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Histopathological and bacteriological studies of vegetative valvular endocarditis in a dromedary camel (*Camelus dromedarius*)

Naresh Kumar Sharma, S. Rani, Manisha Mehra and Shesh Asopa

Abstract

Vegetative valvular endocarditis is one of the rare primary cardiac diseases in dromedary camel. The incidence of this condition is poorly reported. The present study was described the gross and histopathological alterations in vegetative valvular endocarditis observed in a 15-year-old (Approx.) male camel during postmortem. Grossly, the heart appeared firm on palpation. When it was opened, a mild grade of left ventricular hypertrophy was observed. Irregular vegetation was presented on the left atria-ventricular valve, cause stenosis of valvular space. The vegetation was yellow-red and usually covered by a thin clot of blood. Microscopically, the affected valve was severe thickened and covered with a mixture of degenerated inflammatory cells, leucocytes, necrotic cellular debris, fibrin and bacterial colonies. *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae* were isolated organisms from vegetative growth in present study.

Keywords: Vegetative endocarditis, camel, gross and histopathology

1. Introduction

Cardiac diseases are rarely reported in veterinary cases [1] and the diagnosis is normally made almost at the terminal stage with poor prognosis and generally reported during post mortem in farm animals [2]. Vegetative endocarditis in camels is usually caused by bacterial infection, whether the infection gains entrance by direct adhesion to undamaged endothelium or by hematogenous route [3]. Bacteria-induced vegetative valvular endocarditis is one of the main cardiac disorders in cattle [4]. It is often linked to a primary source of infection and the presence of other infectious lesions, such as mastitis, metritis, arthritis, or liver abscesses [1]. Chronic active infection may lead to sustained or recurrent bacteremia, predispose to animals for development of bacterial endocarditis [5]. The most common isolates from bovine endocarditis are *Arcanobacterium pyogenes*, *E. coli*, *Klebsiella pneumoniae* and *pseudomonas* in cattle. [3, 6]

2. Material and Methods

In present study, a total (n=35) samples of heart were examined, out of them 3 samples showing cauliflower like gross lesion were collected in 10 per cent formal saline for further gross and histopathological examination. The tissues were processed for paraffin embedding by acetone and benzene technique [7]. The tissue sections of 4-5 µm thickness were cut by the help of manual microtome and stained with hematoxylin and eosin staining method [8]. The possible results were recorded by both grossly and histopathologically. A tissue swabs (n=3) were taken from cauliflower mass in nutrient broth for microbiological culture. Maccallum-Good pasture stain used for identification of bacteria's in tissue section of vegetative lesion [8]. All three samples inoculated onto nutrient agar plates, aerobically for 24-48 hours at 37°C and identified by MALDI - TOF MS. (VITEK MS RUO)

3. Result

3.1 Histopathological results

This condition was encountered in 8.57 per cent cases among camels in present study

Grossly: Irregular vegetation was presented on the atria-ventricular valve. The vegetation was yellow-red or yellow grey and usually covered by a thin clot of blood, which were easily

peeled off. The surface of vegetation was friable and small vegetation easily removed to leave a granular, eroded surface on the valve (figure.1). The ulceration was common on the commissars and free margins of valves.



Fig 1: Gross photograph of heart showing yellow-red, small rough vegetative lesion on atria-ventricular valves.

Microscopically: The lesions were consisted of accumulated layers of fibrin and numerous embedded bacterial colonies (Gram positive bacilli) underlined by a zone of neutrophilic infiltration (figure. 2 & figure. 3). Proliferation of connective tissue occurred in chronic cases. The lumen of heart revealed septic thrombus which was attached with the basal valvular endocardium along with the fibrous tissue proliferation. Individual morphology of bacterial colonies were visible and showed bunches of blue colored spherical bacilli (Macullum-Good pasture stain) (figure.4).

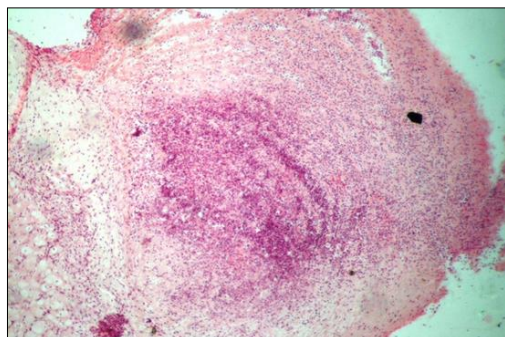


Fig 2: Microphotograph of vegetative endocarditis showing accumulated layers of fibrin and numerous embedded bacterial colonies (Gram positive bacilli) with neutrophilic infiltration. H&E 100x

