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# Pathological changes associated with bacterial infection in black bear

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**Research**

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**Abstract**
Two captive American black bears (male and female), male black bear showed sudden death, meanwhile female black bear suffered from depression, anorexia, frothy salivation, weakness and nervous sign. This study performed to reveal the role of pathology in diagnosis of zoo animal medicine by analysis clinical, hematological, biochemical and histopathological investigation to diagnoses and evaluate disorders in captive wild animals and determine the etiology of death.
Tissues samples were internal organs (kidney, liver and intestine) from male bear, vomitus and fecal swab from female bear.
No viral infection could be detected. The results of bacteriological investigation revealed positive *Clostridium perfringens*-alpha toxin as detected by PCR techniques in samples of female bear.
Histopathological examination of male black bear, renal tissue revealed that most of the epithelial cells lining Renal tubules were degenerated associated with interstitial hemorrhages. Glomerular oedema, hyalinization of glomerular tuft and/or glomerular hemorrhages (rupture of glomerular tuft). While hepatic tissue showed disorganization of hepatocytes accompanied with oedema (dilated sinusoids). Intestinal tissue seen degeneration of two third of intestinal villi accompanied with round cells infiltration. Many villi showed pluntted edges with activation of glandular epithelium.
Hemogram in female black bear demonstrated dehydration state and marked leucopenia.
This study provides information about histopathological lesions and hemogram interpretation of two captive bears as reference values and baseline for future understanding the clinical cases of wild animals.

***Keywords:*** *American black bear, Clostridium perfringens, endotoxins, histopathology, hematology, blood chemistry.*

## Introduction

Management, medicine and nutrition considered as fundamental roles for wild animals in captivity. Zoos holding animals, with each being housed separately, two animals or even if a large number is in the collection, that differ than nature of wild life.
Zoonoses can be acquired from birds, the most common ones are bacterial, also the list includes those with viral, fungal and parasitic causes **(Fowler, 1999).**
Giant pandas are subjected to many of infections diseases of other carnivorous as tuberculosis, canine distemper and Escherichia coli septicemia **(Qui and Mianka, 1993).**
**Zachary (2017)** mentioned that pathogenesis of focal encephalomalacia begins as an enterotoxemia caused by *Clostridium perfringens* in small intestine. Acute coagulative necrosis of cells and tissues by bacterial toxins is the

mechanism of injury. Gross lesions include bilaterally symmetrical malacia (represented in acute coagulative necrosis of neuron cell bodies) and liquefactive necrosis of basal ganglia and thalamus with edema and hemorrhage. Cerebral edema leads to indistinct sulci and flattened gyri. The sequence of steps causing focal symmetric encephalomalacia occurs in first phase or early in the second phase of enterotoxemia before toxin-induced massive necrosis of intestine. Also, acute coagulative necrosis of neurons cell bodies which attributed toxin-induced micro-thrombosis of capillaries, leading to neuronal ischemia. Experimental studies suggest that bacterial toxins diffuse across mucosae may injury firstly endothelial cells in capillaries of lamina propria before attachment of bacterial to apical surface of enterocytes. Potent cytotoxins produced by C. perfringens include alpha, beta, epsilon and iota, which consider as enterocyte membrane toxins.

