli* mixed infection involved in the formation of abscess. It was also reported that immunocompromised animals are more prone to the Klebsiella infection (Boucher & Nouaille, 2002; Nemet *et al.,* 2011), This could be one of the reasons it was seen in such young rabbits and having compromised immune status.

The histological observation confirmed the subcutaneous abscess by its characteristic central area of necrosis with pyrogenic member and peripheral connective tissue capsule membrane that serves as a barrier and limiting its further spread as shown in Figure 1. D, E (Vegard, 2007). The histopathological findings are very well corroborated with PET/CT image analysis. This case was incident during the quarantine period, hence quarantine practice is must and it will decrease or eliminate the exposure risk to colony animals and create a safe environment in the animal house for the investigator working with animals.

Conclusion: In conclusion, the quarantine procedure is the key procedure to keep laboratory animal house environments safe for animals as well as researchers. This study clearly demonstrates that the use of non-invasive PET-CT imaging in combination with routine cytology and microbiological impression could enhance the accuracy of the diagnosis and confirmation of the lesion. PET/CT imaging can be promoted as a tool for early and differential diagnosis of lumps, nodules, inflammatory lesions in laboratory veterinary practices.

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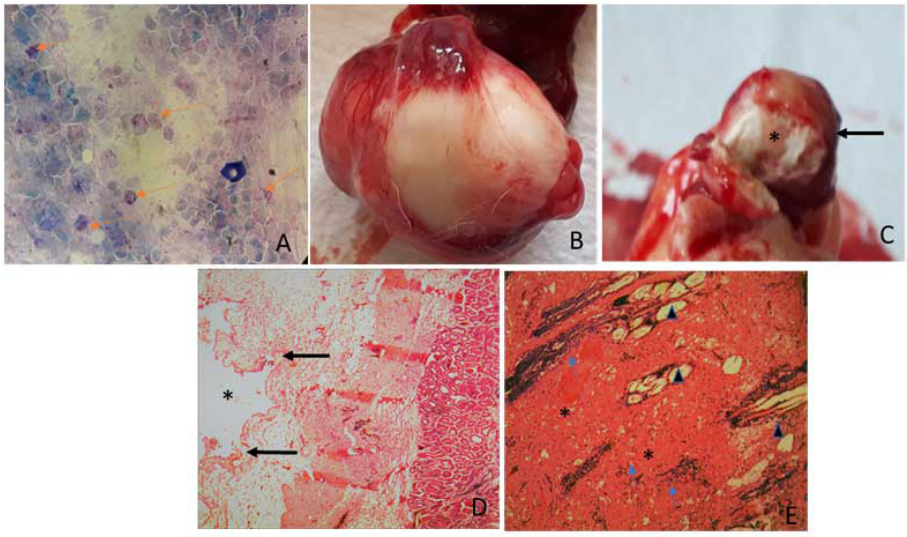
Figure.1. Cell cytology and Histopathology of the nodule:



A) Degenerated heterophiles(arrow) with few RBCs stained with Geimsa stain100x.,

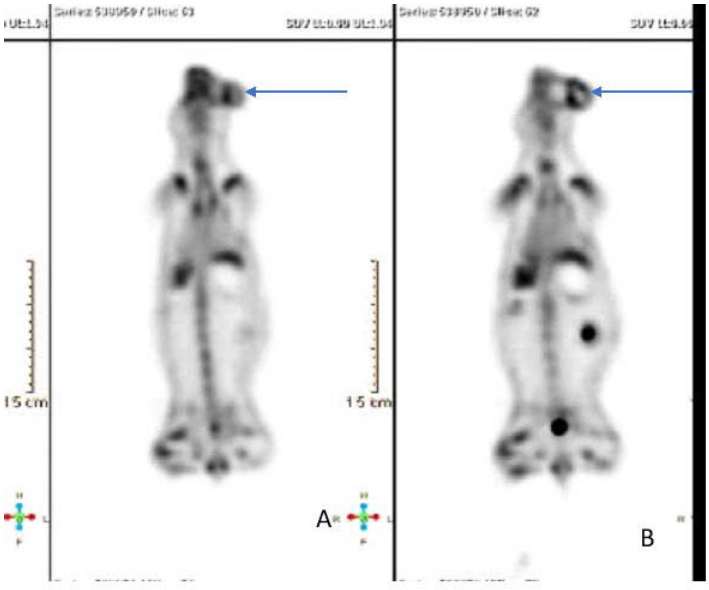
B) Nodule on grossly round.,

C) Grossly cut surface (black arrow) with cream white coloured pus inside (*).,

D) Nodules histopathology showed pyrogenic membrane with connective tissue (black arrow), central cavity of dead tissue (*) stained with H&E stain, 10X.,

E) Involvement of hair follicles (Triangle) with infiltration of polymorphonuclear cells(arrow) and necrotic area (*) H&E stain,5X.

Figure.2. PET-CT imaging of rabbit skin nodule:



A) Subcutaneous nodule with peripheral uptake of ^{18}F-FDG.,

B) Image showed central liquified necrotic tissue area with active peripheral zone (arrow