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Article

Case Report: The First Record of zoonotic Enterococcus gallinarum in She Camel feces (Camelus dromedaries)

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Abstract: Enterococcus gallinarum is a well-known normal flora in the human and animal digestive systems. It is an opportunistic microorganism that increased recently due to the massive use of antibiotics. This study aimed to describe the four years she-camel presented to Al-Muthanna Veterinary Hospital suffering from severe watery diarrhea mixed with blood and pus, dehydration, and anorexia. Other clinical signs were dyspnea and icterus mucous membranes. A fecal swab was collected from rectum in a sterile swab and sent to the laboratory for microbiological investigation and bacterial antibiotic sensitivity test. Fecal swabs were culture in selective medium for bacterial isolation. The isolated strain was identified in AL Hussein Teaching Hospital in Samawah using VITEK® system for fast, accurate, microbial identification and antibiotic susceptibility testing and also routine traditional biochemical tests. According to VITEK® system and biochemical test, E. gallinarum was isolated as pure culture from fecal swab. The isolated microorganism was sensitive for Levofloxacin= 0.25, Erythromycin <= 0.25, Linezolid] 2, Teicoplanin < =0.5, Tigecycline <= 0.12, Fostomycin = 32, while it was resistant to Vancomycin= 2, tetracycline >=16. For our knowledge this is the first report that isolated E. gallinarum from diarrheic she-camel. The intestinal bacterial disease showed diarrhea that was accompanied with massive portions of inflammatory exudate which contains blood, pus, and proteinaceous material. Precautionary measures need to be taken to avoid the severe draw back consequences of E. gallinarum infection.

Keywords: Camelus dromedaries, Enterococcus gallinarum, Camels, VITEK, Iraq, Al-Muthanna

Introduction

11.24 million camels were documented in the Arab world, representing nearly 61% of camel numbers (Al-Yasari, et al., 2023; Al-Yasari, Alsalih & Alsaadawi, 2024). The digestive tract can be an ideal habit for many infectious pathogenic agents (Thwiny et al., 2022; Hameed et al., 2022). Enteric

infections in camels tend to be more prevalent in spring (Luyt et al., 2014). The diarrheic animals showed massive inflammatory exudate, blood, pus, and proteinaceous material in feces (Fairbrother and Nadeau 2019). Diarrhea could be one of the most important killing factors of camel calves up to 6 months of age (Gao et al,2019). Enterococcus infects the gastrointestinal tract of humans and animals (Van et al., 2014). Enterococcus is a lactic acid microorganism of the phylum Firmicutes. Enterococci are gram-positive cocci that frequently manifest in pairs (diplococci) or quick chains, and it is hard to distinguish from streptococci (Gilmore,2002). No data showed that E. gallinarum was recorded in camel feces yet. This case report aimed to explain the new record of Enterococcus in diarrheic female camel for the first time in Al-Muthanna Province in Iraq.

Materials and Methods

A methodology for this study was designed to isolate and identify, using traditional microbiological techniques and advanced diagnostic tools, a diarrheic camel that harbored an Enterococcus gallinarum strain. In sterile conditions, a rectal swab of the camel was collected which was inoculated into sterile brain heart infusion broth, then incubated at 36.5°C for 24 hours. Bacterial growth was promoted and further identification allowed by sub-cultivation of the cultured colonies onto both blood agar and Bile Esculin Azide Agar as selective media. Gram staining reactions, motility and morphological characteristics of the bacteria were observed microscopically.

The isolate was then subjected to further analysis with the VITEK® system for the precise microbial identification and antibiotic susceptibility profiling. This advanced diagnostic system generated adequate data to determine the strain's sensitivity to different antibiotics including Levofloxacin and Linezolid and resistance to the others such as Vancomycin and Tetracycline. All steps in the workflow were in line with standard protocols and consequently reliable and reproducible results were obtained.

Integration of classical culture techniques with modern molecular diagnostics allowed the methodology to successfully detect E. gallinarum in camel excreta in Iraq for the first time. Results indicate that combined use of advanced diagnostic tools will complement traditional methods for identification and effective management of zoonotic pathogens. This method gives insights into resistance of microbes and informs the public health and veterinary strategies to mitigate the risk.

Results

The clinical symptoms alongside She camel have been recorded constantly which were depression, restlessness, diarrhea, partial alopecia (Figure 1 Left Panel) and jaundiced paleness of mucous membranes (Figure 1 Right Panel). Rectal swabs were collected from a 4-year-old female camel in clean, sterilized containers. The sample then was inoculated in brain heart infusion broth and incubated at 36.5 °C for 24 hours (Balakrishna *et al*., 2017). The growing colonies on the broth were sub-cultivated in blood agar and Bile Esculin Azide Agar. The cultivated bacteria were examined under the microscope for their motility, morphology, and staining reactions to gram stain (Danchenko,2019). Consequently, the isolated bacteria were sent to AlHussein Hospital at Al-Muthann University for VITEK examination. This equipment offers high microbial identification and antibiotic susceptibility testing results. The isolate was stored according to Hoefman *et al*., (2012).

VITEC results altogether with culture features showed that the isolated bacteria were *E. gallinarum* (Figur3). It was also clear that the bacterium was sensitive to Levofloxacin, Erythromycin, Linezolid, Teicoplanin, Tigecycline and Fosfomycin. However, *E. gallinarum* was resistance to Vancomycin and Tetracycline.

Figure 1: Clinical signs of She camel.
Left Panel: The main outer signs were depression, restlessness, diarrhea, and partial alopecia. Right Panel: Clinical signs of mucous membrane, the paleness of mucous membranes, and the jaundice were recorded (double arrow).