Myelocytes have a key role in granulocyte production. It is believed that such production is expanded by increasing in the number of myelocytic division and by shortening of generation time **(Coles, 1986).**
Neutrophils and eosinophils contained numerous nonmembrane-bound inclusions in cytoplasm which contained lipids (lipid bodies). These findings suggest that in the brown bear, peripheral white blood cells also are involved in lipid metabolism as agents of transport and storage **(Musiani *et al.,* 1996)** in Apennine brown bears.
A unique mononuclear cell, with a moderately indented nucleus and shared ultrastructural and cytochemical characteristics of lymphocytes and monocytes, was discovered in this species. The combined cytochemical, immunocytochemical (CD3) and ultrastructural features of these unique cells more resemble those of monocytes, but the definitive cell lineage remains unknown at this time **(Kehoe *et al.,* 2020)** in giant panda.
Well-established normal values were recorded in human for water intake and output
Through different routes. Although there is data on water balance for most of domestic animals, these data vary from species to others and are valid for the specific experimental conditions under which they were collected. Koala in its native state (Australia) obtains all its water from leaves of specific species of eucalyptus trees which is its entire diet. Dehydration because of water restriction with and without heat stress, has been studied in different animal species **(Kaneko *et al.,* 2018).**
Dehydration as a disturbance of water may occur when loss of body water exceeds intake. This results from decreased intake with normal water loss or excessive water loss without compensatory increased intake. The principal compensatory mechanism is consumption of water in amounts necessary to reduce the dehydrated state. In addition, antidiuretic hormone (ADH) is controlled water loss through the kidney in response to plasma osmotic pressure**.**
Also, plasma protein concentration increases if there is water loss from extracellular fluid (ECF). Values of blood urea nitrogen frequently follow the same pattern of plasma protein levels **(Coles, 1986).**

The aim of the work: An attempt in this investigation to study the mortality pattern among sudden death and endotoxemia causing by *Clostridium perfringens*- alpha toxin in two captive American black bears from clinicopathological feature to contribute in diagnosis and rapid treatment for emergency cases of wild zoo animals.

## Materials and Methods

### Case history:

Two captive American black bears (male and female) at Giza zoo, male bear was died at age 22 years, while female one at age 20 years. Male black bear showed sudden death, Meanwhile, female black bear suffered from depression, off food, frothy saliva, weakness, nervous signs and isolation from nature. Female bear was died two weeks after sampling. Male black bear was died two weeks after female one during the year of 2021.

### Sample collection:

Tissue specimens for histopathological examination were collected from internal organs (Kidney, liver and Intestine) of male black bear for bacteriolgical and histopathological examinations. Tissues for pathological investigation

fixed in 10% neutral buffer formalin. They were routinely processed by standard paraffin embedding technique, sectioned at 4 microns and stained with hematoxylin and Eosin **(Suvarna *et al.*, 2012).**

Samples from female bear (vomitus and fecal swab) were collected for bacteriological and virological investigations.

Two blood samples were collected from female black bear. Hematological results were obtained from the EDTA-anticoagulated blood and the other one without anticoagulant for separation of serum for biochemical analysis.

Hemogram including erythrocyte count (RBCs), hemoglobin (Hb), packed cell volume (PCV), total and differential leucocytic counts (WBCs), as well as values of red blood indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and Giemsa stained blood film for leucocyte differentiation were determined according to **Kerr (2002).**

Serum biochemical analysis for alanin - amino transferase (ALT) was determined colorimetrically according to **Reitman and Frankel method (1957),** total protein (TP) was determined according to Burtis and Ashwood (1999), albumin according to Douman et al. (1971), globulin was determined by difference between TP and albumin and albumin globulin ratio (A/G ratio). Urea concentration in serum was measured according to **Tietz (1995) and creatinine according to Kroll *et al.* (1987).**

Virological investigation of the suspected specimens was performed at the virology department, Animal Health Research Institute.

Bacteriological investigation was done at Avian reference lab (Animal Health Research Institute) by PCR.

## Results

### Pathogenic identification:

Bacteriological isolation from internal organs (kidney, liver and intestine) of male black bear indicates negative results for clostridium species.

### No viral infection could be detected:

As reported by the Avian Reference Lab (Animal Health Research Institute), the results of the bacteriological investigation revealed positive *Clostridium perfringens*- alpha toxin as detected by PCR technique in samples of female bear.

### Histopathological results:

Concerning kidney, the histopathological examination revealed that most of the epithelial cells lining Renal tubules were degenerated associated with interstitial hemorrhages. Glomerular oedema, hyalinization of glomerular tuft and glomerular hemorrhages (rupture of glomerular tuft) (Fig. 1 a, b, c).