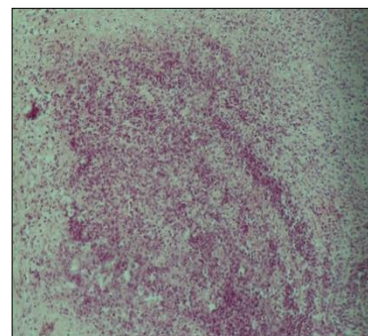


Fig 3: Higher magnification of fig. 2. H&E 200x

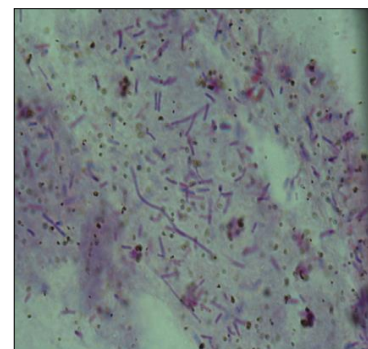


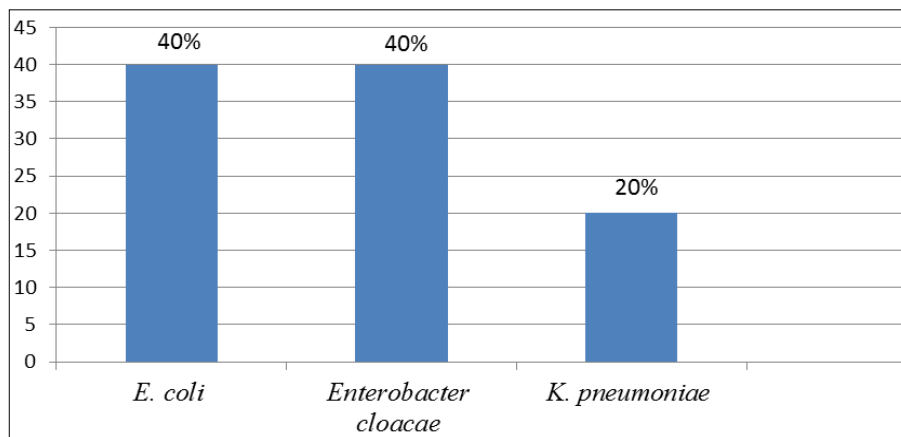
Fig 4: Microphotograph of vegetative endocarditis showing bunches of gram positive bacilli (blue colored). Macullum-Good pasture stain (Oil immersion)

3.2 Bacteriological result

All three samples (n=3) were bacteriological positive, when inoculated onto nutrient agar plates, aerobically for 24-48 hours at 37 °C and identified by MALDI - TOF MS. A total of 5 isolates, belong to only 3 genera were identified. The most predominant microorganisms were *E. coli* and *Enterobacter cloacae* (40 per cent each) followed by *K. pneumoniae* 20 per cent. More than one organism was reported from two samples 66.66 per cent. The Gram negative bacilli were only isolated organisms from vegetative growth in present study.

Table 1: Percentage of microorganisms isolated from vegetative growth in camel

Isolates	No of isolates (n=3)	Percentages
<i>Escherichia coli</i>	2	40
<i>Enterobacter cloacae</i>	2	40
<i>Klebsiella pneumoniae</i>	1	20
Total	5	100



The percentage of microorganisms isolated from vegetative growth in camel.

4. Discussion

This condition was reported as (8.57 per cent) cases in present study. The gross findings of this condition characterized by a mass of cauliflower like growth on atrio-ventricular valve. It was previously noticed by Gutierrez *et al.* ^[6] and Firshman *et al.* ^[9] in camel and alpacas, respectively. Microscopic changes were severely thickened valve and covered with a mixture of degenerated, inflammatory cells, leukocytes, necrotic cellular debris, fibrin and bacterial colonies. These findings were in agreement with earlier research by Gutierrez *et al.* ^[6] in camel. Reef and Mcguirk, ^[5] stated that chronic active infection predisposing to animals for development of chronic bacteremia. The most predominant microorganisms were *E. coli* and *Enterobacter cloacae* (40 per cent each) followed by *K. pneumonia* (20 per cent) reported. It is agreement with previous report by Gutierrez *et al.* ^[6] in camels.

In camel, chronic bacteremia might be associated with jugular venopuncture, naval ill, saddle injury, puncture wound, thorny injury, external and internal abscess, and chronic subclinical internal lesions. Bacteria that are normal constituent of body system, could gain access to generalized circulation, colonizing to organ or system particularly in immune suppressive animals Gutierrez *et al.* ^[6]. Power and Rebhun, ^[10] reported that post-partum uterine infections, mastitis, arthro-sinovites, and liver abscesses act as predisposing factors for chronic bacteremia in immunosuppressive animals.

It is usually caused by bacterial infection entrances by direct adhesion to undamaged endothelium or hematogenous route (Rodasitis *et al.* ^[3]). Bacteria sequestered within the lesion are safe from antibiotic therapy because thickness of lesion and furry wall.

5. Conclusion

In the present study, 35 heart samples examined for vegetative lesions. Out of them 3 samples were showed cauliflower like growth on atrio-ventricular valve. *E. coli*, *Enterobacter cloacae* and *K. pneumoniae* were common isolates from lesion. Naval ill, saddle injury, puncture wound, thorny injury, external and internal abscess were predisposing factors for chronic bacteraemia in immunosuppressive animals and contributed to vegetative lesion.

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