مديرية صحة المثنى/ قسم المختبرات

Microbiology cart report

Patient name: Ali Husein Ali

Printed Jan 15.2021. 9.45

Lab ID: 1302

Isolate number: 1

selected organism: *Enterococcus galinarum*

Source urine:

Collected

COMMENTS	

Identification information	Analysis time: 4:37 hours
Selected organism	98% *Enterococcus galinarum* Blonumber:536002365733671

Susceptibility information	Analysis time :18.30 hours			State :final	
Antimicrobial	Mic	interperation	Antimicrobial	MIC	Interperation
Cefoxitin screen			Teicoplanin	<=0.5	S
Oxacilin			Vancomycin	2	R
Gentamicin			Tetracyclin	>=16	R
Tobramycin			Tigecyclin	<=0.12	S
Levofloxacin	0.25	S	Fostomycin	32	S
Moxifloxacin			Nitrofuranin		
Inducble clindamycin resistance			Mupinocin		
Erythromycin	<=0.25	S	Rifampicin		
Clindamycin			Trimethprin		
linezold	2	s			

+= Deduced drug *= AES modified **= User modified

AES Finding	
Confidence	Consistent

Bacteriology

Figure 2: The special report of the Central Public Health Laboratory/Al-Hussein Hospital of *Enterococcus galinarum* isolate..

Discussion

DISCUTIONS:

Clinical symptoms, cultivated features of bacteria and VIETIK diagnostic results were used to diagnose *Enterococcus galinarum* in fecal samples. This result is considered as the first record of *Enterococcus galinarum* in camel feces. The *E.gallinarum* infections are associated with serious disorders and can cause acute signs in human such as hepatobiliary or oncohematological issues (Jacopo 2018). *E.gallinarum* can induce other harmful microorganism growth thus worsening the clinical case of the patient (Alsaadawi et al., 2019). The reported signs of this bacteria in camel (by this study) showed that *E. gallinarum* infections were also associated with acute enteric symptoms. The source of infection of *E. gallinarum* can be gastrointestinal or the genitourinary tract (Dargere *et al*., 2002).

The increasing occurrence of *Enterococcus gallinarum*, as a microorganism in the use of antibiotics raises significant concerns for both animal and public health.

Clinical Importance of *E. Gallinarum*

While *E. Gallinarum* is typically considered a part of the gut flora in humans and animals contributing to gut health its emergence as a microbe in certain situations such as weakened immune systems or after antibiotic therapy poses a grave threat. Evading the immune system is a progressive increasing technique to establish infections (Alsaadawi et al., 2021). The case discussed in this research is remarkable because it represents the known instance of *E. Gallinarum* being isolated from a she camel with diarrhea suggesting a change, in how this species can cause harm.

The observed clinical symptoms of diarrhea, with blood and pus, dehydration, and loss of appetite indicate the presence of serious intestinal infections caused by opportunistic pathogens suggesting that *E. Gallinarum* could play a role in worsening gastrointestinal illnesses in livestock (Cecil, Goldman & Schafer, 2012).

Our suggestions focus on two aspects. Firstly, it is important to enhance the accuracy and advancement of techniques, in Iraq for detecting new occurrence of pathogens infections as what happened in the case of COVID-19 to avoid pandemic infections. The government has equipped institutions, with state-of-the-art machines and kits to identify the agent (Alsalih et al. 2021). Secondly, there is a need for treatment programs to consider incorporating remedies that have shown success in treating dangerous pathogens (Al Khafaji et al., 2021; Al Safar et al., 2023; Saeed et al., 2024).

Virulence Mechanisms

E. Gallinarums virulence can be attributed to factors such as its ability to survive conditions and its potential for developing resistance to antibiotics. Enterococci, including *E. Gallinarum* are known for their resilience in environments enabling them to thrive when exposed to antibiotics. The research findings on susceptibility and resistance patterns provide insights into potential treatment strategies for infections caused by this organism. Although the isolate showed sensitivity to antibiotics like levofloxacin and linezolid it displayed resistance to vancomycin and tetracycline. Essential drugs for treating infections. This resistance pattern highlights the importance of the use of antibiotics to prevent the emergence of strains of multiple drugs as emphasized by Wanger et al. (2017).

AntibioticPublic Health Implications

The increase in antibiotic enterococci poses a concern, in both veterinary and human healthcare sectors. The pressure exerted by usage has led to the development of resistant strains complicating treatment choices and raising the risk of severe infections .

The discovery of *E. Gallinarum*, in an environment especially concerning resistance sparks concerns regarding potential transmission between animals and humans and its impact on public health. Since enterococci can pass from animals to humans it is crucial to monitor and control infections in livestock to reduce the risks associated with antibiotic bacteria [Dubin, & Pamer, 2017].

Conclusion

Finding *E. Gallinarum* in a camel has significant implications for both veterinary medicine and public health. This case not only underscores the ability of *E. Gallinarum* to become a pathogen but also stresses the urgent need for continuous surveillance of antibiotic resistance patterns in animal and human populations alike. Future studies should concentrate on unraveling the mechanisms behind *E. Gallinarums* pathogenicity and devising strategies for prevention and treatment in settings. Adopting approaches that promote antibiotic usage track resistance trends and enhance knowledge about microbial interactions within the gut microbiome will be vital, in addressing the challenges posed by this opportunistic pathogen.

Competing interests

No competing interests were disclosed

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Author contribution

The designing of the study, doing the experiments, and writing the manuscript were done by AA and IA. Rewriting the article and revising the whole manuscript and statistical analysis was done by TK.

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