The histopathological examination of the liver revealed disorganization of hepatic cells (Fig. 2) accompanied with oedema (dilated sinusoids).

As detected by histopathological examination, different parts of Intestine showed degeneration of two third of intestinal villi accompanied with round cells infiltration specially at the base of villi (Fig. 3a). Some other specimens showed slouching of the epithelium, pluntted edges accompanied with activation of glandular epithelium (Fig. 3b).

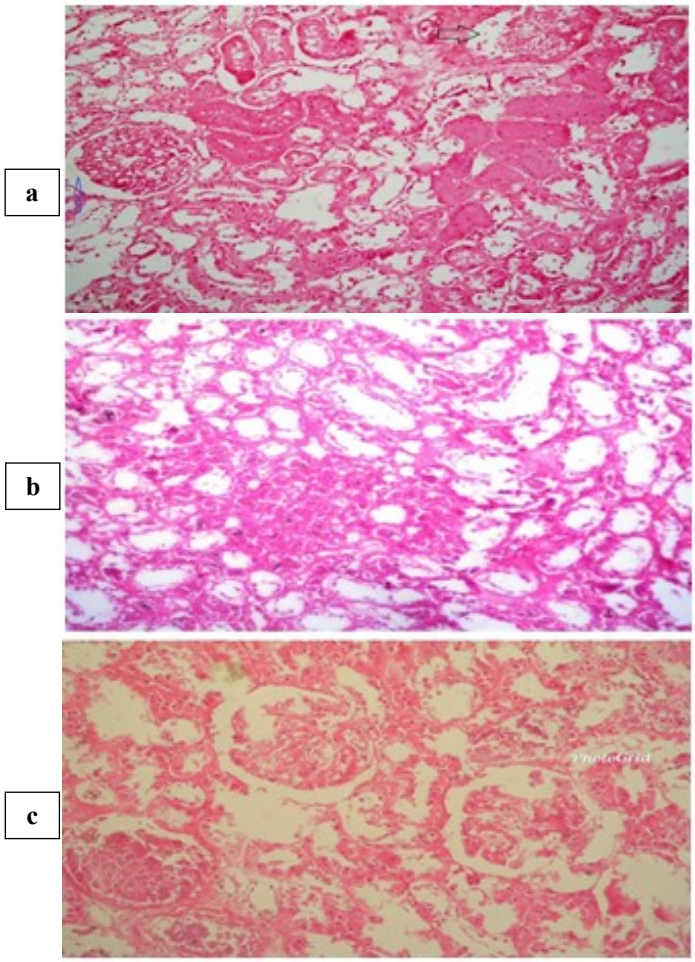



**Fig. (1a):** Kidney showing massive desquamation of tubular lining epithelium accompanied with coagulative necrosis of many renal tubules in addition to glomerular hemorrhages (black arrow) and oedema (blue arrow) are obvious (H&E, X 400).
**Fig. (1b):** Kidney showing degeneration of the epithelial cells lining the renal tubules associated with interstitial hemorrhages (H&E, X400).
**Fig. (1c):** Kidney showing glomerular oedema, hyalinization of glomerular tufts and glomerular hemorrhages (Rupture of glomerular tuft) (H&E, X400).

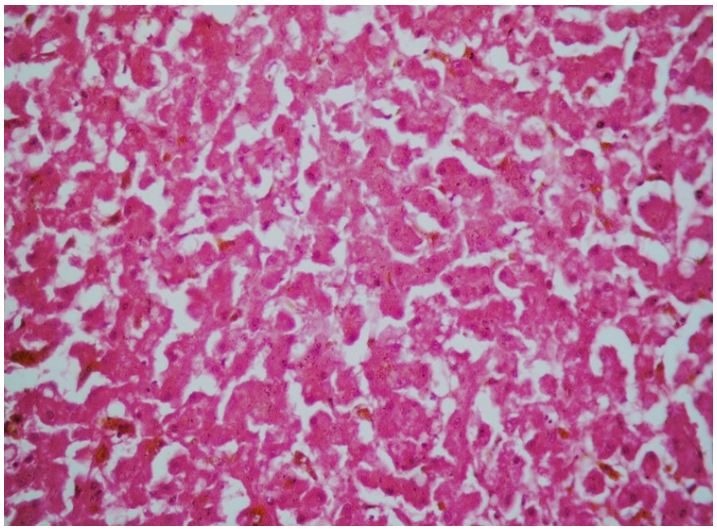
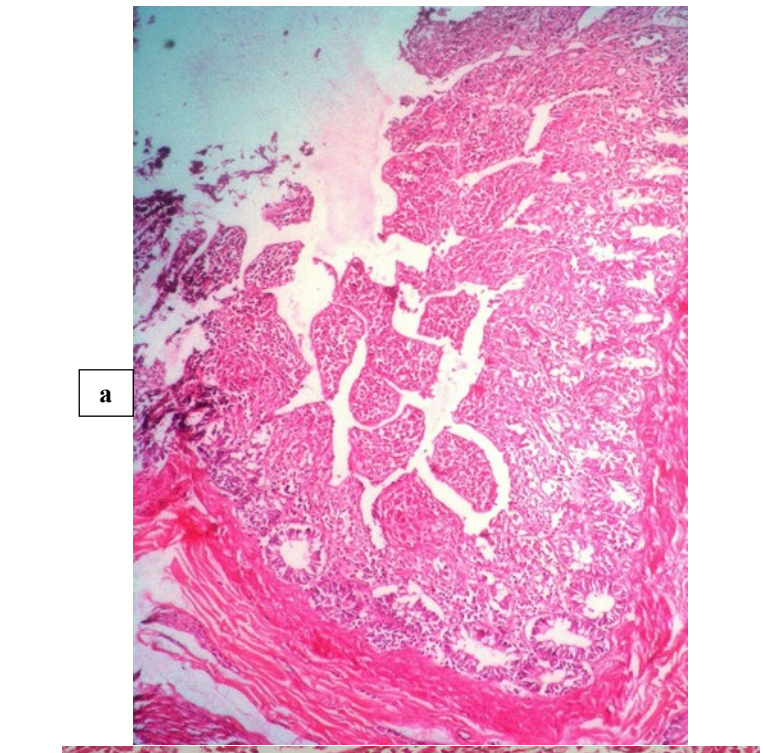


**Fig. (2):** Liver showing disorganization of hepatic cells accompanied with oedema (dilated sinusoids) (H&E, X 400).



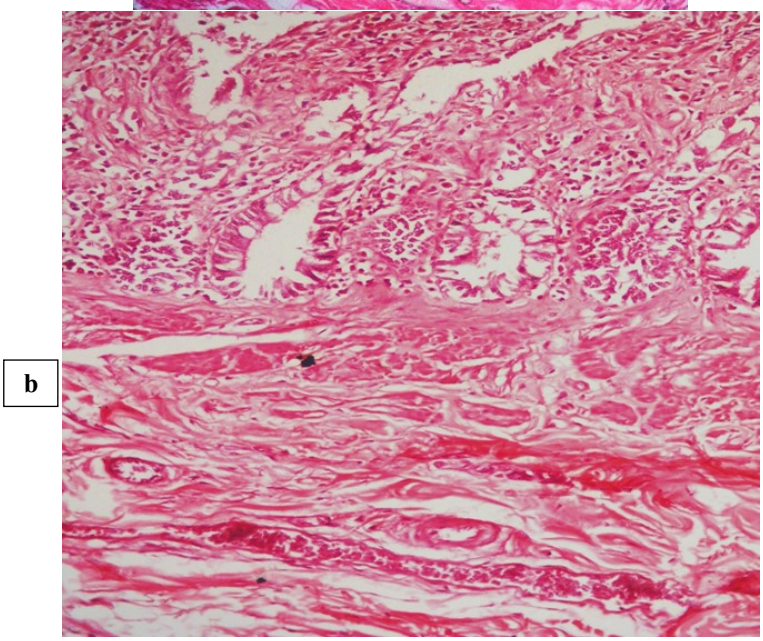




**Fig. (3a):** Intestine showing degeneration of intestinal villi accompanied with round cells infiltration specially at the base of villi (H&E, X 400).
**Fig. (3b):** Intestine showing sloughing of the epithelium, pluntted edges accompanied with activation of glandular epithelium (H&E, X400).
Hematological results:

**Tables (1&2)** Demonstrated changes in the hemogram of female black bear. The erythrogram showed increase of RBCs count with increased in Hb content and PCV value (dehydration state). The leucogram showed high decline level of total leucocytic count, while neutrophils were the predominant cells in Giemsa stained blood film.

**Table (3)** Demonstrated changes in serum biochemical constituent of female black bear. High normal level was observed in total protein value

**Table (1).** Values of erythrogram of infected American black bear (female) with *C. perfringens* (alpha toxin).

| Parameters | Total RBCs ($X10^6$/µl) | Hb (g/dl) | PCV (%) | MCV (fl) | MCH (pg) | MCHC (g/dl) |
|---|---|---|---|---|---|---|
| **American black bear ( Female )** | 12 | 15 | 48 | 40 | 12.5 | 31.25 |

**Table (2).** Values of leucogram (absolute values $X10^3$/ µl and percentage) of infected American black bear (female) with *C. perfringens* (alpha toxin).

| Parameters | Total WBCs | Neutrophils | Lymphocytes | Monocytes | Eosinophils | Basophils |
|---|---|---|---|---|---|---|
| **American Block bear (female)** | 2.95 | 2.21 (75%) | 0.44 (15%) | 0.30 (10%) | 0 (0%) | 0 (0%) |

**Table (3).** Blood chemistry values of infected American black bear (female) with *C. perfringens* (alpha toxin).

| Parameters | ALT (U/L) | Total protein (g/dl) | Albumin (g/dl) | Globulin (g/dl) | A/G ratio | Urea (mg/dl) | Creatinine (mg/dl) |
|---|---|---|---|---|---|---|---|
| **American block bear (female)** | 11.36 | 6.52 | 4.25 | 1.99 | 2.1 | 31.1 | 1.12 |

## Discussion

Bears have important values as a representative species for global wildlife and consider charismatic members of zoological collections worldwide. Conservation efforts and studies describe the data to accurately identify and characterize the Pathological lesion, hematological changes and biochemical alterations to aid in diagnosis in dead tissue and infected animal.

In renal tissue of male bear, some renal tubules showing coagulative necrosis. Massive desquamation of tubular lining epithelium and effusion of erythrocytes within the tubular lumen were observed. Thus, blood may be present in urine (hematuria). **Coles (1986)** reported that hematuria with the presence of intact erythrocytes in urine may occur in association with disease of the genitourinary system. Author added that among the conditions resulting in hematuria are: pyelonephritis, cystitis, pyelitis, neoplasms of kidney and acute nephritis.

Hepatic tissue of male bear revealed disarrangement of hepatic tissue and coagulative necrosis accompanied with dilated hepatic sinusoids by oedema. On my opinion, the detected hepatic lesions may be due to pathological lesion in cardiac tissue which it could be affecting on the flow of the hepatic blood system causing hepato-cardiac disorder that causing sudden death of male bear.

While, the intestinal tissue of male bear showed desquamation of epithelial lining of intestinal villi with inflammatory cells infiltration in submucosa and degeneration of epithelial lining of intestinal glands. The affected

intestinal villi and glands demonstrated a digestive problem, that added more risk on animal.

Hematological characters and serum chemistry are becoming increasingly important diagnostic tools for the living animal.
In this study, blood picture of female black bear demonstrated higher levels of erythrocyte count, hemoglobin content and packed cell volume value as compared with hematological Profiles of Formosan Black Bear (**Chang *et al.* 2006).** These results suggested that Female suffered from dehydration status. **Coles (1986)** recorded that methods for evaluating the status of body fluid are measurements of packed cell volume, hemoglobin level and total erythrocyte count. The author added that total plasma protein concentration is increased, if there is water loss from extracellular fluid (ECF).
In the current study, blood chemistry analysis of female bear illustrated high normal level of total protein value, in comparing with reference intervals of captive Asiatic black bears **(Yang *et al.,* 2017).**
Dehydration raises the risk of blood clots. That may cause blood to thicken and blood vessels to narrow. So dehydration considers one of the risk factors for excessive blood clotting. **Lopez and Martinson (2017)** Recorded that dehydration increase the mucus viscosity which may lead to stopping mucociliary movement.
Dehydration results from inadequate fluid intake in the face of normal or increased fluid losses which shared proportionately by intracellular fluid (ICF) volume and extracellular fluid (ECF) volume and other clinical or clinicopathological disorders will be noted until fluid losses become more severe. Hypernatremia is associated with contraction of ICF volume and the shrinkage of cells. Hyponatremia is best considered as an indication of a relative water excess **(Kaneko *et al.,* 2018).**
In the present study, the possibility to prolonged fever due to *C. perfringens*-alpha toxin. Some conditions may be associated with a lack of water intake include inability to drink or nervous system disturbances as result of clostridial infection. Specially, the female bear bite unusually his grad and revealed frothy saliva.
**Coles (1986)** explained that excessive water loss may occur in one of the following conditions: prolonged vomiting, sequestration of fluids in the digestive tract, prolonged fever, sweating or uncontrolled polyuria if there is inadequate water intake to compensate for the loss. The possibility that there has been a lack of water intake may be associated with lack of access, in ability to drink, nervous system disturbances and failure of operation of normal thirst mechanism.
Loss of water in female bear may be greater than can be compensated for. The case becomes a life - endangering problem. The author reported that many animals that have lost large quantities of body water may have an alteration in sensorium that prevents compensatory water intake.
Differential count of White blood cells of peripheral blood has an importance in the body reaction to pathogens.
Regards to leucogram, female black bear demonstrated marked leucopenia associated with neutropenia and lymphopenia. Neutrophils were the predominant white cells in Giemsa stained blood film, comparing with the levels reported in hematological Profiles of Formosan Black Bear by **Chang *et al.* (2006) and Musiani *et al.* (1996)** in Apennine brown bears. That related to endotoxemia causing by alpha-toxin of *Clostridium perfringens* infection.
**Coles (1986)** explained that neutrophilia is frequently preceded by decrease in total number of circulating leucocytes. Leucopenia occurs if there is a severe microbial infection, viral-induced disease or endotoxemia. Leucopenia occurs as neutrophils move into tissues. When tissue demand is greater the storage pool is depleted and neutropenia occurs. Neutrophilia occurs in systemic infections such as salmonellosis, pasteurellosis, leptospirosis, or other septicemias is not marked. There is also an increase in immature neutrophils. In tissues, phagocytosis of small particles is the primary function of mature segmented neutrophil, although band neutrophil and metamyelocyte have some phagocytic ability. Neutrophils are associated with inflammatory conditions.

In conclusion, the present investigation provides with histopathological, hematological and biochemical information about two cases of captive American black bears that can be

needed to the medical management, assessment and contribute in diagnosis and treatment of wild animals in captivity.

The accurate diagnosis leading to proper treatment, the results of this study considered as reference values which will aid in the diagnosis of pathological cases and in health of these wild animals in captive environment.

A conservation program must be followed to diminish any risk on general health and mortality. Fundamental roles considering nutritional intake, stress, and refine the applications to assess the healthy body condition.

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